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COMMENTARY

Physiological activities of carbon monoxide-releasing molecules: Ça ira

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In this issue of *British Journal of Pharmacology*, Megías and colleagues demonstrate how preincubation of human colonic Caco-2 cells with CORM-2, a carbon monoxide releasing molecule (CO-RM), reduces the expression of inducible nitric oxide synthase, interleukin (IL)-6 and IL-8 caused by proinflammatory cytokines. A role for IL-6 in the regulation of metalloproteinase (MMP)-7 expression by CORM-2 is described. However, it is the demonstration that CORM-2 inhibits MMP-7 or matrilysin expression, which is most intriguing as this small MMP has been implicated in carcinogenesis. Thus, CO-RMs appear to now possess chemoprotective properties and, in this particular case, may influence inflammation-induced colon carcinogenesis via modulation of nuclear factors participating in the transcription of genes implicated in the development of intestinal inflammation and cancer. This report opens yet another door for research involving these exciting molecules and it is now clear that further discoveries of the beneficial properties of CO-RMs will go on.
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Over the last 5 years, the laboratories of Professor Roberto Motterlini and colleagues at the Northwick Park Institute for Medical Research, Middlesex, UK, have developed several transitional metal carbonyls as carbon monoxide (CO)-releasing molecules (CO-RMs), which either contain a heavy metal such as manganese (CORM-1), ruthenium (CORM-2 and -3) or iron (CORM-F3) or are based on boron (CORM-A1) (Motterlini *et al*., 2002, 2003, 2005). The development and experimental use of such CO-RMs have allowed researchers to elucidate the many physiological roles of CO (Chatterjee, 2004; Motterlini *et al*., 2005). CO-RMs also possess anti-ischaemic and anti-inflammatory qualities similar to that of CO (Motterlini *et al*., 2005). The ability of CO to interact with metal centres in proteins and thereby modify their activities has prompted researchers to investigate the effects of CO-RMs on matrix metalloproteinases (MMPs). MMP activation is implicated in ischaemia-reperfusion injury, inflammation, cardiovascular disease and cancer (Sang *et al*., 2006). Previously, CORM-2 had been shown to reduce the expression and activity of MMP-1 and -2 in human lung A549 epithelial cells, which has implications for the therapy of emphysema (Desmard *et al*., 2005). The article by Megías and colleagues (2007) in this issue of *British Journal of Pharmacology* takes this a step further by demonstrating that CORM-2 can reduce the expression of MMP-7 in the human colonic Caco-2 cell line. MMP-7, also known as matrilysin, is one of the smallest members of the MMP family and is important in the maintenance of innate immunity (Burke, 2004). MMP-7 is also implicated in the development of cancer (Ii *et al*., 2006). However, it is the role of MMP-7 in the development of acute and chronic inflammation and inflammatory disorders such as multiple sclerosis and Alzheimer's disease and the potential therapeutic applications of MMP inhibitors that has produced the most excitement (Wielockx *et al*., 2004, Sang *et al*., 2006). Megías and colleagues report that a 30-min preincubation of A549 cells with CORM-2 followed by incubation with a combination of interleukin (IL)-1β, tumour necrosis factor (TNF)-α and interferon (IFN)-γ reduces the expression of inducible NO synthase (iNOS), IL-6, IL-8 and MMP-7 via modulation of the transcription factors nuclear factor-κB, activator protein-1 and CCAT/enhancer-binding protein (Megías *et al*., 2007). Furthermore, using a small interfering RNA protocol, the authors show that CORM-2 regulates MMP-7 expression via IL-6.

A couple of important points arise from this study. First is the possibility that CORM-2 or the CO it releases could induce the expression of haeme oxygenase (HO)-1. Indeed, an earlier study by Sawle and colleagues demonstrated the ability of CORM-2 (50 and 100 μM) to induce HO-1 protein expression and activity in murine RAW264.7 macrophages (Sawle *et al*., 2005). This could lead to the further degradation of haeme, which may reduce the activity of iNOS (Albakri and Stuehr, 1996). However, Megías and colleagues show that at the concentrations investigated (50, 100 and 150 μM), CORM-2 *did not* modify HO-1 expression in Caco-2 cells. Intriguingly, another recent study has reported that

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lower concentrations of CORM-2 (10 and 50 μM) can actually reduce HO-1 expression in RAW264.7 cells albeit using a different stimulator of inflammation (bacterial lipopolysaccharide) (Srisook *et al*., 2006). Second, Megías *et al*. (2007) report that CORM-2 reduced the expression of iNOS. Again, this is in contrast to the report by Sawle *et al*., 2005 who reported that although CORM-2 reduced iNOS activity, it did not affect iNOS expression and concluded that CO was binding to the haeme within iNOS thereby inhibiting its activity (Sawle *et al*., 2005). This effect of CORM-2 on iNOS activity rather than expression was recently confirmed by investigators studying astrocyte-induced HO-1 expression in microglia (Min *et al*., 2006). However, Megías and colleagues respond that several other studies have reported inhibition of iNOS expression by either CO or CORM-2 itself (Dijkstra *et al*., 2004, Yang *et al*., 2004, Srisook *et al*., 2006). In fact, the authors themselves have recently reported that CORM-2 inhibited iNOS expression in human chondrocytes stimulated with IL-1β (Guillen *et al*., 2006). Nevertheless, this issue certainly requires further investigation.

One final issue for consideration is the potential toxicity of CO-RMs *in vivo*. Although information on adverse effects is sparse and the authors of this study show that concentrations of CORM-2 up to 150 μM did not affect the viability of Caco-2 cells, the solubility of CORM-2 and other CO-RMs in organic solvents such as dimethyl sulphide (DMSO) is still a cause for concern for potential *in vivo* applications. It has recently been reported that both active and inactive CORM-2 were toxic to renal human embryonic kidney (HeK) and Mardin–Darby canine kidney (MDCK) cells at both 20 and 100 μM in contrast to 20 p.p.m CO which did not have any adverse effects (Winburn *et al*., 2006). Cellular toxicity appeared to be because of oxidative stress and, although it is not clear if this was as a direct consequence of CORM-2 itself or because of its vehicle, it would be prudent to perform future experiments with water-soluble CO-RMs such as CORM-3 or CORM-A1 wherever possible.

Overall, this study by Megías and colleagues is the first to suggest that CO-RMs may possess chemoprotective properties in cancer and in this particular case, may modulate inflammation-induced colon carcinogenesis via modulation of nuclear factors, which participate in the transcription of genes implicated in the development of intestinal inflammation and cancer progression. This report opens yet another door for these exciting molecules and it is now clear that discoveries of the benefits of CO-RMs will go on.

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