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COMMENTARY

Water-soluble carbon monoxide-releasing molecules: helping to elucidate the vascular activity of the 'silent killer'

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Carbon monoxide (CO) is formed during the degradation of haeme by haeme oxygenase (HO). As well as being an important signalling molecule and vasodilator, CO also possesses antihypertensive, anti-inflammatory and antiapoptotic qualities and protects against ischaemic tissue injury. Several approaches have been used to investigate the therapeutic potential of CO, ranging from direct administration of CO gas to the use of prodrugs, which generate CO upon metabolism. A novel approach involves the use of specific CO carriers, which will release measurable, controllable and effective amounts of CO into biological systems. Transitional metal carbonyls based around iron, manganese or ruthenium have recently been developed as CO-releasing molecules (CO-RMs) that, under appropriate conditions, will release CO. Such molecules have been shown to provide cardioprotection in both *ex vivo* and *in vivo* experiments. To date, CO-RMs have been largely incompatible with biological systems in that they are only soluble in organic solvents or have to be preactivated either by physical or chemical stimuli. However, the recent development of water-soluble CO-RMs has provided new opportunities to investigate the pharmacological and biological features of CO without such confounding influences. CORM-3, a novel water-soluble CO-RM, has recently been used to confirm the cardioprotective actions of CO. In this issue of *British Journal of Pharmacology*, Foresti and co-workers report that CORM-3 delivers CO, produces aortic vasodilation *ex vivo* and reduces blood pressure *in vivo via* modulation of the same cGMP and potassium channels utilised by endogenous and exogenous CO. These findings suggest that CORM-3 has the potential for use as a modulator of vascular function and hypertension. However, the use of water-soluble CO-RMs raises several questions of their own which will need to be addressed if CO-RMs are to be of future use therapeutically.
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Abbreviations: CO, carbon monoxide; CO-RM, carbon monoxide releasing molecule; DMSO, dimethylsulphoxide; EPO, erythropoietin; HO, heame oxygenase; MABP, mean arterial blood pressure; L-NAME, N^G-nitro-L-arginine methyl ester; NO, nitric oxide

Several years ago, nitric oxide (NO) underwent an amazing transformation from a noxious, colourless gas to become one of the body's key chemical messengers – a change in image that any 'PR' firm would be jealous of and one which made NO the 'Molecule of the Year' in 1992 and earned several researchers the Nobel Prize in Medicine in 1998. Around the same time, carbon monoxide (CO), a gas normally associated with pollution, poisoning and suicide – 'the silent killer' (Blumenthal, 2001; Townsend & Maynard, 2002), was undergoing a similar, although not as popular, change of face (Marks *et al*., 1991). It was already known for several decades that the human body will produce CO upon decomposition of haemoglobin, followed by the discovery that haeme oxygenase (HO) was the enzymatic source of CO. However, the discovery in the early 1990s that CO was an endogenous neurotransmitter (Verma *et al*., 1993) allowed some to predict that new discoveries of the physiological roles of CO would mean that '…this gas is likely to provide fuel to run plenty of labs.' (Barinaga, 1993). Since then, many biological activities of CO have been revealed, in investigations involving diverse strategies ranging from pharmacological induction of HO by haemin and transfection of the HO gene through to direct administration of CO gas and the use of prodrugs that generate CO upon hepatic metabolism (Thiemermann, 2001; Chauveau *et al*., 2002; Abraham, 2003).

Understanding the physiological and pathophysiological functions of NO has been facilitated by the development of a variety of organic compounds, which can spontaneously release NO. However, until recently, similar compounds that release CO have not been available. Prodrugs such as methylene chloride will release CO upon catabolism by hepatic enzymes (Chauveau *et al*., 2002); however, such agents require adequate liver function for their activation, which may be compromised (e.g. during haemorrhagic shock). Recently, transitional metal carbonyls have been identified as potential CO-releasing molecules (CO-RMs) with the potential to facilitate the pharmaceutical use of CO by delivering it to tissues and organs (Motterlini *et al*., 2003). Such complexes contain a heavy metal such as nickel, cobalt, iron or ruthenium surrounded by carbonyl groups as coordinated ligands

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(Motterlini *et al*., 2002; 2003). *In vitro* studies have demonstrated that such complexes can release measurable and controllable levels of CO either in the presence of certain ligands or upon exposure to light. Specifically, Motterlini *et al*. (2002) identified that iron pentacarbonyl [$Fe(CO)_5$], dimanganese decacarbonyl [$Mn_2(CO)_{10}$] and tricarbonyldichlororuthenium (II) dimer [$Ru(CO)_3Cl_2]_2$ could release CO in a concentration-dependent manner upon appropriate stimulation and elicit specific vascular responses reminiscent of those mediated by the HO/CO pathway. All three agents could convert deoxymyoglobin to carbonmonoxymyoglobin, indicating that CO was indeed released from these metal complexes. In biological experiments, *via* the release of CO, these CO-RMs caused sustained vasodilation in rat aortic rings precontacted with phenylephrine, attenuated coronary vasoconstriction caused by N^G-nitro-L-arginine methyl ester (L-NAME) in rat hearts *ex vivo* and significantly reduced acute hypertension caused by L-NAME *in vivo* (Motterlini *et al*., 2002). These effects were mimicked by haemin, which was used to increase endogenous levels of CO *via* activation of HO-1. CO released from [$Ru(CO)_3Cl_2]_2$ has been shown to promote the production and angiogenic activity of vascular endothelial growth factor, an effect that was again replicated by the activation of HO-1 by haemin or overexpression of HO-1 (Józkowicz *et al*., 2003). Recently, CO released from [$Ru(CO)_3Cl_2]_2$ has been shown to inhibit endothelin-1 release from serum-stimulated human pulmonary artery smooth muscle cells (Stanford *et al*., 2004).

Although the results of these early studies were promising, the use of these compounds *in vivo* caused several problems. First, these agents were only soluble in organic solvents such as dimethylsulphoxide (DMSO). Second, chemical or physical stimuli were required to promote the dissociation of CO from these complexes, in the form of steric ligands or light. For example, although [$Ru(CO)_3Cl_2]_2$ would spontaneously release CO in the presence of DMSO, [$Fe(CO)_5$] and [$Mn_2(CO)_{10}$] only released CO by photodissociation and, therefore, exposure of Krebs buffer containing [$Mn_2(CO)_{10}$] to a cold light source was required before it could be used in experiments. To overcome this, Motterlini and co-workers have recently developed tricarbonylchloro(glycinato)ruthenium(II) ([$Ru_9CO)_3Cl$(glycinate)]) as a water-soluble CO-RM. [$Ru_9CO)_3Cl$(glycinate)] was also given the more user-friendly name 'CORM-3', with [$Mn_2(CO)_{10}$] being relabelled CORM-1 and [$Ru(CO)_3Cl_2]_2$ CORM-2, respectively (Clark *et al*., 2003). CORM-3 was specifically designed to be soluble in aqueous solutions by positioning a biologically compatible ligand (glycine) onto the metal centre of the complex and which would release a quantifiable amount of CO into physiological solutions without activation. Subsequently, CORM-3 was shown to protect myocardial cells and tissues against ischaemia–reperfusion injury as well as cardiac allograft rejection (Clark *et al*., 2003). More recently, CORM-3 has been shown to attenuate infarct size in mice subjected to myocardial ischaemia–reperfusion when administered at the time of reperfusion and without producing any effect on mean arterial blood pressure (MABP), heart rate or plasma carboxyhaemoglobin levels (Guo *et al*., 2004).

In this issue of *British Journal of Pharmacology*, Foresti *et al*. (2004) have investigated the vasoactive properties of CORM-3 on rat vasculature. Specifically, the effects of CORM-3 on aortic vessel tone and blood pressure have been explored and, using *ex vivo* (rat aortic rings) and *in vivo* (MABP measurement in rats) animal models, the authors demonstrate that CORM-3 can modulate vessel tone and blood pressure *via* mechanisms used by CO. Specifically, the authors show that CORM-3 delivers CO and produces aortic vasodilation and reduces blood pressure *via* modulation of cGMP and potassium channels that are utilised by endogenous and exogenous CO. Intriguingly, the authors also demonstrate that inhibiting NO production (using L-NAME) or physically removing the endothelium in aortic rings significantly reduced vasodilation afforded by CORM-3, suggesting that factors produced by the endothelium modulate the vasoactive response to CORM-3. Three possible reasons have been highlighted in this article: (1) that CO released from CORM-3 displaces NO from an intracellular store which then contributes to vasodilation, (2) the endothelium produces a factor which synergises with CO to augment its vasodilatory activity or (3) that one or several endothelium-derived factors such as NO may interact with the ruthenium in CORM-3 to facilitate the release of CO. As the authors state (Foresti *et al*., 2004), this final hypothesis seems valid as it is known that ruthenium complexes have a high affinity for NO and can effectively scavenge NO in biological systems (Mosi *et al*., 2002) and that metal carbonyls are used as a starting material for metal nitrosyl complexes (Miranda *et al*., 1997). Another reasonable possibility for consideration is that CORM-3 may need to be metabolised or activated by the endothelium after which it is able to facilitate the release of NO.

The development of a water-soluble CORM is no doubt a significant step forward in elucidating the physiological roles of CO *in vivo*. The findings reported here by Foresti and co-workers suggest that these recently synthesised water-soluble CO-releasing metal carbonyls could eventually be used to deliver CO for the modulation of vascular function and prevention of hypertension. However, although such agents may allow us to elucidate the role of CO in biological systems, their use raises several questions of their own which will need to be addressed before CO-RMs can be used therapeutically. For example, is there any toxicity associated with the use of CO-RMs? Although the toxicity of exogenous CO has been recognised for many years (Blumenthal, 2001; Townsend & Maynard, 2002), it is interesting to note that CO can also modulate the activity of genes that may well be protective (Dulak & Józkowicz, 2003). For example, under hypoxic conditions, CO can inhibit the induction of the erythropoietin (EPO) gene (Huang *et al*., 1999), which is particularly intriguing in the light of recent reports that EPO may, *via* pleiotropic actions, provide protection against tissue injury under such conditions (Gassmann *et al*., 2003). Finally, the apparent tachyphylaxis in the vasodilator response produced by CORM-3 remains to be addressed.

References

ABRAHAM, N.G. (2003). Therapeutic applications of human heme oxygenase gene transfer and gene therapy. *Curr. Pharm. Des.,* **9,** 2513–2524.

BARINAGA, M. (1993). Carbon monoxide: killer to brain messenger in one step. *Science,* **259,** 309.

BLUMENTHAL, I. (2001). Carbon monoxide poisoning. *J. R. Soc. Med.,* **94,** 270–272.

CHAUVEAU, C., BOUCHET, D., ROUSSEL, J.C., MATHIEU, P., BRAUDEAU, C., RENAUDIN, K., TESSON, L., SOULILLOU, J.P., IYER, S., BUELOW, R. & ANEGON, I. (2002). Gene transfer of heme oxygenase-1 and carbon monoxide delivery inhibit chronic rejection. *Am. J. Transplant.,* **2,** 581–592.

CLARK, J.E., NAUGHTON, P., SHUREY, S., GREEN, C.J., JOHNSON, T.R., MANN, B.E., FORESTI, R. & MOTTERLINI, R. (2003). Cardioprotective actions by a water-soluble carbon monoxide-releasing molecule. *Circ. Res.,* **93,** e2–e8.

DULAK, J. & JÓZKOWICZ, A. (2003). Carbon monoxide – a 'new' gaseous modulator of gene expression. *Acta Biochim. Pol.,* **50,** 31–47.

FORESTI, R., HAMMAD, J., CLARK, J.E., JOHNSON, T.R., MANN, B.E., FRIEBE, A., GREEN, C.J. & MOTTERLINI, R. (2004). Vasoactive properties of CORM-3, a novel water-soluble carbon monoxide-releasing molecule. *Br. J. Pharmacol.,* **142,** 453–460.

GASSMANN, M., HEINICKE, K., SOLIZ, J., OGUNSHOLA, O.O., MARTI, H.H., HOFER, T., GRIMM, C., HEINICKE, I. & EGLI, B. (2003). Non-erythroid functions of erythropoietin. *Adv. Exp. Med. Biol.,* **543,** 323–330.

GUO, Y., STEIN, A.B., WU, W.J., TAN, W., ZHU, X., LI, Q.H., DAWN, B., MOTTERLINI, R. & BOLLI, R. (2004). Administration of a CO-Releasing molecule at the time of reperfusion reduces infarct size *in vivo*. *Am. J. Physiol. Heart Circ. Physiol.,* **286,** H1649–H1653.

HUANG, L.E., WILLOMRE, W.G., GU, J., GOLDBERG, M.A. & BUNN, H.F. (1999). Inhibition of hypoxia-inducible factor 1 activation by carbon monoxide and nitric oxide. Implications for oxygen sensing and signalling. *J. Biol. Chem.,* **274,** 9038–9044.

JÓZKOWICZ, A., HUK, I., NIGISCH, A., WEIGEL, G., DIETRICH, W., MOTTERLINI, R. & DULAK, J. (2003). Heme oxygenase and angiogenic activity of endothelial cells: stimulation by carbon monoxide and inhibition by tin protoporphyrin-IX. *Antioxid. Redox. Signal.,* **5,** 155–162.

MARKS, G.S., BRIEN, J.F., NAKATSU, K. & McLAUGHLIN, B.E. (1991). Does carbon monoxide have a physiological function? *Trends Pharmacol. Sci.,* **12,** 185–188.

MIRANDA, K.M., BU, X., LORKOVIC, I. & FORD, P.C. (1997). Synthesis and structural characterisation of several ruthenium porphyrin nitrosyl complexes. *Inorg. Chem.,* **36,** 4838–4848.

MOSI, R., SEGUIN, B., CAMERON, B., AMANKWA, L., DARKES, M.C. & FRICKER, S.P. (2002). Mechanistic studies on AMD6221: a ruthenium-based nitric oxide scavenger. *Biochem. Biophys. Res. Commun.,* **292,** 519–529.

MOTTERLINI, R., CLARK, J.E., FORESTI, R., SARATHCHANDRA, P., MANN, B.E. & GREEN, C.J. (2002). Carbon monoxide-releasing molecules. Characterisation of biochemical and vascular activities. *Circ. Res.,* **90,** e17–e24.

MOTTERLINI, R., MANN, B.E., JOHNSON, T.R., CLARK, J.E., FORESTI, R. & GREEN, C.J. (2003). Bioactivity and pharmacological actions of carbon monoxide-releasing molecules. *Curr. Pharm. Des.,* **9,** 2525–2539.

STANFORD, S.J., WALTERS, M.J. & MITCHELL, J.A. (2004). Carbon monoxide inhibits endothelin-1 release by human pulmonary artery smooth muscle cells. *Eur. J. Pharmacol.,* **486,** 349–352.

THIEMERMANN, C. (2001). Inhaled CO: Deadly gas or novel therapeutic? *Nature,* **7,** 11–12.

TOWNSEND, C.L. & MAYNARD, R.L. (2002). Effects on health of prolonged exposure to low concentrations of carbon dioxide. *Occup. Environ. Med.,* **59,** 708–711.

VERMA, A., HIRSCH, D.J., GLATT, C.E., RONNETT, G.V. & SNYDER, S.H. (1993). Carbon monoxide: a putative neural messenger. *Science,* **259,** 381–384.

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