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Enzyme-triggered CO-releasing molecules (ET-CORMs): towards tissue-specific delivery of carbon monoxide

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Carbon monoxide (CO) is an ambivalent gasotransmitter endogenously generated during physiological degradation of haem. While being highly toxic at elevated concentrations, the gaseous molecule holds promising properties at low and physiological concentrations. Application of the exogenous equivalent can mimic these beneficial characteristics including cytoprotective and anti-proliferative effects as well as anti-inflammatory properties.

Enzyme-triggered CO-releasing molecules (ET-CORMs) are a novel class of transition metal carbonyl complexes that offer several advantages over previous forms of exogenous CO application. While inhalation of CO and other CORMs predominantly deliver carbon monoxide via unspecific passive diffusion to cells or tissue, ET-CORMs allow for an intracellular administration of carbon monoxide. More precisely, esterase dependent, enzymatic cleavage of the chemical bonds leads to intracellular release of carbon monoxide, ferric iron and the mother compound. Previously published data demonstrates that the biological properties of ET-CORMs strongly depend on their chemical structure, the type and position of the ester substituent as well as the mother compound from which they are derived.

In this work a series of structurally different ET-CORMs (*rac*-**1**, *rac*-**4** and *rac*-**8**) were investigated in human umbilical vein endothelial cells: the data showed that the cytotoxic effect of ET-CORMs relies on the speed or extent of carbon monoxide release. The disparity in cytotoxicity is unlikely to be mediated by different intracellular uptake of the investigated compounds based on the notion that the cellular uptake of the purpose-built cyclodextrin encapsulated ET-CORMs is equal and the cytotoxic profile remained unaltered. At toxic concentrations, there was no indication that released ferric iron or the mother compounds contribute noticeably to the cytotoxic effect of ET-CORMs. In this case (i.e., toxic concentrations of CO), it is expected that CO binds to intracellular haem containing proteins leading to an impairment of cell respiration. The collected data demonstrates that ATP depletion promotes cell damage, thus supporting the hypothesis of a causal connection between the cytotoxicity of ET-CORMs and cellular respiration. On the contrary, treatment with ET-CORMs at low concentrations significantly increases intracellular ATP levels. This is in agreement with previous studies on exogenous CO application suggesting that the increase in ATP levels might be mediated by activation of soluble guanyl cyclase, which in turn indicates an enhancement in oxidative phosphorylation.

In terms of anti-inflammatory modulation, the investigated ET-CORMs effectively inhibited TNF-α induced VCAM-1 expression and may mediate the inhibition of VCAM-1 via inhibition of NFκB, presumably in an IκBα independent manner. Additionally, ET-CORMs showed activation of the Nrf2 signaling pathway leading to induction of the antioxidant protein HO-1. It is well known that both the NFκB and Nrf2 signaling pathway are crucial for the cellular response and regulation to inflammatory stimuli.

The mother compound of *rac*-**1** and *rac*-**4** itself, i.e., 2-cyclohexenone (**L1**), inhibited VCAM-1 and induced HO-1 expression. Accordingly, **L1**-derived ET-CORMs can act as bifunctional complexes in a way that the beneficial effects of the by-products summate. This opens up various opportunities for the complementary delivery of ET-CORMs and a second agent.

Finally, enantiomers of ET-CORMs displayed differences in the efficacy to mediate a biological response, providing essential structural information. Cis-enantiomers showed a pronounced biological activity in terms of cytotoxicity, anti-inflammation and cold preservation injury when compared to trans-enantiomers. This information can be employed to chemically modify and thus optimize existing ET-CORMs.

Although intracellular esterases are ubiquitous and tissue-targeted delivery of CO based on ET-CORMs cannot be guaranteed at this point, this work set a first foundation on the road to tissue-specific delivery of carbon monoxide generating fundamental insights for designing ET-CORMs with differential CO release and biological activities.