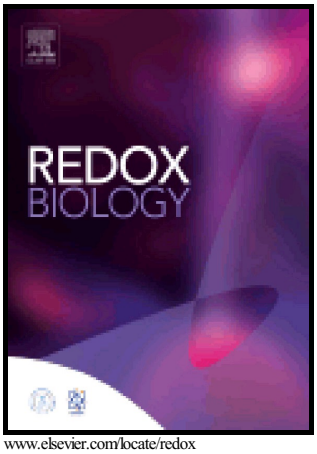

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PII: S2213-2317(18)30459-2
DOI: https://doi.org/10.1016/j.redox.2018.06.008
Reference: REDOX942

To appear in: *Redox Biology*

Received date: 30 May 2018
Accepted date: 23 June 2018

Cite this article as: Hannah M. Southam, Thomas W. Smith, Rhiannon L. Lyon, Chunyan Liao, Clare R. Trevitt, Laurence A. Middlemiss, Francesca L. Cox, Jonathan A. Chapman, Sherif F. El-Khamisy, Michael Hippler, Michael P. Williamson, Peter J.F. Henderson and Robert K. Poole, A Thiol-Reactive Ru(II) Ion, not CO Release, Underlies the Potent Antimicrobial and Cytotoxic Properties of CO-Releasing Molecule-3, *Redox Biology,* https://doi.org/10.1016/j.redox.2018.06.008

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ABSTRACT

Carbon monoxide (CO)-releasing molecules (CORMs), mostly metal carbonyl compounds, are extensively used as experimental tools to deliver CO, a biological 'gasotransmitter', in mammalian systems. CORMs are also explored as potential novel antimicrobial drugs, effectively and rapidly killing bacteria in vitro and in animal models, but are reportedly benign towards mammalian cells. Ru-carbonyl CORMs, exemplified by CORM-3 ($Ru(CO)_3Cl$(glycinate)), exhibit the most potent antimicrobial effects against *Escherichia coli*. We demonstrate that CORM-3 releases little CO in buffers and cell culture media and that the active antimicrobial agent is Ru(II), which binds tightly to thiols. Thus, thiols and amino acids in complex growth media – such as histidine, methionine and oxidised glutathione, but most pertinently cysteine and reduced glutathione (GSH) - protect both bacterial and mammalian cells against CORM-3 by binding and sequestering Ru(II). No other amino acids exert significant protective effects. NMR reveals that CORM-3 binds cysteine and GSH in a 1:1 stoichiometry with dissociation constants, K_d, of about 5 μM, while histidine, GSSG and methionine are bound less tightly, with K_d values ranging between 800 - 9000 μM. There is a direct positive correlation between protection and amino acid affinity for CORM-3. Intracellular targets of CORM-3 in both bacterial and mammalian cells are therefore expected to include GSH, free Cys, His and Met residues and any molecules that contain these surface-exposed amino acids. These results necessitate a major reappraisal of the biological effects of CORM-3 and related CORMs.