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CO-Releasing Binuclear Rhodium Complexes as Inhibitors of Nitric Oxide Generation in Stimulated Macrophages

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Supporting Information

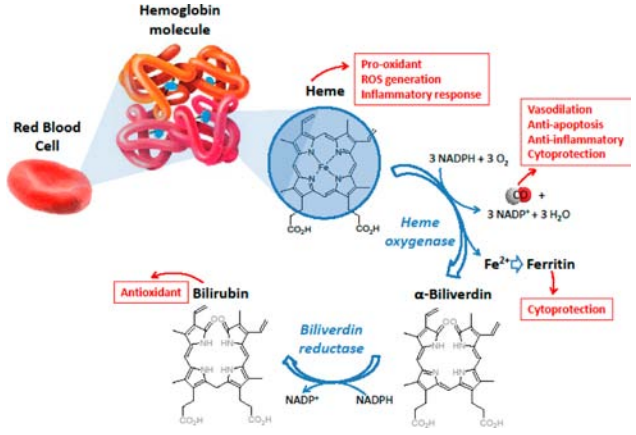
ABSTRACT: Nontoxic CO-releasing dirhodium complexes act as inhibitors of NO in stimulated macrophage cells, suggesting that novel antiinflammatory treatments could involve the use of these types of binuclear complexes.

Within the last 10 years, the reputation of carbon monoxide (CO) has started to shift from a noxious, polluting gas to an essential molecule involved in the modulation of several biological processes[1] as a signaling pathways regulator, fundamental for almost all living organisms including humans. These vital roles of CO, including regulation of neurotransmission,[2] vascular tone,[3] inflammation,[4] cell proliferation,[5] in vitro and in vivo programmed cell death,[6] mitochondrial biogenesis,[7] and autophagy,[8] make CO gas promising in molecular medicine as experimental and clinical therapeutic.[9]

Production of this gaseous molecule in the organism is mediated by heme oxygenase (HO-1) activity.[10] This enzyme catabolizes heme to iron, biliverdin, and CO, which mediates many of the biological effects of induced HO-1 (Scheme 1). The protective role of the HO-1/CO pathway has been demonstrated in numerous experimental models of inflammation, tissue damage, and cardiovascular diseases.[11] In inflammatory and immune conditions, the expression of HO-1 by many cell types could be part of an adaptive mechanism against stress through scavenging of reactive oxygen or nitrogen species, regulation of cell proliferation, and prevention of apoptosis.[12] The therapeutic benefit of CO inhalation has been shown in a number of preclinical animal models of lung and vascular diseases.[13] Inhaled CO diffuses rapidly across alveolar and capillary membranes, forming a tight-binding complex with the oxygen carrier protein hemoglobin to form carboxyhemoglobin (COHb). To avoid CO poisoning, the COHb levels associated with CO inhalation must be heeded. In spite of the probed benefits obtained, the administration route for gaseous compounds is restricted to inhalation through the lung, with difficulty in controlling the absorption, distribution, and specificity of CO. In this context, the biological effects of CO can be reproduced by the administration of CO-releasing molecules (CO-RMs), which are capable of carrying and delivering small amounts of CO in biological systems.[14] The use of CO-RMs represents a good alternative to CO gas in terms of controlled delivery through all possible routes of administration and also reduces COHb to nondangerous levels (<10%). The beneficial effect of these molecules has been shown after administration in various experimental models of arthritis.[15] In these conditions, CO-RMs reduce several proinflammatory parameters including nitric oxide (NO)[16] and prostaglandin E_2 (PGE_2) produced by activation of inducible NO synthase (iNOS) and cyclooxygenase-2, respectively.

CO-RMs have been extensively studied over the last years and can be classified into several types.[17] In particular, the most common CO-RMs are organometallic carbonyl complexes (mainly of chromium, molybdenum, manganese, rhenium, iron, and ruthenium).[18] However, also α,α-dialkylaldehydes, oxalates, boroncarboxylates, and silacarboxylates have recently been studied for their application in CO-release processes. Among all of these types of CO-RMs, metal carbonyls are perhaps the most well-suited for their use in pharmaceutical applications.[17] Employment of aldehydes as CO-RMs is limited by their slow release rate and medium toxicity.[19] Moreover, the CO delivery rate from oxalates is too slow,[20] whereas

Scheme 1. Schematic Representation of Metabolites Derived from HO-1 Activity and Their Physiological Effects



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silicacarboxylates need an activator (fluoride, methoxide, or *tert*-butoxide anions) to induce CO release.[21] On the other hand, boron carboxylates show a limited scope for chemical transformation that makes them unsuitable for the generation of compounds with appropriate pharmaceutical characteristics.[22]

From another point of view, a family of binuclear rhodium complexes of the general formula $[Rh_2\{(XC_6H_3)P(XC_6H_4)_2\}_n(O_2CR)_{4-n}]\cdot L_2$ containing one or two, in a head-to-tail arrangement, metalated phosphine ligands and different equatorial and axial ligands had recently been used by us as chromogenic probes for CO detection.[23] In this context, these ideal sensing systems not only display sensing features at low concentration but also show reversible binding with the target CO molecule.

On the basis of these observations and interest in the design of new CO-RMs, it occurred to us that CO-containing binuclear rhodium complexes could be used as suitable systems as physiological CO donors. In this context, we report herein, as far as we know for the first time, the use of binuclear rhodium complexes bearing coordinated CO molecules as CO-RMs and have tested them in the generation of inflammatory mediators NO and PGE_2 in murine macrophage cell line RAW 264.7.

In particular, we used for this study the complexes $[Rh_2\{(C_6H_4)P(C_6H_5)_2\}_2(O_2CCH_3)_2]\cdot 2CO$ (**1**·2CO) and $[Rh_2\{(m\text{-}CH_3C_6H_3)P(m\text{-}CH_3C_6H_4)_2\}_2(O_2CCH_3)_2]\cdot 2CO$ (**2**·2CO) (see Figure 1). The starting complexes, i.e., $[Rh_2\{(C_6H_4)P(C_6H_5)_2\}_2(O_2CCH_3)_2]\cdot 2CH_3CO_2H$ (**1**·$2CH_3CO_2H$) and $[Rh_2\{(m\text{-}CH_3C_6H_3)P(m\text{-}CH_3C_6H_4)_2\}_2(O_2CCH_3)_2]\cdot 2CH_3CO_2H$ (**2**·$2CH_3CO_2H$), were obtained by refluxing the corresponding phosphines, $P(C_6H_5)_3$ for **1**·$2CH_3CO_2H$ and $P(m\text{-}CH_3C_6H_4)_3$ for **2**·$2CH_3CO_2H$, with $[Rh_2(O_2CCH_3)_4]$ in toluene/acetic acid. The molar ratio phosphine−dirhodium(II) tetraacetate was fixed to 2:1. Both complexes **1**·$2CH_3CO_2H$ and **2**·$2CH_3CO_2H$ (simplified as #·$2CH_3CO_2H$) contain two orthometalated arylphosphines and two acetates as bridging ligands.[23] The design of CO-RM derivatives involves the use of these binuclear rhodium(II) cyclometalated complexes and the well-documented labile coordination ability in their axial sites. Moreover, coordination studies on adduct formation between dirhodium compounds and different Lewis bases[24] have demonstrated that even systems such as dirhodium(II) tetra-μ-carboxylate are very effective in π-back-bonding to the axial ligands, which is a very interesting feature for the design of CO binding complexes. Besides, when biscyclometalated compounds are compared with dirhodium tetracarboxylate derivatives, a higher ability of the former for π-back-donation to the axial ligand has been reported. In fact, the simple exposure of solutions of **1**·$2CH_3CO_2H$ and **2**·$2CH_3CO_2H$ in methanol to a CO stream allowed one to obtain the corresponding **1**·2CO and **2**·2CO complexes. Moreover, the formation of **1**·2CO and **2**·2CO from **1**·$2CH_3CO_2H$ and **2**·$2CH_3CO_2H$ can easily be monitored through the naked eye because the color of the complexes changes from purple (#·$2CH_3CO_2H$ absorbance at ca. 560 nm) to orange [#·(CH_3CO_2H,CO) absorbance at ca. 510 nm] to yellow (#·2CO absorbance at ca. 390 nm) upon reaction with CO.[23a] Moreover, when the yellow complexes **1**·2CO and **2**·2CO were left in an open-air atmosphere, the color changed slowly (in about 15 h) to purple because of the release of axially coordinated CO molecules. This release of axial CO molecules from **1**·2CO and **2**·2CO complexes was also studied by thermogravimetry, and in both cases, the weight changes correspond to the release of 2 equiv of CO per molecule (see the Supporting Information). Bearing in mind the adequate uptake and release features of both complexes, we intend to use them as CO-RMs. For this purpose, the cytotoxicity of both **1**·2CO and **2**·2CO complexes was first evaluated.

In particular, RAW 264.7 macrophages were treated with **1**·2CO and **2**·2CO at different concentrations over a 60 min period at 37 °C, and the cell viability was determined by MTT assay. The obtained results are shown in Figure 2a. As seen, both complexes are essentially nontoxic in the concentration tested (1 and 10 μM). In fact, absorbance values after treatments were not statistically different with respect to the stimulated control (C; see the Supporting Information for details).

Once the nontoxicity of both complexes was demonstrated, the inhibition of nitrite and PGE_2 in RAW 264.7 macrophages in the presence of **1**·2CO and **2**·2CO was studied. Incubation of RAW 264.7 macrophages with lipopolysaccharide (LPS)

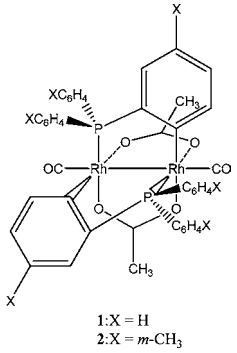

Figure 1. Structure for dirhodium complexes **1**·2CO and **2**·2CO with the general formula $[Rh_2\{(XC_6H_3)P(XC_6H_4)_2\}_2(O_2CCH_3)_2]\cdot 2CO$.

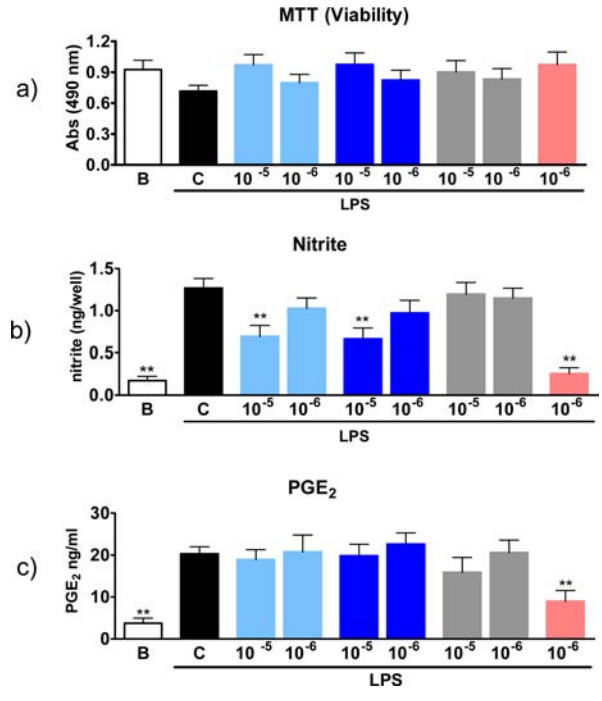

Figure 2. (a) MTT assay, (b) nitrite release, and (c) PGE_2 generation. Data are expressed as mean ± SEM (n = 12−18). **$p < 0.01$. Compared to stimulated control cells (C) and nonstimulated or blank cells (B); (light blue) complex **1**·2CO (30-days stock); (sky blue) complex **1**·2CO (recently prepared); (gray) complex **2**·2CO; (salmon) dexamethasone.

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produced the expression of iNOS and a consequent increase of the nitrite levels (Figure 2b), which were significantly inhibited after preincubation with complex **1**·2CO (10 μM) and the reference compound dexamethasone (1 μM). In contrast, complex **2**·2CO had no effect at this level. As shown by the results, complex **1**·2CO was able to maintain its inhibitory properties even 30 days after preparation (maintained under a CO atmosphere), thus demonstrating its stability and ability to donate CO molecules even after this time period. In a parallel assay, complex **1**·2CH_3CO_2H (without coordinated CO molecules) was tested in the same conditions, demonstrating that this compound had no antiinflammatory properties per se (data not shown). Finally, none of the tested compounds showed an inhibitory effect with respect to the generation of PGE_2 in stimulated macrophages (Figure 2c).

In summary, we have reported herein, for the first time, the potential use of cyclometalated binuclear rhodium complexes containing CO molecules as axial ligands as CO-releasing systems. This was demonstrated using complex **1**·2CO, which was found to clearly inhibit the nitrite levels in biological assays carried out in mouse macrophage cells where inflammation had been stimulated by LPS. We believe that this first proof-of-concept may open new studies in the use of binuclear rhodium complexes as potential CO-RMs able to carry and deliver small amounts of CO in biological systems and may allow advancement in the study of the pathophysiological and therapeutical role of CO.

■ ASSOCIATED CONTENT

Ⓢ Supporting Information

Materials and methods, general considerations, synthesis of the rhodium complexes, cell culture, cell viability assay, determination of nitrite and PGE_2, statistical analysis, diffuse reflectance UV−vis and thermogravimetric studies. This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

The authors declare no competing financial interest.

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