Focus on carbon monoxide: a modulator of neutrophil oxidants and elastase spatial localization?

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GASEOUS SIGNAL MEDIATORS include nitric oxide (NO), hydrogen sulfide, and carbon monoxide (CO) (3, 20, 35). Both NO and hydrogen sulfide play important roles in inflammation, with the existing evidence supporting them as components of the normal biochemical milieu that stabilize the vasculature against leukocyte adhesion, extravasation, and vascular permeability (25, 35). Unlike G protein-coupled receptors, gasotransmitters are membrane-permeant gases that modulate several forms of cell signaling by chemically binding numerous intracellular targets (25, 26, 35, 36). CO is formed in several tissues through the action of two types of hemooxygenases (HOs): an inducible form HO-1 and constitutive forms HO-2 and -3 (HO-3 is involved in O2 sensing) (19). HO-1 is the 32-kDa form of HO, synthesized in response to heat stress (hence, heat shock protein-32), oxidants, cytokines, and environmental stress (e.g., metals). HOs catabolize heme to biliverdin (rapidly converted to bilirubin by biliverdin reductase), Fe2+, and CO. While biliverdin and bilirubin formed by HO-1 and -2 are major antioxidants, with potency comparable with glutathione, many studies suggest that the induction of HO-1 expression in inflammation is adaptive through CO-mediated effects.

CO is a potent physiological regulator of vascular tone, like NO, and exhibits some similar properties. For example, CO and NO both bind Hb, but NO binds Hb ~1,500× more avidly than CO. NO also enhances the binding of CO to heme (the rate of carboxy-Hb formation is 10–15× greater at equivalent levels of CO and NO). NO also induces >30× more cGMP than CO and sensitizes guanylate cyclase to CO stimulation (3, 29). Whereas NO is a highly reactive free radical that forms NO-metal complexes, CO is a stable, water soluble gas that resists oxidation and reduction reactions. Thus CO and NO interact in toxicity and cell signaling, and the concept of CO/NO synergy is gaining acceptance as an NO-1-NO-CO axis (3, 17).

CO activates several important signaling systems besides guanylate cyclase (22), including p38 MAPK, phosphatidylinositol 3-kinase/Akt, STATs, hypoxia-inducible factor-1α, Ca2+-activated K+ channels (40), and Toll-like receptor-2, -5, and -9 (24). CO binds cytochromes in mitochondrial complex IV (3a), reducing electron (e−) transfer to produce O2 and other oxidants (45). CO also indirectly modulates peroxisome proliferator-activated receptor-γ activity (2). CO exerts diverse and cell-specific effects on several physiological processes and pathological phenomena including circadian rhythms (via neuronal pas domain protein 2/brain and muscle ARNT-like protein 1) (13), inhibition of PDGF-induced smooth muscle proliferation (via reduced ERK1/2 and cyclin-D activity) (32), neurotransmission/long-term potentiation (by activating cGMP-dependent kinases in locus coeruleus neurons) (18, 28, 34), platelet inhibition (5), apoptosis (by inhibiting caspase-3 and -7 and increased STAT-3) (42, 43), and inflammation. CO protects the vasculature against many forms of inflammatory injury including vasoconstriction and atherosclerosis (19, 23), cardiac and graft rejection (30, 31), arteriosclerosis (27), and LPS-induced cytokine expression (1, 16, 41). Paradoxically, CO may activate anti-inflammatory/preconditioning pathways by oxidant-dependent signals (1, 41). The current study by Mizuguchi et al. (22a) shows that septic lung injury (cecal ligation/puncture) leads to pulmonal entrapment and infiltration of neutrophils (polymorphonuclear leukocyte (PMN)). This injury was blocked by the ruthenium-based CO-releasing molecule (CORM-3). The effect of CORM-3 was CO and cell specific since only PMN infiltration was blocked; these effects were not observed with inactive CORM-3, and the macrophage density in lungs was unaltered.

CO can activate guanylate cyclase and cGMP/PKG signaling and may regulate several PMN behaviors including migration, apoptosis, and myeloperoxidase release (6, 33, 38, 39). A recent study by Ciuman et al. (11) shows that activated PMNs lose soluble guanylate cyclase and PKG, presumably decreasing CO-induced cGMP signaling (11). Moreover, PKG can phosphorylate and inhibit soluble guanylate cyclase activity (44), suggesting that other signaling pathways may be involved in the observed effects of CO. Whereas CO at high concentrations is toxic, lower levels of CO only slightly reduce mitochondrial respiration, yielding nontoxic amounts of “signal” oxidants that may trigger adaptation and preconditioning. PMNs are thought of as having few mitochondria, deriving most of their energy from glycolysis, allowing them to function in low ambient O2 and conserve O2 for respiratory burst (14). Studies by Fossati et al. (14) suggest that PMNs actually possess an intricate mitochondrial network and that mitochondrial membrane potential is an essential regulator of PMN motility, cytokeskeleton, and apoptosis. CO-induced intracellular reactive oxygen species (ROS) might reflect CO-mediated uncoupling of mitochondrial e− flow (12), leading to the production of signal oxidants (3); at least some of these signal pathways may be anti-inflammatory (1, 4). When PMN e− flow is disrupted (e.g., by an uncoupler like FCCP) or by CO, PMNs also undergo morphologic/cytoskeletal reorganization, decrease chemotaxis (formyl-methionyl-leucylphenylalanine and LPS), and initiate apoptosis. The protective effects of CO produced by CORM-3 (reduced lung injury, solute permeability, PMN binding, and infiltration) are associated with increased intracellular and extracellular oxidants measured using two different reporters: L-012 (extracellular O2) and dihydroorhodamine (cytotoxic OH/ONOO−). Using L-012, Mizuguchi et al. (22a) show that SOD-inhibitable O2 production by PMN is increased by CO; previous studies show that neither the PMN respiratory burst nor phagocytic capacity is blocked by mitochondrial inhibition (e.g., by CO), more consistent with
NADPH oxidase activity. CO treatment of endothelial cells could uncouple endothelial mitochondria to promote ROS; however, CO does not apparently increase endothelial ROS as is seen in PMNs (15, 37). CO also appears to protect against elastase-mediated tissue injury, but mechanisms underlying this are less clear. CORM-3 decreased alveolar protein leakage and PMN elastase in the lung airspace, effects related to lower PMN infiltration. CO effects on PMN migration were also shown to be endothelial independent since only CORM-3 pretreatment of PMNs (not endothelium) prevented chemotaxis. CORM-3 also blocked PMN rolling and adhesion, on endothelial monolayers, also shown to be PMN-specific responses.

PMN elastase [neutrophil elastase (NE)] is a “double edged” protease, with central roles in host immune defense and inflammation. The broad substrate specificity of NE includes extracellular matrix molecules (elastin, collagens, laminins, and fibronectin), lung surfactant, and junction components [vascular endothelial (VE)-cadherin]. We previously found VE-cadherin to be a target of PMN elastase (7) and that elastase inhibition prevents VE-cadherin degradation during PMN-mediated lung injury. Though controversial, NE has also been implicated in PMN migration to sites of inflammation. The migration of PMNs across several biological barriers including endothelium, basement membranes, and epithelium may be facilitated by the action of NE. Previous studies by this group (8, 9, 21) have shown that PMN elastase is positioned at the migrating front of PMNs, where it appears to help remodel junctions during PMN transit.

In this issue of the American Journal of Physiology-Heart and Circulatory Physiology, Mizuguchi et al. (22a) show that elastase membrane localization, rather than its activity, is controlled by CO (using the ruthenium-based CO donor CORM-3). When CO is chemically released by CORM-3, the elastase-tipped pseudopodia shed active elastase. In this way, elastase activity is reduced by dilution, a unique, rapid, and specific mechanism for reducing elastase activity without proteolysis, secretion, or binding of elastase inhibitors (e.g., SerpinA1/a1-AT, SLPI). The loss of elastase from the PMN surface, rather than a change in its activity, appears to underlie the beneficial effect of CORM-3-derived CO (inactive CORM-3 lacks this effect). Whether and how the elastase undocking at the PMN membrane induced by CO is related to oxidant signaling from mitochondria or downstream cell signals, e.g., p38 MAPK, is not yet known. Parallel with this relocation of elastase from the active front of PMNs, the other PMN inflammatory behaviors (rolling and adhesion), blocked by CO may be unrelated to elastase, suggesting that CO activates a host of behaviors in arresting PMN-dependent inflammation. Thus, whereas increased oxidants are often considered cytotoxic and inflammatory, CO-mediated elevation of signal components: role of elastase.

REFERENCES


