

A *cis*-Acting Region Regulates Oxidized Lipid-Mediated Induction of the Human Heme Oxygenase-1 Gene in Endothelial Cells

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Objective—Several proatherogenic agents including oxidized LDL and its major component, 13-hydroperoxyoctadecadienoic acid (13-HPODE), upregulate heme oxygenase-1 (HO-1). Our previous studies have demonstrated that 13-HPODE-mediated HO-1 induction occurs via transcriptional mechanisms. The purpose of this study was to evaluate the molecular regulation and identify the signaling pathways involved in 13-HPODE-mediated HO-1 induction in human aortic endothelial cells.

Methods and Results—The half-life of HO-1 mRNA after stimulation with 13-HPODE was \approx 1.8 hours. Antioxidants such as *N*-acetylcysteine, iron chelation with deferoxamine mesylate, and protein kinase C inhibition with Gö6976 blocked HO-1 induction. Using promoter constructs up to 9.1 kb, no significant reporter activity was observed in response to 13-HPODE. A 13-HPODE-inducible DNase I hypersensitive site was identified that maps to a region \approx 10 to 11 kb from the transcription start site of the human HO-1 gene. Based on the DNase I analysis, a -11.6 -kb human HO-1 promoter construct was generated and elicited a 2.5-fold increase in reporter activity, indicating that 13-HPODE-mediated human HO-1 induction requires, at least in part, sequences that reside between 9.1 and 11.6 kb of the human HO-1 promoter.

Conclusions—Elucidation of the molecular mechanisms which control HO-1 gene expression will allow us to develop therapeutic strategies to enhance the cytoprotective potential of HO-1 in atherosclerosis. (*Arterioscler Thromb Vasc Biol.* 2003;23:1416-1422.)

Key Words: atherosclerosis ■ heme oxygenase-1 ■ gene transcription ■ chromatin structure ■ oxidized LDL

Modified lipoproteins such as oxidized LDL (oxLDL) have been implicated in the initiation and development of atherosclerosis.^{1,2} OxLDL consists of several chemically distinct prooxidant components, including fatty acid hydroperoxides and phospholipids, that serve as specific redox signals in the expression of growth factors³ and cell adhesion molecules⁴ in endothelial cells. A major component of oxLDL, the lipoxxygenase product of linoleic acid, 13-hydroperoxyoctadecadienoic acid (13-HPODE or LAox), is abundantly detectable in human atherosclerotic plaques.⁵ Oxidant stress associated with atherosclerotic lesions influences vascular cell gene expression, resulting in the induction of antioxidant defense mechanisms to counteract and prevent additional oxidative damage.⁶ One such defense mechanism is the induction of heme oxygenase-1 (HO-1), an adaptive and beneficial response to oxidative stress.⁷⁻⁹ Most relevantly, an abundance of HO-1 mRNA and protein exists in animal and human atherosclerotic plaques.¹⁰

The protective effects of HO-1 induction in atherosclerosis is suggested by reports that upregulation of HO-1 by hemin,

a potent inducer of the enzyme, inhibits atherosclerotic lesion formation in LDL-receptor knockout mice whereas inhibition of HO-1 enzyme activity by tin protoporphyrin leads to accelerated atherosclerosis in these mice as well as in Watanabe heritable hyperlipidemic rabbits.^{11,12} The most direct evidence is derived from studies where overexpression of HO-1 using intracardiac administration of an adenoviral HO-1 vector inhibited the development of atherosclerosis in apolipoprotein E-deficient mice.¹³ More recently, the presence of oxLDL, endothelial injury, fatty streaks, and fibrous plaques in the aorta of a patient with HO-1 deficiency was also reported.^{14,15}

HO-1, a 32-kDa microsomal enzyme, catalyzes the rate-limiting step in the degradation of heme yielding biliverdin, carbon monoxide (CO), and free iron.¹⁶ HO-1 is induced by a wide variety of injurious stimuli including heme, heavy metals, growth factors, nitric oxide (NO), peroxyxynitrite, oxLDL, and cytokines, all of which impose a significant shift in cellular redox.^{7,9} Several proatherogenic agents induce HO-1, including lipid metabolites,¹⁷⁻¹⁹ proinflammatory cy-

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tokines,²⁰ peroxynitrite,²¹ and heme.²² In addition to the breakdown of the toxic, prooxidant heme moiety,²³ the products of the heme oxygenase reaction have beneficial effects. Biliverdin is subsequently converted by biliverdin reductase into bilirubin, an antioxidant capable of scavenging peroxy radicals and inhibiting lipid peroxidation.²⁴ The unbound iron liberated during the HO-1 reaction is safely sequestered by ferritin, an intracellular repository for iron.²⁵ The regulation of intracellular iron levels has been suggested as a mechanism for the cytoprotective effects of HO-1 expression.²⁶ CO, a vasodilator that may be beneficial in an impaired blood vessel, has been shown to exhibit potent antiapoptotic and anti-inflammatory effects as well.²⁷ The protective effects of HO-1 overexpression have also been attributed to the upregulation of the cell-cycle regulatory protein p21.²⁸

The molecular mechanisms of oxLDL-mediated HO-1 induction are not known. It has been previously shown that oxLDL and at least 3 of its constituents, 13-HPODE, lyso-phosphatidylcholine (lyso-PC), and oxidized 1-palmitoyl-2-arachidonoyl-sn-glycero-3-phosphorylcholine (oxPAPC), up-regulate the HO-1 gene in endothelial and vascular smooth muscle cells, macrophages, and renal tubular epithelial cells.^{17–19} 13-HPODE causes the most robust induction of HO-1 mRNA levels, and this induction is dependent on de novo transcription.¹⁹ The purpose of this study was to elucidate the signaling pathway and the molecular mechanism involved in 13-HPODE-mediated HO-1 gene induction using specific inhibitors of predicted pathways along with DNase I hypersensitive site and human HO-1 promoter analyses in human aortic endothelial cells.

Methods

Reagents

Tissue culture media, FBS, and media supplements were obtained from Clonetics. Linoleic acid, lipoxygenase (type V, soybean), hemin, actinomycin-D, *N*-acetylcysteine (NAC), and deferoxamine mesylate (DFO) were purchased from Sigma Chemical Co. 13(S)-hydroxyoctadecadienoic acid (13-HODE) was purchased from Cayman Chemical. Lyso-PC, PD98059, SB203580, SB202190, Gö6976, KT5720, and chelerythrine chloride were obtained from Calbiochem. DNase I was purchased from Worthington Biochemicals.

Cell Culture

Human aortic endothelial cells (HAECs) (derived from segments of human aorta obtained from heart transplant donors and previously characterized by positive staining for von Willebrand factor antigen and acetylated LDL²⁹) were grown in endothelial basal medium (Clonetics) supplemented with 10% FBS, gentamicin (50 $\mu\text{g}/\text{mL}$), amphotericin B (50 $\mu\text{g}/\text{mL}$), hydrocortisone (1 $\mu\text{g}/\text{mL}$), human epidermal growth factor (10 ng/mL), and bovine brain extract (6 $\mu\text{g}/\text{mL}$). Studies were performed on cultures over a range of 3 to 6 passages. All cells were grown at 37°C in 95% air and 5% CO₂.

Preparation of 13-HPODE

13-HPODE was freshly prepared as previously described.^{4,19}

Northern and Western Analysis

Total RNA and protein were extracted from cultured cells as described previously.¹⁹

Plasmid Constructs and Transient Transfection Analysis

The 5' flanking region of the human HO-1 gene, extending from -9.1 kb to the +80-bp position, was generated by long-range polymerase chain reaction from a human BAC clone (accession No. Z82244) using rTth DNA polymerase (Perkin-Elmer). *SalI* sites were incorporated in the primers to enable subcloning into the *XhoI* site of pGL3 to generate pHOGL3/9.1. A -11.6-kb HO-1 promoter fragment was also constructed in pGL3. First, a -4.5-kb human HO-1 promoter construct (pHOGL3/4.5) was digested with *NheI* to generate pHOGL3/3.4, then an 8.2-kb *NheI/NheI* fragment (from -3.4 to -11.6 kb from the BAC clone) was gel purified and ligated into pHOGL3/3.4, thereby generating pHOGL3/11.6.

A batch transfection protocol was performed in 15-cm plates using diethylaminoethyl-dextran for HAECs as previously described.¹⁹ Equimolar amounts of plasmid DNA were transfected to control for effective length of the constructs. The day after transfection, cells were split into smaller plates and then allowed to recover for 24 hours before stimulation in 1% FBS in complete media with or without 13-HPODE (25 $\mu\text{mol}/\text{L}$) for 16 hours. Cell lysates were collected and luciferase activity was measured according to the manufacturer's instructions (Promega).

Cell Permeabilization and DNase I Analysis of Chromatin Structure

The method was modified from that described by Mellot et al.³⁰ Details of the method are provided in the attached supplement, which can be found at <http://atvb.ahajournals.org>.

Data Analysis

Results are derived from 2 to 3 independent experiments performed in triplicate each time. Data are expressed as mean \pm SEM. Statistical analysis was performed using Student's *t* test or ANOVA and the Student Newman-Keuls test. All results are considered significant at $P < 0.05$.

Results

13-HPODE-Mediated HO-1 Induction Is Not Dependent on mRNA Stability

We had previously shown that HO-1 mRNA was induced (5- and 16-fold) by 13-HPODE (10 and 25 $\mu\text{mol}/\text{L}$, respectively) at 4 hours and that this increase was dependent on de novo gene transcription.¹⁹ To determine optimal treatment time, HAECs were exposed to 13-HPODE (25 $\mu\text{mol}/\text{L}$) from 0 to 12 hours. Maximum induction was observed at 4 hours of exposure (Figure 1A). We also examined the effects of 13-HODE, another oxidized lipid component of oxLDL. Exposure of HAECs to 13-HODE (1, 10, and 25 $\mu\text{mol}/\text{L}$) for 0, 2, 4, 6, and 8 hours did not induce HO-1 mRNA (data not shown). To determine message half-life and to examine the contribution of mRNA stability in 13-HPODE-mediated HO-1 induction, HAECs were incubated with 13-HPODE (25 $\mu\text{mol}/\text{L}$) for 4 hours, washed with PBS, and then exposed to media containing actinomycin D (4 $\mu\text{mol}/\text{L}$) in the absence or presence of additional 13-HPODE. As shown in Figure 1B, no significant change in the half-life of HO-1 mRNA was observed in the actinomycin D-treated cells in the presence or absence of the second dose of 13-HPODE, demonstrating that the induction is dependent solely on de novo transcription as previously implied by nuclear run-on studies.¹⁹

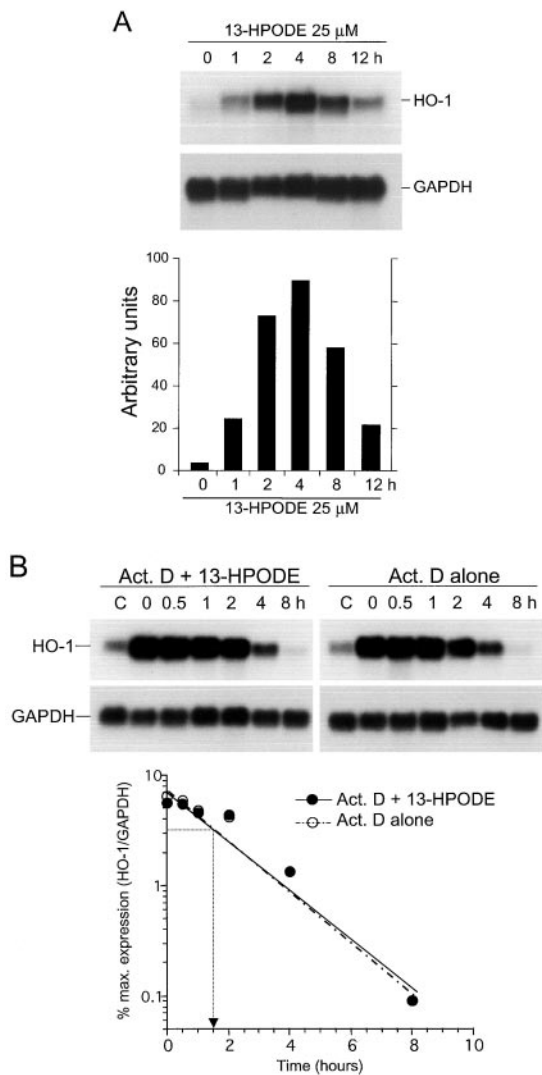


Figure 1. 13-HPODE-mediated HO-1 induction in HAECs. A, Confluent HAECs were incubated in 1% FBS-containing medium with 13-HPODE (25 μmol/L) for the times indicated. B, HAECs were preincubated with 13-HPODE (25 μmol/L) in 1% FBS-containing medium for 4 hours, washed, and exposed to actinomycin D (4 μmol/L) in the absence (○, dashed line) or presence (●, solid line) of additional 13-HPODE (25 μmol/L). Total RNA was isolated and processed at the times indicated. Northern analysis was performed using ³²P-labeled human HO-1 and GAPDH cDNA probes as described in Methods.

13-HPODE-Mediated HO-1 mRNA Induction Is Dependent on Reactive Oxygen Species

Previous studies have shown that NAC, which scavenges reactive oxygen species (ROS) and increases cellular glutathione levels, blocks sodium arsenite-mediated, cadmium chloride-mediated, and hydrogen peroxide-mediated HO-1 induction. Similarly, the iron chelator DFO blocks HO-1 induction by hyperoxia and oxLDL, with no effect by either reagent on transforming growth factor-β (TGF-β) or curcumin-mediated HO-1 induction.^{17,31–33} These agents were used to investigate the possible involvement of ROS in the induction of HO-1 mRNA during 13-HPODE treatment. HAECs were pretreated with NAC for 30 minutes in 1%

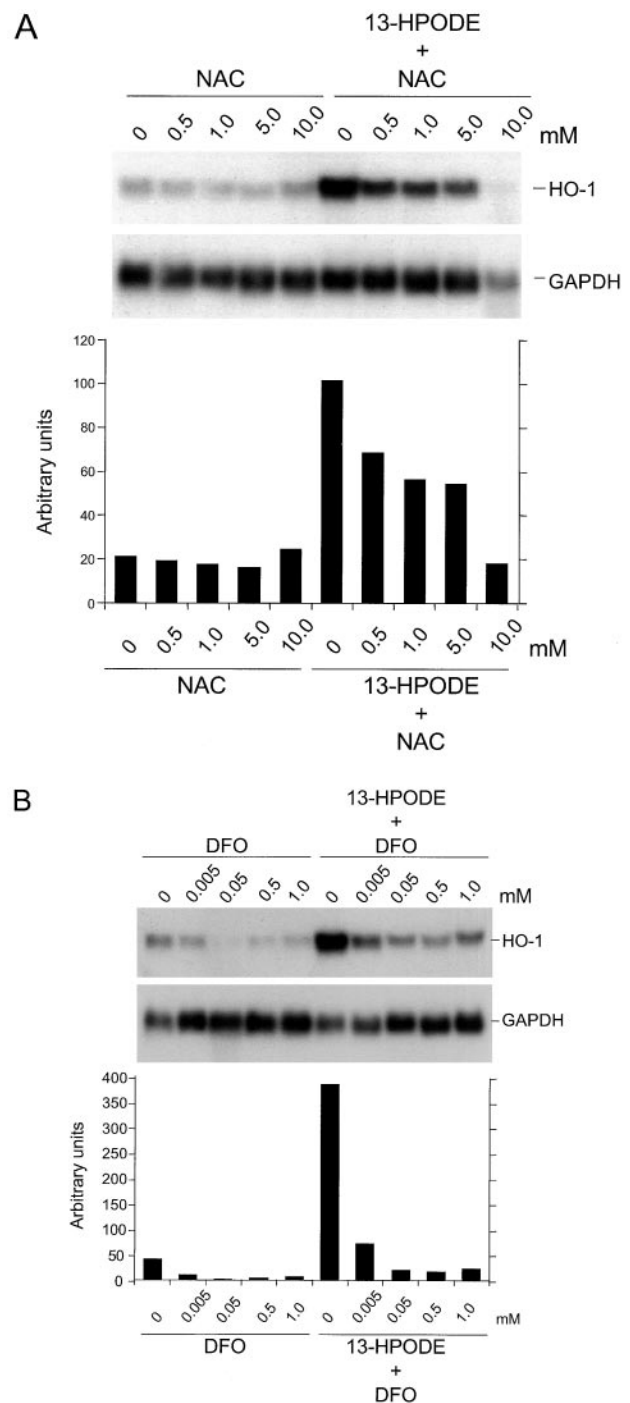


Figure 2. Effects of NAC and DFO on 13-HPODE-mediated induction of HO-1 mRNA. Confluent HAECs in 1% FBS-containing medium were pretreated with NAC (0.5 to 10.0 mmol/L) for 30 minutes followed by cotreatment of NAC with 13-HPODE (25 μmol/L) for 4 hours (A) or with DFO (0.005, 0.05, and 5.0 mmol/L) overnight followed by cotreatment of DFO with 13-HPODE (25 μmol/L) for 4 hours (B). Total RNA was isolated, and Northern analysis was performed as described in Methods.

FBS-containing medium or DFO overnight. NAC (0.5 to 10.0 mmol/L) and DFO (0.005 to 1.0 mmol/L) prevented HO-1 induction in HAECs exposed to 13-HPODE (Figures 2A and 2B), implicating the involvement of ROS in 13-HPODE-mediated HO-1 upregulation.

Role of Kinase Pathways in the Induction of HO-1 by 13-HPODE

Activation of mitogen-activated protein kinases (MAPKs), protein kinase A, and protein kinase C (PKC) is involved in the signal transduction of HO-1 by several stimuli.^{34–37} Therefore, inhibitors of these pathways were used to assess their potential involvement in the induction of HO-1 mRNA by 13-HPODE. The general PKC inhibitor chelerythrine chloride (0.5 to 5 $\mu\text{mol/L}$) failed to inhibit HO-1 induction by 13-HPODE, and, in fact, treatment with 3 and 5 $\mu\text{mol/L}$ chelerythrine chloride alone significantly induced HO-1 mRNA and protein (Figures 3A and 3C). However, pretreating HAECs with Gö6976 (1 to 10 $\mu\text{mol/L}$), which selectively inhibits Ca^{2+} -dependent PKC α -isozyme, PKC β_1 , and PKC μ , attenuated HO-1 mRNA and protein induction by 13-HPODE (Figures 3B and 3C). In contrast, no inhibition was observed when HAECs were pretreated with SB203580 and SB202190 (2 different p38 MAPK inhibitors, 0.1 to 20 μM), PD98059 (an ERK inhibitor, 50 μM), or KT5720 (protein kinase A inhibitor; 1 $\mu\text{mol/L}$) (data not shown). Most strikingly, it seems that some isoforms of PKC inhibit, whereas other isoforms negatively regulate, 13-HPODE-mediated HO-1 induction.

cis-Acting Regions Beyond the –9.1-kb Human HO-1 Promoter Are Responsible for 13-HPODE-Mediated HO-1 Induction

Li et al³⁸ have suggested that an antioxidant response element, located at –4.0 kb of the mouse HO-1 promoter, confers oxidized phospholipid-mediated HO-1 induction in murine macrophages. We had previously shown that a –4.5-kb human HO-1 promoter fragment does not contain 13-HPODE-responsive elements.¹⁹ Alam et al³⁹ have described 2 distal promoter regions, E1 (or SX2) and E2 (or AB1) at –4.0 and –10 kb, respectively, that are required for induction of the mouse HO-1 gene in response to multiple agents. In the human HO-1 gene, 2 regions, A and B, at \approx –4.0 and \approx –9.0 kb, respectively, contain sequences similar to the mouse E1 and E2, respectively (Figure 4A). Based on these findings, we generated a –9.1-kb human HO-1 promoter fragment (pHOGL3/9.1, Figure 4B) and tested this construct in transient transfections in HAECs. As shown in Figure 4C, no significant increase in luciferase activity was observed after 13-HPODE stimulation, suggesting that induction of the human HO-1 gene by 13-HPODE requires sequences outside of the 9.1-kb promoter fragment.

Identification of a 13-HPODE-Dependent DNase I Hypersensitive Site

Because promoter analysis of up to 9.1 kb of the human HO-1 promoter failed to respond to 13-HPODE, DNase I hypersensitive studies were performed to identify 13-HPODE-specific regulatory elements that may exist 5' to this region. A 225-bp single copy probe that abuts the *EcoRI* site at –1.4 kb from the transcription start site was used to evaluate by DNase I analysis a region extending to –21.1 kb. As shown in Figure 5, an inducible hypersensitive site was identified that maps to a region \approx 9.0 kb from the 3' *EcoRI* site or \approx 10 to 11 kb from the transcription start site of the human HO-1 gene.

Cis-Acting Regions Between –9.1 and –11.6 kb of the Human HO-1 Promoter Are Required for 13-HPODE-Mediated HO-1 Induction

A –11.6-kb HO-1 promoter construct (pHOGL3/11.6, Figure 6A) was generated and evaluated in transient transfection assays based on the presence of the 13-HPODE-dependent DNase I hypersensitive site at \approx 10 to 11 kb from the transcription start site of the human HO-1 gene (Figure 6A). As shown in Figure 6B, 13-HPODE elicited a 2.5-fold increase in luciferase activity in cells transfected with pHOGL3/11.6. Because Gö6976 inhibited 13-HPODE-mediated HO-1 expression, we tested its effects on 13-HPODE-mediated activation of pHOGL3/11.6. As shown in Figure 6B, Gö6976 inhibited 13-HPODE-inducible promoter activity, additionally implicating the PKC pathway. These results demonstrate that sequences between the 9.1- and 11.6-kb human HO-1 promoter are required, at least in part, for 13-HPODE-mediated HO-1 induction and additionally illustrate the unique molecular mechanisms associated with the regulation of the human HO-1 gene.

Discussion

In the present study, we demonstrate that 13-HPODE-mediated HO-1 induction has a half-life of approximately 1.8 hours and is not dependent on mRNA stability, results consistent with our previous findings that 13-HPODE-mediated HO-1 induction occurs via increased *de novo* transcription.¹⁹ The induction of the HO-1 gene by several, but not all, stimuli occurs through changes in cellular oxidative stress.^{7,32,33} In contrast, HO-1 induction by curcumin and TGF- β , for example, requires mechanisms that are independent of changes in the cellular redox state.^{32,33} Oxidative stress, however, does play a role in oxLDL- and hyperoxia-mediated HO-1 induction.^{17,31} Similarly, we observed that 13-HPODE-mediated upregulation is dependent on ROS, because NAC, as well as DFO, blocked the induction of HO-1.

Several inducers of HO-1 are also activators of MAPK, including 13-HPODE, lipopolysaccharide, growth factors, and phorbol esters.^{34,40} We therefore examined the potential role of these pathways in HO-1 induction by 13-HPODE. Our data indicate, however, that this induction is independent of the ERK and p38 MAP kinase pathways. The involvement of the MAPK in HO-1 induction has been highly variable, depending on stimuli and cell type. For instance, ERK and p38 MAPK are involved in NO-mediated and sodium arsenite-mediated induction of HO-1.^{34,41} By contrast, Masuya et al³⁶ have demonstrated that tyrosine kinases rather than MAPK are involved in the regulation of HO-1 gene expression by various inducers, including sodium arsenite, in HeLa cells.

Interestingly, we observed that PKC may be important in the induction of HO-1 by 13-HPODE. As with MAPK, the involvement of PKC in HO-1 induction has been highly variable, depending on stimuli and cell type.^{37,42} Terry et al³⁷ demonstrated that in human vascular endothelial cells, TNF- α and interleukin-1 α induction of HO-1 requires PKC-mediated phosphorylation. Analogously, the PKC signaling pathway is necessary for 13-HPODE-mediated HO-1 induction as demonstrated by inhibition of HO-1 induction by Gö6976, an

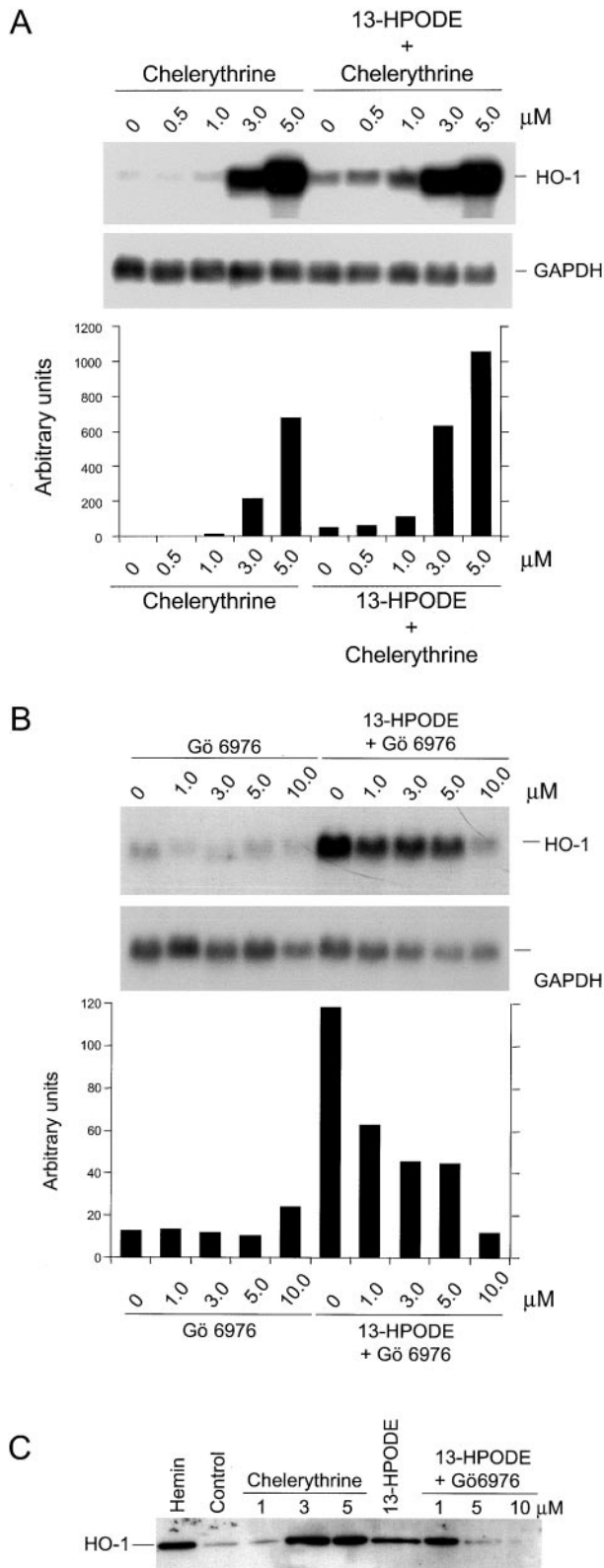


Figure 3. Effect of PKC inhibitors on 13-HPODE-mediated induction of HO-1 mRNA and protein. Confluent HAECs were pretreated with chelerythrine chloride (0.5 to 5.0 $\mu\text{mol/L}$) (A and C) and with Gö6976 (1 to 20 $\mu\text{mol/L}$) (B and C) in 1% FBS-containing medium for 30 minutes followed by cotreatment of inhibitors with 13-HPODE (25 $\mu\text{mol/L}$). Total RNA (A and B) was isolated at 4 hours, and Northern analysis was performed as described in Methods. Total protein (C) was isolated at 16

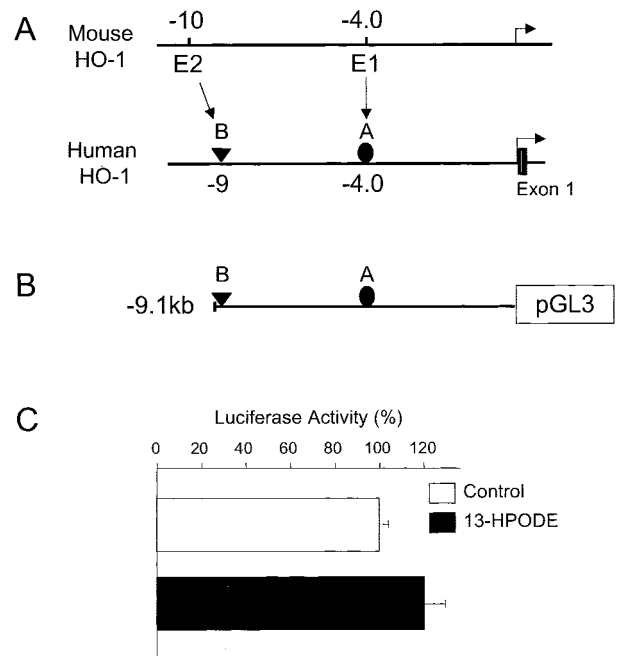


Figure 4. Analysis of the 9.1-kb HO-1 promoter-reporter gene in response to 13-HPODE. A, Location of 2 distal promoter regions, E1 (or SX2) and E2 (or AB1), at -4.0 and -10 kb, respectively, of the mouse HO-1 gene and the 2 regions, A and B, at ≈ -4.0 and ≈ -9.0 kb, respectively, of the human HO-1 gene. B, The promoter-reporter gene construct containing a 9.1-kb promoter fragment of the human HO-1 gene and a luciferase (pGL3) plasmid (pHOGL3/9.1). C, pHOGL3/9.1-transfected HAECs were exposed to 13-HPODE (25 $\mu\text{mol/L}$, dark bar, $n=6$) or PBS (control, open bar, $n=6$) for 16 hours, lysed, and assayed for luciferase, as described in Methods.

inhibitor of α -, β -, and μ -PKC isozymes. Interestingly, blockade of the overall PKC pathway with chelerythrine chloride resulted in a significant upregulation of HO-1, suggesting that other PKC isoforms may function as repressors of HO-1 gene expression. Additional studies to evaluate the role of PKC isoforms in 13-HPODE-mediated HO-1 induction using dominant-negative inhibitors would be of interest.

Thus far, most of our knowledge regarding the molecular regulation of the HO-1 gene is derived from extensive evaluation of the mouse HO-1 gene. Alam et al^{39,43,44} have described 2 distal promoter regions (E1 and E2) at -4.0 and -10 kb, respectively, that are required for induction of the mouse HO-1 gene in response to multiple stimuli. These regions contain sequences that recognize the Nrf2 transcription factor.⁴³ It was proposed that all of the stimuli activate the mouse HO-1 gene exclusively via E1 or E2. In the human gene, 2 regions (A and B) at ≈ -4.0 and ≈ -9.0 kb, respectively, contain sequences similar to the mouse E1 and E2, respectively. In addition, an antioxidant response element, located at -4.0 kb of the mouse HO-1 promoter, mediates oxidized phospholipid-mediated HO-1 induction in murine macrophages.³⁸

hours, and Western analysis was performed using a rabbit polyclonal HO-1 antibody (Stressgen, Vancouver, Canada) as described in Methods. Cells treated with hemin (5 $\mu\text{mol/L}$) were used as a positive control. HO-1 was identified as a band at 32 kDa.

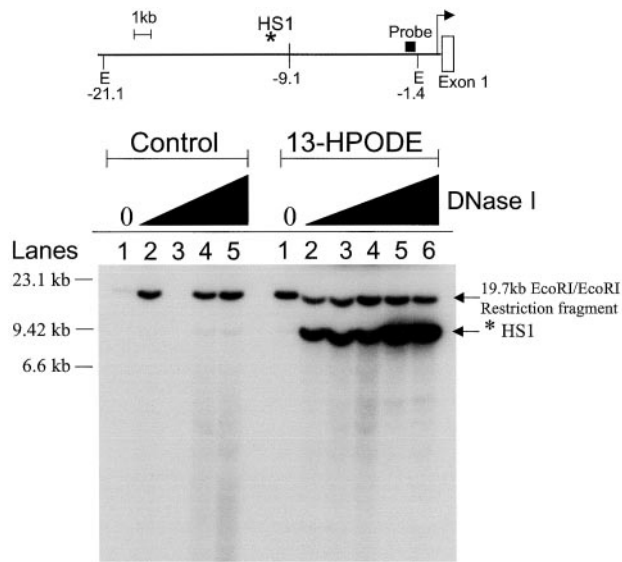


Figure 5. Identification of a 13-HPODE-inducible DNase I hypersensitive site in the human HO-1 promoter. The restriction fragment is a 19.7-kb E/E (*EcoRI*) fragment of the HO-1 promoter (−1.4 to −21.1 kb). After exposure to PBS (control) or 13-HPODE (25 μ mol/L) for 4 hours, cells were permeabilized with lyso-PC and treated with increasing concentrations of DNase I (0, 4, 8, 12, 16, and 32 U/mL) (lanes 1 through 6, respectively). Genomic DNA was isolated and digested with *E*, and Southern blot analysis was performed using a 225-bp probe that abuts the 3' *E* site at −1.4 kb, as shown. Arrow, Transcription start site. *Hypersensitive site (HS1) that maps to \approx 10 to 11 kb from the transcription start site.

Based on the mouse HO-1 data, we generated multiple human HO-1 promoter constructs (up to −9.1 kb) that include the corresponding response elements described in the mouse gene and incorporated them into reporter vector systems to identify potential regions in the human gene responsible for human HO-1 induction. We have observed that the regions A and B in the human HO-1 gene are only partially responsible for heme, NO, and cadmium-mediated HO-1 gene induction (unpublished observations), and unlike the mouse HO-1 gene, these regions in the human HO-1 promoter do not respond to other stimuli such as oxidized lipids, hyperoxia, hydrogen peroxide, or TGF- β .^{19,31,32} Similarly, we show here that promoter constructs of the human HO-1 gene, up to −9.1 kb, were not activated by 13-HPODE. That human HO-1 is regulated differently than the mouse gene is also indicated by the presence of a GT repeat region in the human HO-1 gene, which is absent in the mouse HO-1 gene; length polymorphisms of this region correlates with disease development of atherosclerosis,⁴⁵ emphysema,⁴⁶ and vascular restenosis.⁴⁷ Clearly, differences exist in the regulation of the mouse versus the human HO-1 gene, and the molecular mechanisms involved in the activation of the human HO-1 gene need to be further explored.

To identify 13-HPODE-specific regulatory elements in the human HO-1 promoter, DNase I hypersensitive studies were performed to evaluate changes in chromatin structure. A 13-HPODE-inducible hypersensitive site was revealed that maps to a region \approx 10 to 11 kb from the transcription start site of the human HO-1 gene. A −11.6-kb human HO-1 promoter construct was therefore generated, and a 2.5-fold increase in reporter

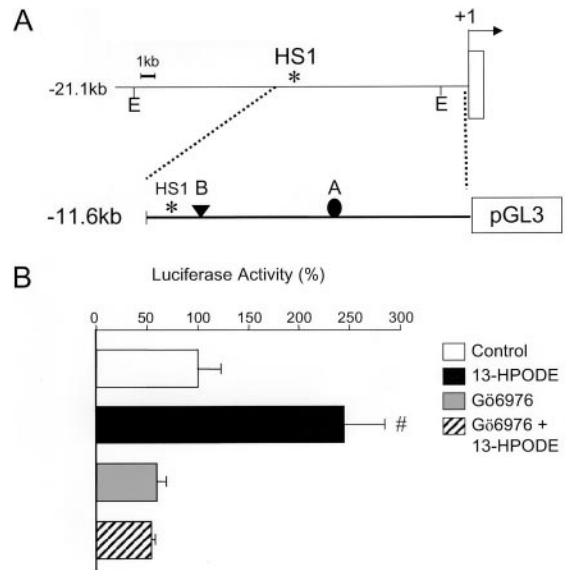


Figure 6. Analysis of the pHOGL3/11.6 in response to 13-HPODE. pHOGL3/11.6 was transfected into HAECs by the diethylaminoethyl-dextran method as described in Methods. A, *EcoRI* restriction fragment of human HO-1 with 13-HPODE-mediated DNase I-HS (HS1) site and the promoter-reporter gene construct (pHOGL3/11.6) containing an 11.6-kb promoter fragment of the human HO-1 gene is illustrated. B, pHOGL3/11.6-transfected HAECs were exposed to control (PBS), 13-HPODE (25 μ mol/L), Gö6976 (5 μ mol/L), or 13-HPODE (25 μ mol/L) plus Gö6976 (5 μ mol/L) for 16 hours, lysed, and assayed for luciferase, as described in Methods. Luciferase activity is plotted for control (open bar, n=3) and cells treated with 13-HPODE (dark bar, n=4), Gö6976 (gray bar, n=6), and 13-HPODE plus Gö6976 (hatched bar, n=12). #*P*<0.001.

gene activity was observed. Similar to endogenous HO-1 expression, PKC inhibition with Gö6976 also blocked promoter activity of this construct. It seems that 13-HPODE-mediated HO-1 induction requires sequences that reside between 9.1 and 11.6 kb of the human HO-1 promoter. Although this induction does not completely recapitulate levels of induction seen with the endogenous gene, it is possible that additional sequences outside this region will also be required for 13-HPODE-mediated HO-1 induction. Additional studies to identify such potential regulatory elements will be important.

The physiological relevance of oxLDL and HO-1 in atherogenesis is highlighted by the recent demonstration of the presence of highly oxidized LDL in an HO-1-deficient patient.¹⁴ The patient exhibited extensive endothelial damage, and LDL isolated from the plasma of this patient was cytotoxic to endothelial cells in vitro, thus strengthening the concept of the cytoprotective nature of HO-1.^{14,15} Additional analysis of the molecular mechanisms involved in oxidized lipid-mediated HO-1 gene induction could aid in exploiting the cytoprotective effects of HO-1 in the pathogenesis of atherosclerosis.

Acknowledgments

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