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Original Contribution

HNE increases HO-1 through activation of the ERK pathway in pulmonary epithelial cells

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Abstract

Heme oxygenase-1 (HO-1) is a key cytoprotective enzyme and an established marker of oxidative stress. Increased HO-1 expression has been found in the resident macrophages in the alveolar spaces of smokers. The lipid peroxidation product 4-hydroxynonenal (HNE) is also increased in the bronchial and alveolar epithelium in response to cigarette smoke. This suggests a link between a chronic environmental stress, HNE formation, and HO-1 induction. HNE is both an agent of oxidative stress in vivo and a potent cell signaling molecule. We hypothesize that HNE acts as an endogenously produced pulmonary signaling molecule that elicits an adaptive response culminating in the induction of HO-1. Here we demonstrate that HNE increases HO-1 mRNA, protein, and activity in pulmonary epithelial cells and identify ERK as a key pathway involved. Treatment with HNE increased ERK phosphorylation, c-Fos protein, JNK phosphorylation, c-Jun phosphorylation, and AP-1 binding. Whereas inhibiting the ERK pathway with the MEK inhibitor PD98059 significantly decreased HNE-mediated ERK phosphorylation, c-Fos protein induction, AP-1 binding, and HO-1 protein induction, inhibition of the ERK pathway had no effect on HNE-induced HO-1 mRNA. This suggests that ERK is involved in the increase in HO-1 through regulation of translation rather than transcription.

Keywords: 4-Hydroxynonenal; Oxidative stress; HO-1; MAPK signaling; Free radicals

Heme oxygenase-1 (HO-1) is an important cytoprotective enzyme and widely accepted marker of oxidative stress. HO-1 catalyzes the first and rate-limiting step in the catabolism of heme, which generates equimolar amounts of biliverdin, ferrous iron, and carbon monoxide [1–3]. Although heme is the major substrate of HO-1, a variety of non-heme-containing agents are also strong inducers of HO-1. HO-1 has been shown to respond to a number of proinflammatory cytokines, including TNF-α, IL-1, and IL-11 [4]. HO-1 is also induced by a variety of agents that cause oxidative stress and/or in response to environmental stress [5–8]. For example, increased HO-1 expression has recently been found in the alveolar spaces in the resident macrophages of smokers [9]. Once induced, HO-1 provides protection against multiple types of tissue injury [10–13]; specific to the lung, overexpression of HO-1 in pulmonary epithelial cells has been shown to protect against oxygen toxicity [14]. In contrast, HO-1 deficiency in mice (HO-1$^{-/-}$) increases susceptibility to inflammatory lung injury [15], as does HO-1 deficiency in humans [16]. Although the

Abbreviations: AP-1, activator protein-1; EMSA, electrophoretic mobility shift assay; EpRE, electrophile response element; ERK, extracellular signal-regulated kinase; HNE, 4-hydroxynonenal; HO-1, heme oxygenase-1; HRP, horseradish peroxidase; JNK, c-Jun N-terminal kinase; MAPK, mitogen-activated protein kinase; NFDM, nonfat dry milk; PD, PD98059; SB, SB202190; SP, SP600125; StRE, stress response element; T-TBS, Tris-buffered saline, with 0.05% Tween 20.

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precise mechanisms by which HO-1 exerts its cytoprotective effects are not known, there is mounting evidence that carbon monoxide plays a key role in this process. For thorough reviews of the recent literature see [17,18].

Another characteristic of oxidative stress is the formation of the lipid peroxidation product 4-hydroxy-2-nonenal (HNE). HNE has been shown to increase HO-1 in the cell [19,20], but the mechanism(s) has not been clearly defined. HNE is an α,β-unsaturated aldehyde that is formed from the reaction of oxygen species with arachidonate in cellular membranes during many forms of environmental stress, including exposure to cigarette smoke [9,21,22]. HNE is known to impact the cell in many ways, which include inactivation of enzymes, depletion of intracellular glutathione, and inhibition of DNA and protein synthesis [23–25]. Recent data suggest that HNE may also modulate biological responses by triggering intracellular signal transduction pathways [26–28].

The mitogen-activated protein kinases (MAPK) are among the signal transduction pathways activated by HNE. As such, the MAPK pathways may play a significant role in mediating the many cellular functions that are affected by HNE. The MAPK are activated by dual phosphorylation on specific tyrosine and threonine residues [29]. The MAPK include the extracellular signal-regulated kinases 1 and 2 (ERK1/2), p38 MAP kinases, c-Jun N-terminal kinases (JNK), and the big MAPK. The MAPK are activated in response to various stressors, including oxidative stress. They phosphorylate and activate key transcription factors, thereby regulating the transcription of many genes. For a comprehensive review of MAPK-redox signaling see [29]. Members of the MAPK family are critical regulators of the transcription factor complex AP-1 (activator protein-1). AP-1 consists of proteins of the Jun, Fos, and ATF families that can form homo- and heterodimers. Activation of AP-1 involves expression of AP-1-driven genes that include c-Jun. c-Jun may also form part of the stress response (StRE) and electrophile response elements (EpRE), also key regulators of redox-sensitive genes [30,31].

HO-1 gene and protein expression is also modulated by MAPK activation. Seminal studies support a role for these kinases as mediators of HO-1 induction in a broad range of tissues and cell types. Studies have shown that HO-1 is induced via the ERK pathway by LPS + IFN-γ [32], through p38 MAPK in murine macrophages in response to IL-1β, and in human lung epithelial cells by TGF-β1 [33,34]. However, care must be taken not to generalize, as the signaling pathways that are activated are both agonist- and species-specific.

In this study, we set out to determine if HNE induces HO-1 in lung pulmonary epithelial cells, and, if so, the pathway or pathways involved. As HNE and HO-1 are increased in the bronchial and alveolar epithelium in response to chronic environmental insults such as cigarette smoke, mechanistically, this raises the possibility that such insults increase HNE, which in turn induces HO-1. We tested this hypothesis in vitro, by treating lung epithelial cells with HNE, assaying the effects on HO-1 induction, and then determining which of the MAPK pathways are involved.

Materials and methods

Materials

HNE was purchased from Cayman Chemical (Ann Arbor, MI, USA). SB202190 (SB), PD98059 (PD), SP600125 (SP), and the JNK inhibitor peptide (JNKi) were purchased from Calbiochem. TRIzol reagent was purchased from Life Technologies (Grand Island, NY, USA). The antiserum to the small Maf proteins was a gift from Volker Blank (McGill University, Montréal, QC, Canada). All other antibodies were obtained from Santa Cruz Biotechnology (Santa Cruz, CA, USA). All chemicals were at least of analytical grade and were purchased from Sigma–Aldrich unless otherwise noted.

Cell culture and treatments

L2 cells are a rat alveolar epithelial type II-like cell line that was purchased from the ATCC. Cultures were maintained in F12-K nutrient mixture supplemented with 1% penicillin/streptomycin and 10% BSA in a humidified incubator at 37°C with 5% CO_2. Cells were grown to near confluence and then treated as outlined below.

HNE was dissolved in ethanol; the inhibitors SB, PD, and SP were dissolved in dimethyl sulfoxide (DMSO); and the JNKi was dissolved in water. The final concentrations of ethanol and DMSO were 0.05 and 0.1%, respectively. Treatments were performed when the cells reached 90–95% confluence. For inhibitor studies cells were pretreated with 25 μM SB, 50 μM PD, 20 μM SP, 20 μM JNKi, or the dilution buffer (containing DMSO) for 30 min and then incubated with 20 μM HNE for increasing lengths of time as outlined under Results. After treatments, cells were washed once with 1× phosphate-buffered saline (PBS) and harvested with a cell scraper in 1× PBS. Cells were centrifuged at 500 g for 5 min, trace PBS was removed, and the pellets were either processed immediately or stored at −80°C.

Western blotting analysis

Western blotting was done as described previously [35,36]. Briefly, cell lysates were prepared using the M-PER reagent (Pierce, Rockford, IL, USA) following the manufacturer's instructions. Between 20 and 50 μg of protein was electrophoresed under denaturing conditions on a 10% Tris–glycine acrylamide gel (Invitrogen), transferred to a polyvinylidene difluoride membrane (Immobilon P; Millipore), and then blocked in nonfat dry milk (NFDM) at