Carbon Monoxide–Releasing Molecules
Characterization of Biochemical and Vascular Activities

Roberto Motterlini, James E. Clark, Roberta Foresti, Padmini Sarathchandra, Brian E. Mann, Colin J. Green

Abstract—Carbon monoxide (CO) is generated in living organisms during the degradation of heme by the enzyme heme oxygenase, which exists in constitutive (HO-2 and HO-3) and inducible (HO-1) isoforms. Carbon monoxide gas is known to dilate blood vessels in a manner similar to nitric oxide and has been recently shown to possess antiinflammatory and antiapoptotic properties. We report that a series of transition metal carbonyls, termed here carbon monoxide–releasing molecules (CO-RMs), liberate CO to elicit direct biological activities. Specifically, spectrophotometric and NMR analysis revealed that dimanganese decacarbonyl and tricarbonyldichlororuthenium (II) dimer release CO in a concentration-dependent manner. Moreover, CO-RMs caused sustained vasodilation in precontracted rat aortic rings, attenuated coronary vasoconstriction in hearts ex vivo, and significantly reduced acute hypertension in vivo. These vascular effects were mimicked by induction of HO-1 after treatment of animals with hemin, which increases endogenously generated CO. Thus, we have identified a novel class of compounds that are useful as prototypes for studying the bioactivity of CO. In the long term, transition metal carbonyls could be utilized for the therapeutic delivery of CO to alleviate vascular- and immuno-related dysfunctions. The full text of this article is available at http://www.circresaha.org. (Circ Res. 2002;90:1045–1053.)

Key Words: carbon monoxide • transitions metal carbonyls • heme oxygenase-1 • vascular function • gene expression

Although it has been known for a long time that carbon monoxide (CO) is generated in the human body, only in recent years have scientists begun to explore the possible biological activities of this gaseous molecule. The main endogenous source of CO is heme oxygenase, which exists in constitutive (HO-2 and HO-3) and inducible (HO-1) isoforms; heme serves as substrate for HO-1 and HO-2 in the formation of CO, free ferrous iron, and biliverdin, the latter being rapidly converted to bilirubin by biliverdin reductase. There is general consensus, supported by extensive published reports, that HO-1 represents a pivotal inducible defensive system against stressful stimuli, including UVA radiation, carcinogens, ischemia-reperfusion damage, endotoxic shock, and several other conditions characterized by production of oxygen-derived free radicals. As part of its physiological and cytoprotective actions, heme oxygenase-derived CO appears to play a major role as neurotransmitter, regulator of sinusoidal tone, inhibitor of platelet aggregation, and suppressor of acute hypertensive responses. In addition, exogenously applied CO has been shown to protect against lung injury in vivo, prevent both production of proinflammatory cytokines and endothelial cell apoptosis, and suppress graft rejection in mouse-to-rat cardiac transplants; all these effects are simulated by transfection of the HO-1 gene. Thus, consistent findings reveal a series of important cellular functions that support a versatile and previously unidentified role for CO. It is interesting that many of the novel properties pertaining to CO have strong analogies with the well-established biological activities elicited by nitric oxide (NO), another gaseous molecule produced intracellularly by a family of ubiquitous enzymes known as NO synthases. Research in the field of NO has been largely facilitated by the development of a variety of organic compounds that spontaneously release NO and can reproduce a physiological or pathophysiological function of NO; however, no attempts have been made so far to identify compounds that are capable of carrying and delivering CO into biological systems.

Our laboratory has been working for the past few years on the characterization of transition metal carbonyls as potential CO-releasing molecules (CO-RMs). These complexes are compounds that contain a heavy metal such as nickel, cobalt, or iron surrounded by carbonyl (CO) groups as coordinated ligands. From inorganic chemical studies conducted on
these substances in vitro, it is known that certain ligands in a metal complex can promote, either sterically or electronically, dissociation of CO. Furthermore, photodissociation and consequent elimination of the CO group(s) following exposure to light has been reported in the case of specific metal carbonyls. The discovery that carbonyl complexes possess such interesting and promising features in vitro prompted us to examine the ability of some of these compounds to promote a physiological response in biological systems. We report here that dimanganese decacarbonyl and tricarbonyldichlororuthenium (II) dimer release CO under appropriate conditions and can elicit specific vascular activities that are reminiscent of those mediated by the HO-1/CO pathway.

Materials and Methods

Reagents

Iron pentacarbonyl ([Fe(CO)5]), dimanganese decacarbonyl ([Mn2(CO)10]), tricarbonyldichlororuthenium (II) dimer ([Ru(CO)3Cl2]), and ruthenium (III) chloride hydrate (RuCl3) were purchased from Sigma-Aldrich Company, Ltd. Tri-dimethyl sulfoxide-dichlororuthenium (II) dimer ([Ru(DMSO)3Cl2]), and ruthenium (III) chloride hydrate (RuCl3) was synthesized as previously described. Hemin (tertirippotrophophyrin IX chloride) and tin protoporphyrin IX (SnPPIX) were from Porphyrin Products, Inc. The guanylate cyclase inhibitor, [1H-2,12-0xidazole[4,3-a]quinoxalin-1-one] (ODQ), was obtained from Alexis Corporation, and polyclonal rabbit anti-HO-1 antibodies were purchased from Stressgen. Horse heart myoglobin, Nω-nitro-L-arginine methyl ester (L-NAME), and all other reagents were from Sigma, unless otherwise specified.

Detection of CO Release

The release of CO from metal carbonyl complexes was assessed spectrophotometrically by measuring the conversion of deoxymyoglobin (deoxy-Mb) to carboxymyoglobin (MbCO). The amount of MbCO formed was quantified by measuring the absorbance at 540 nm (extinction coefficient = 15.4 mol L⁻¹ cm⁻¹). Myoglobin solutions (66 μM final concentration) were prepared fresh by dissolving the protein in 0.04 mol L⁻¹ phosphate buffer (pH 6.8). Sodium dithionite (0.1%) was added to convert myoglobin to deoxy-Mb prior to each reading. Because CO loss from [Fe(CO)5] and [Mn2(CO)10] occurs only by photodissociation, the release of CO was induced by exposing these metal carbonyl complexes to a cold light source and allowing the gas to diffuse through a membrane before reacting with myoglobin (see Figure 1A). In contrast, CO released from [Ru(CO)3Cl2] was quantified by adding aliquots of stock solutions (10 μL) of the carbonyl complex in DMSO directly to the myoglobin solution. All the spectra were measured using a Helios α spectrophotometer.

Nuclear Magnetic Resonance Analysis

The release of CO from metal carbonyl complexes was also assessed by nuclear magnetic resonance (NMR) analysis. All the spectra were recorded at room temperature and measured using a Bruker AMX400 NMR spectrometer. [RuCl3(CO)2] (0.020 g) was dissolved in d6-DMSO (0.5 mL) and the 13C(NMR) spectrum run immediately. The 13CO signals were detected after 23 minutes of signal accumulation. The sample was then left for the 13C NMR spectrum run immediately. In order to compare chemical shifts with the literature and make the assignment of the signals clearer, [RuCl3(CO)2] was first dissolved in DMSO (50 μL), and the solution further diluted in 0.6 mL CDCl₃ prior to NMR analysis. The signals were referenced to the central signals of d₆-DMSO at δ 39.5 and CDCl₃ (deuterated chloroform) at δ 77.0, respectively.

Cell Viability Assay

Bovine vascular smooth muscle cells were obtained from the Coriell Cell Repository (Camden, NJ) and grown in Dulbecco’s Minimal Essential Medium (MEM) as previously described. Confluent cells were treated with different concentrations of [Ru(CO)3Cl2] for various times and cell viability was assessed using a colorimetric assay kit from Promega as previously described.

Immunohistochemistry and Northern Blot Analysis

For immunohistochemistry analysis, sections of heart muscles (5 μm thickness) were treated with 0.3% H2O2 in methanol to block endogenous peroxidase activity. Immunohistochemical staining was performed using rabbit polyclonal antibody against HO-1 (1:1000 dilution) as previously described. Total RNA was then extracted using a modification of the method described by Chomczynski and Sacchi. RNA was run on a 1.3% denaturing agarose gel containing 2.2 mol L⁻¹ formaldehyde and transferred onto a nylon membrane overnight. The membrane was hybridized using [α-32P]dCTP-labeled cDNA probes to rat HO-1 and GAPDH genes, and bands were analyzed using a densitometer as previously described.

Isolated Aortic Ring Preparation

Transverse ring sections of thoracic aorta were isolated from male Lewis rats and suspended under a 2-g tension in an organ bath containing oxygenated Krebs-Henseleit buffer at 37°C, as previously described by our group. The relaxation response to cumulative doses of [Ru(CO)3Cl2] was assessed in aortic rings precontracted with phenylephrine (3 μmol L⁻¹) in the presence of various agonists. Control rings were similarly treated by adding equal doses of DMSO (vehicle) to the organ bath.

Isolated Rat Heart Preparation

Langendorff preparations were performed using male Lewis rat hearts (300 to 350 g) as previously described by our group. Coronary perfusion pressure (CPP), a parameter indicative of coronary vessel contractility, was continuously measured by a pressure transducer connected to the aortic cannula and data analyzed with an AcqKnowledge software (BIOPAC System Inc). Hearts removed either from control rats (vehicle-treated) or from animals that were pretreated with hemin (75 μmol kg⁻¹ IP) the day before were initially equilibrated for 20 minutes on the Langendorff apparatus and then perfused with L-NAME (25 μmol L⁻¹ final concentration) to elicit vasoconstriction. The extent of CPP increase by L-NAME was also monitored over time in hemin-treated animals that received a heme oxygenase inhibitor (SnPPIX, 40 μmol kg⁻¹ IP) 1 hour prior to heart excision and in control hearts that were perfused with buffer supplemented with [Mn2(CO)10] (13 μmol L⁻¹ final concentration). Because [Mn2(CO)10] releases CO only by photodissociation, Krebs buffer containing [Mn2(CO)10] was exposed to a cold light source immediately before entering the aortic cannula.

Animal Studies

Lewis rats (280 to 350 g) were anesthetized by intramuscular injection of 1 mL kg⁻¹ Hypnorm. Specially designed femoral artery and venous catheters were then surgically implanted as previously described and blood pressure monitored continuously using a pressure transducer connected to the aortic cannula and data analyzed with an AcqKnowledge software (BIOPAC System Inc). Hearts removed either from control rats (vehicle-treated) or from animals that were pretreated with hemin (75 μmol kg⁻¹ IP) 24 hours prior to blood pressure monitoring were administered to animals 1 hour or 20 minutes prior to L-NAME injection, respectively.
Statistical Analysis

All data are expressed as mean±SEM. Differences between the groups analyzed were assessed by the Student’s 2-tailed t test, and an analysis of variance (ANOVA) was performed where more than 2 treatments were compared. Results were considered statistically significant at P<0.05.

Results

Characterization of CO-Releasing Molecules (CO-RMs): Detection of CO Liberated From Transition Metal Carbonyl Complexes and NMR Studies

Direct addition of iron pentacarbonyl ([Fe(CO)\textsubscript{5}]) or dimanganese decacarbonyl ([Mn\textsubscript{2}(CO)\textsubscript{10}]) to myoglobin solutions did not result in any appreciable formation of carbonmonoxy myoglobin (MbCO) over time (data not shown). This is consistent with the notion that these two transition metal carbonyl complexes do not release CO unless stimulated by...
Therefore, a source of cold light was utilized to promote CO release from carbonyls by photodissociation. The schematic representation of the device used for measuring CO from [Fe(CO)₅] and [Mn₂(CO)₁₀] is shown in Figure 1A. On illumination, [Fe(CO)₅] and [Mn₂(CO)₁₀] produced a change in the absorbance spectrum of myoglobin; in both cases the distinctive identified spectra were the ones typical of MbCO, as confirmed by bubbling CO gas to a solution of deoxy-Mb (Figure 1B). A gradual increase in MbCO formation was observed over time with [Fe(CO)₅] and [Mn₂(CO)₁₀], respectively (Figures 1C and 1D). Interestingly, and in contrast to the above studied compounds, a different metal carbonyl complex, tricarbonyldichlororuthenium (II) dimer ([Ru(CO)₃Cl₂]₂ in DMSO), rapidly elicited MbCO formation when added directly to a deoxy-Mb solution (Figure 2B). The amount of MbCO formed was dependent on the concentration of [Ru(CO)₃Cl₂]₂ used; a fitting analysis of the saturation curve of MbCO revealed that for each mole of [Ru(CO)₃Cl₂]₂, approximately 0.7 moles of CO are liberated (Figure 2C). The ¹³C NMR spectrum showed that [Ru(CO)₃Cl₂]₂ freshly dissolved in DMSO does not exist as a dimer; in fact, 2 distinct peaks corresponding to tri-carbonyl (1) and di-carbonyl (2) monomers could be identified (Figure 3). The NMR analysis reveals that, during the solubilization process, DMSO acts as a coordinated ligand to ruthenium, thereby promoting the formation of the monomers. Interestingly, the detection of di-carbonyl monomers demonstrates that CO is liberated; the ¹³C NMR spectrum also suggests that the ratio between 1 and 2 is 40:60.

Effect of [Ru(CO)₃Cl₂]₂ on Cell Viability

As there are no previous studies on the use of metal carbonyl complexes in biological systems, it was necessary to evaluate the potential cytotoxic effect of these compounds. Because we noticed that exposure of [Fe(CO)₅] to light gradually resulted in deposition of a green-brown precipitate, we decided to abandon the studies on this metal carbonyl. Treatment of vascular smooth muscle cells for 3 hours with [Ru(CO)₃Cl₂]₂ (0 to 420 μmol/L final concentration) did not promote any detectable cytotoxicity (Figure 4B). Similarly,
cell viability was well-preserved after exposure to this metal carbonyl for 3 hours followed by an additional 21-hour incubation in complete medium. A pronounced cytotoxic effect (50% loss in cell viability) was only apparent after prolonged exposure (24 hours) to very high concentrations (400 μmol/L) of [Ru(CO)3Cl2]. Treatment of cells with the same amounts of vehicle (DMSO) or equivalent molar concentrations of ruthenium (RuCl3) did not cause any appreciable decrease in cell viability over time (Figures 4A and 4C, respectively). In the case of [Mn2(CO)10] (0 to 100 μmol/L), no major cytotoxicity on smooth muscle cells was detected after exposure for 24 hours in complete medium (data not shown).

Vasodilatory Effect of CO Released From [Ru(CO)3Cl2]2
To investigate whether CO released from CO-RMs evokes specific biological activities, we first assessed the effect of [Ru(CO)3Cl2] on vessel contractility using the isolated aortic ring model. Consecutive additions of [Ru(CO)3Cl2] to aortic rings precontracted with phenylephrine elicited a rapid and significant vasodilatation (Figure 5); the extent of relaxation was already pronounced after the first addition of the compound (45% more than control). Interestingly, after extensive washing, the phenylephrine-induced contraction was completely restored in control but not in [Ru(CO)3Cl2]-treated vessels, indicating that CO-RMs produces long-lasting effects (Figure 5). The vasodilatory response mediated by CO-RMs was almost totally abolished when reduced Mb, which avidly binds CO, was added to the buffer. Because CO modulates signal transduction mechanisms also via increased production of cGMP, we investigated the effect of a selective inhibitor of guanylate cyclase (ODQ, 10 μmol/L) on vessel contractility. As expected, ODQ considerably reduced the vasodilatation observed after the first 2 additions of [Ru(CO)3Cl2]; however, it is of interest that the third addition of CO-RM still elicited a substantial vasodilatory action despite the presence of ODQ.

Attenuation of Vasoconstriction by CO-RMs In Vivo: Comparison With Endogenously Generated CO
Additional experiments were conducted to determine the biological activity of CO-RMs on vascular function in vivo and compare it with HO-1–derived CO. Changes in coronary perfusion pressure (CPP) of isolated rat hearts were monitored to examine the effect of [Mn2(CO)10] on coronary vessel contractility. Infusion with the NO synthase inhibitor, NG-nitro-L-arginine methyl ester (L-NAME), caused a time-dependent increase in CPP, which reached a maximum (3-fold) after 30 minutes of perfusion (Figure 6C). Notably, perfusion of hearts with light-stimulated [Mn2(CO)10] (13 μmol/L) significantly delayed vasoconstriction and maintained CPP at much lower levels at the end of perfusion. When the buffer containing [Mn2(CO)10] was not exposed to light, thus omitting the CO-induced release process, the extent of constriction mediated by L-NAME was unaffected (data not shown); in addition, perfusion with manganese...
chloride (negative control) had no effect on myocardial CPP (data not shown). The effect observed with [Mn₂(CO)₁₀] could be similarly reproduced by induction of HO-1 in heart tissue. We previously reported that treatment of rats with hemin results in increased production of cardiac bilirubin, which is equimolar to the endogenously generated CO. Figure 6A and 6B shows that HO-1 protein and mRNA are highly expressed in hearts 24 hours after hemin treatment; interestingly, the immunostaining for HO-1 protein was primarily confined to the vessels of cardiac muscle (Figure 6A, right panel). As anticipated above, the rise in CPP mediated by L-NAME in hemin-treated hearts was markedly attenuated (*P*, 0.05) to an extent similar to that produced by [Mn₂(CO)₁₀] (Figure 6C); predictably, the effect of hemin was completely reversed by tin protoporphyrin IX (SnPPIX), a heme oxygenase inhibitor. In vivo experiments were also conducted to evaluate the usefulness of CO-RMs in modulating mean arterial pressure (MAP) and attenuating the acute hypertensive response. As shown in the Table, intravenous administration of increasing concentrations of [Ru(CO)₃Cl₂]₂ (5, 10, and 20 µmol/kg) produced a transient decrease in blood pressure, which was significant at the highest dose and returned to basal levels within 30 minutes of injection. Interestingly, treatment of animals with [Ru(DMSO)₃Cl₂], which does not contain carbonyl groups (negative control), was totally ineffective, indicating that CO is effectively released from CO-RM in vivo. Oxyhemoglobin saturation remained unchanged throughout the various treatments (data not shown). When rats were infused with L-NAME (30 µmol/kg IV) a rapid and significant increase in blood pressure was observed (*P<0.05); this effect was markedly suppressed by pretreatment of animals with a single infusion of [Ru(CO)₃Cl₂]₂ (20 µmol/kg) prior to L-NAME administration (Figure 6D). Moreover, and in analogy with the data on coronary vasoconstriction in isolated hearts, treatment of animals with hemin resulted in a significant suppression of the L-NAME–mediated hypertensive responses, which once again was totally reversed by blockade of the heme oxygenase pathway with SnPPIX (Figure 6D).

**Discussion**

Because of the strong experimental evidence implicating the heme oxygenase pathway and its products, bilirubin and CO, in the modulation of physiological and pathological processes, we were attracted by the idea of developing substances that could mimic the pharmacological action of heme oxygenase-derived CO. We initially explored the literature to ascertain whether chemical compounds capable of carrying carbonyl (CO) groups had already been described. Interestingly, we found that transition metal carbonyl complexes, which are used as catalysts in organic synthesis, possessed such a property and thus appeared excellent candidates to be tested as CO-releasing molecules (CO-RMs) in biological systems. Our studies focused on iron pentacarbonyl ([Fe(CO)₅]), dimanganese decacarbonyl ([Mn₂(CO)₁₀]), and tricarbonyldichlororuthenium (II) dimer ([Ru(CO)₃Cl₂]₂).
3 compounds had the ability to convert deoxy-Mb to MbCO, implying that CO was liberated from the metal complexes. Unlike [Fe(CO)5] and [Mn2(CO)10], which released CO on exposure to light (see Figure 1A for details), [Ru(CO)3Cl2]2 in DMSO spontaneously promoted MbCO formation after addition to the myoglobin solution. That CO is effectively dissociating from [Ru(CO)3Cl2]2 was confirmed by NMR analysis; indeed, the NMR spectrum revealed the presence of 2 major peaks, which were identified as fac-[RuCl2(CO)3(DMSO)] and cis,cis,trans-[RuCl2(CO)2(DMSO)2], respectively, the tri-carbonyl and di-carbonyl monomers of [Ru(CO)3Cl2]. The existence of di-

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<th>Effect of [RuCl2(CO)3]2 on Hemodynamic Parameters</th>
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<td>Mean Arterial Pressure, mm Hg</td>
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<tr>
<td>Heart Rate, beats/min</td>
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<td>0 min</td>
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<tr>
<td>Vehicle (DMSO)</td>
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<td>78±6</td>
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<tr>
<td>[RuCl2(CO)3]2</td>
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<td>5 μmol/kg</td>
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<td>RuCl3(DMSO)2</td>
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Freshly prepared solutions of [RuCl2(CO)3]2 or RuCl3(DMSO)2 (negative control) were injected intravenously at the doses indicated. Oxyhemoglobin saturation remained unchanged throughout the various treatments (data not shown). Data represent the mean±SEM (n=4). *P<0.05 vs 0 min; †Recording ended after no change.
Carbonyl species clearly indicates that CO can be liberated during the formation of monomers; at present, we cannot exclude the occurrence of a possible equilibrium between the metal and CO in solution, but future studies using ligands different from DMSO will give us more information about the mechanism by which CO is released. The encouraging findings on the biochemical properties of these carbonyl carriers enabled us to design specific experiments and address important questions on the possible biological activity of CO-RMs in the cardiovascular system. Because [Mn2(CO)10] and [Ru(CO)3Cl2] did not exhibit apparent cytotoxicity in a concentration range of 40 to 210 μmol/L, we further investigated whether these 2 metal complexes could modulate vessel contractility ex vivo and blood pressure in the whole animal and analyzed a possible contribution of the guanylate cyclase pathway to this effect. This was a plausible approach because we have previously demonstrated that increased endogenous CO as a result of HO-1 induction in rat aortas markedly attenuates vasoconstriction in vitro and in vivo by increasing vascular cGMP levels.10,11

We report here that [Ru(CO)3Cl2] elicits profound vaso-dilatation when added to precontracted aortic rings. The relaxation process was abolished in the presence of myoglobin and was significantly attenuated by a guanylate cyclase inhibitor (ODQ), confirming that the pharmacological effect mediated by CO liberated from CO-RMs is, at least in part, cGMP-dependent. Despite the presence of ODQ, substantial vasodilation was still elicited after the third addition of [Ru(CO)3Cl2] to aortas, suggesting that CO released from CO-RMs may have accumulated in sufficiently high levels to displace ODQ from guanylate cyclase. Alternatively, elevated CO amounts could circumvent guanylate cyclase and exert relaxation via other independent cellular pathways.30 Notably, the effect of CO appears to be long-lasting as aortic rings previously treated with [Ru(CO)3Cl2] failed to regain contraction on a second challenge with phenylephrine.

The experiments conducted using the isolated heart model confirmed the vasoactive properties of another CO-RM, [Mn2(CO)10]. This metal carbonyl markedly attenuated L-NAME–mediated increase in coronary perfusion pressure; notably, this effect could only be achieved when [Mn2(CO)10] was stimulated by light to release CO. Hearts expressing high HO-1 in the vasculature following treatment of animals with hemin also displayed reduced contractility when challenged with L-NAME, and inhibition of hem oxygenase activity abolished the effect; this confirms the important role of endogenously produced CO in vascular control.10,11 Thus, augmented HO-1–derived CO can profoundly modulate cardiac vessel functions, and this effect can be mimicked by exogenously applied CO-RMs. Finally, our findings on the antihypertensive action of [Ru(CO)3Cl2] in animals support the possible utilization of CO-RMs in vivo. Consistent with a previous report from our group,10 we found that induction of the HO-1 system by hemin pretreatment considerably suppressed the increase in mean arterial pressure elicited by intravenous administration of L-NAME; as observed for the isolated aortic ring and heart preparations, SnPPIX restored the vasoconstrictor responses to L-NAME. Interestingly, infusion of [Ru(CO)3Cl2] strongly attenuated L-NAME–induced hypertension, indicating that CO-RMs do liberate CO once administered in vivo and can reproduce the effects of HO-1–derived CO.

The present study identifies a novel group of substances that are capable of carrying and delivering CO. In a similar fashion to endogenous HO-1–derived CO, CO-RMs also exert biological activities by eliciting vascular relaxation and mitigating both coronary vasoconstriction and acute hypertension. Recent evidence has highlighted the beneficial role of inhaled CO in rescuing HO-1–deficient mice from lethal ischemic lung injury.13 Moreover, potent antiinflammatory and antiapoptotic effects of endogenous CO gas have been described in models of endotoxin-induced inflammation and acute vascular rejection.14,15 The potential of using CO as a therapeutic agent in a variety of disease states is, therefore, strongly emerging. In this context, the newly discovered CO-RMs could facilitate the progression toward pharmaceutical applications as they could be engineered to liberate CO with distinct kinetics and highly selective delivery to target tissues. The accessibility to and utilization of CO-RMs will greatly enhance our understanding on the chemistry of CO, its interaction with intracellular components, and its biological importance in physiology and disease.

Acknowledgments

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References


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