A New Road to Induce Heme Oxygenase-1 Expression by Carbon Monoxide

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Heme oxygenase (HO)-1 catalyzes the rate-limiting step in the metabolic conversion of heme to bilirubin, iron, and carbon monoxide (CO).1 Ample literature has demonstrated that exogenous or endogenously-produced CO possesses signaling properties affecting numerous critical cellular processes including inflammation, cellular proliferation, and apoptotic cell death.1,2 CO and the bile pigments biliverdin and bilirubin play important physiological roles in the circulation. The production of CO in vascular cells modulates blood flow and blood fluidity by regulating vasomotor tone and inhibiting smooth muscle cell proliferation and platelet aggregation. CO also maintains vessel wall integrity by directly blocking apoptosis and inhibiting the release of proinflammatory cytokines from the vessel wall. These effects of CO are mediated via multiple pathways, including activation of soluble guanylyl cyclase, p38 mitogen-activated protein kinase (MAPK), and potassium channels.1,2 Induction of HO-1 gene expression and the subsequent release of CO and bile pigments are observed in numerous vascular disorders providing an adaptive response to preserve homeostasis at sites of vascular injury. Hence, the formation of CO and bile pigments in vascular cells may function as an important vasoprotective system.1,2

Thus far, several transcriptional factors (TF) have been implicated in HO-1 gene expression. Among these TFs, the heat-shock, nuclear factor-κB (NF-κB), nuclear factor-erythroid 2-related factor 2 (Nrf2), and activator protein–1 families are the most important regulators of cellular stress responses. Nrf2 is activated by various xenobiotics and oxidants and regulates genes encoding proteins with antioxidant and xenobiotic detoxification activities.3 Besides exogenous stimuli, physiological effectors such as hormones and nitric oxide (NO) in the body also induce HO-1 to perform their biological duties. Recently Zuckerbraun et al have demonstrated that CO has a cytoprotective activity by inducing iNOS via the NF-κB pathway in the liver and hepatocytes.4 Released NO by iNOS activity is in part responsible for the beneficial effects of CO through the subsequent induction of HO-1, implying the antiapoptotic crosstalk between CO and NO. In hepatocytes in vitro Lee et al expanded the concept that CO directly induces the expression of HO-1 in a MAPK/Nrf2-dependent fashion (Figure 1).5

In this issue of Circulation Research, Kim et al report the induction of HO-1 by exogenous CO administration in human endothelial cells.6 HO-1 activity, in turn, protected the cells from endoplasmic reticulum (ER) stressors. The novelty of the present study is that protein kinase R–like endoplasmic reticulum kinase (PERK)–dependent activation of Nrf2 by CO is critical for cell survival signal during ER stress. Likewise, they not only added another transcription factor to HO-1 by CO-releasing molecules (CORM)7 or CO gas, but also suggested a positive feedback loop of the signaling pathway in HO-1/CO axis (Figure 1). Scavenging CO by hemoglobin completely blocked PERK activation, implying the specificity of CO gas. Activated PERK triggered nuclear trafficking of Nrf2 and increased HO-1 transcription.6 Under basal states, Nrf2 is localized to the cytoplasm through an interaction with Keap1.8 PERK-dependent phosphorylation of Nrf2 leads to release from the tethering complex, nuclear accumulation of Nrf2, and increased transcription of Nrf2 target genes including HO-1. In line with these observations, cells deficient in Nrf2 displayed increased apoptosis and decreased survival in response to tunicamycin treatment.9,10 Taken together, these results confirmed again that Nrf2 activity is critical for cell survival after ER stress. Data demonstrated by Kim et al clearly provide a novel link between PERK-Nrf2 signaling cascade and autonomous induction of HO-1 by its metabolite, CO (Figure 2). These findings provide insight into the evolutionary pressures produced by noxious environments that may have facilitated the development of more efficient cytoprotective circuits, like the HO-1/CO positive feedback loop.

It is apparent that one of the earliest cellular responses to cellular stress is inhibition of protein synthesis and growth arrest.11,12 This process is accompanied by phosphorylation of eukaryotic translation initiation factor (eIF2)-α which modulates global rates of protein synthesis in all eukaryotic cells (Figure 2). PERK belongs to an eIF2α kinase family that includes the interferon-inducible, RNA-dependent protein kinase R (PKR), the heme-regulated kinase (HRI), and general control nonrepressed (GCN)2. Among these, PERK function appears to be specifically required for cellular response to ER stress.13 Accumulation of misfolded proteins in the ER activates the ER membrane...
kinases PERK and IRE1 leading to the unfolded protein response (UPR, Figure 2). After UPR activation, PERK-dependent phosphorylation of eIF2α at Ser51 attenuates translation of most cell proteins while promoting increased translation of select target proteins, including ATF4 and the proapoptotic transcription factor CCAAT/enhancer-binding protein homologous protein CHOP/GADD153. Using CORM, Kim et al demonstrated that CO selectively activated PERK and its downstream eIF2α. In addition, the activation of PERK is only responsible for Nrf2 nuclear trafficking, which is eIF2α-independent. Rather, PERK-eIF2α activation may be involved in growth arrest by HO-1/CO in endothelial cells. Even though they did not determine the upstream kinases of PERK activated by CO, a potential candidate is p38 MAPK because the kinase inhibitor derepressed the proapoptotic CHOP protein. Specifically in ER stress models, however, PERK is likely to act as an upstream kinase for stress activated protein kinases (JNK and p38 MAPK). The mechanism by which PERK selectively facilitates the nuclear shuttling of Nrf2 but not eIF2α should also be investigated further.

Based on these data CO appears to provide cytoprotection not only by inducing HO-1 expression and concurrent production of antiapoptotic CO and bile pigments, but also by suppressing proapoptotic CHOP. It is of note to consider the expression of GADD34 which is regulated by ATF4 transcriptional activation and functions as a negative modulator of CHOP. By forming a phosphatase-complex with protein phosphatase (PP) 1c, CREP, and Nck, GADD34 is also involved in modulating eIF2α phosphorylation (Figure 2). Given the importance of controlling eIF2α phosphorylation in a timely manner, elucidation of the role of CO in GADD34 activity would shed light on drawing a broad picture for cytoprotective activity of the gas molecule in endothelial cells. In line with this hypothesis, Boyce et al have reported that a selective inhibitor of dephosphorylation of eIF2α, salubrinal, protects cells from ER stress. Collectively, present work done by Kim et al demonstrated that CO could induce HO-1 expression, thereby generating a positive feedback loop in the liver and vascular endothelial cells. In these processes, PERK-Nrf2 signaling played a critical role in endothelium, whereas MAPK-Nrf2 signaling was essential in hepatocytes. These findings should be further confirmed by observing the effects of CO exposure in PERK or Nrf2 knockout mice. Last, it would be interesting to study how the specificity of cell signaling by CO can be obtained in different cell types or organs. In these respects, the differential responses of endothelial and smooth muscle cell (or pericytes) to CO must be investigated further.

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References


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