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Role of Human Heme Oxygenase-1 in Attenuating TNF-α-Mediated Inflammation Injury in Endothelial Cells

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Abstract Heme oxygenase (HO) is the rate-limiting enzyme in the formation of bilirubin, an antioxidant, and carbon monoxide (CO), a cell cycle modulator and a vasodilator. Cyclooxygenase (COX) is a hemeprotein that catalyzes the conversion of arachidonic acid (AA) to various prostanoids, which play an important role in the regulation of vascular endothelial function in normal and disease states. The influence of suppression or overexpression of HO isoforms on COX expression and synthesis of prostanoids is of considerable physiological importance. Consequently, the goal of the present study was to determine whether the heme-HO system regulates COX enzyme expression and activity in vascular endothelial cells in the absence and presence of TNF-α (100 ng/ml). Endothelial cells stably transfected with the retrovirus containing the human HO-1 gene exhibited a several-fold increase in HO-1 protein levels, which was accompanied by an increase in HO activity and a marked decrease in PGE_2 and 6-keto $PGF_{1\alpha}$ levels. We also assessed the effect of retrovirus-mediated HO-1 gene transfer in the sense and antisense orientation on HO-1 expression and cell cycle progression in human endothelial cells. The levels of CO and HO activity were increased in cells transduced with the HO-1 sense and were greatly suppressed in cells transduced with HO-1 antisense as compared to control sham-transduced cells ($P < 0.05$). The percentage of the G_1-phase in cells transduced with HO-1 significantly increased (41.4% ± 9.1) compared with control endothelial cells (34.8% ± 4.9). We measured COX activity by determining the levels of PGI_2 and PGE_2. The levels of PGI_2 decreased in cells transduced with HO-1 sense and increased in cells transduced with HO-1 in antisense orientation. The expression of p27 was also studied and showed a marked decrease in cells transduced with HO-1 sense and a marked increase in the HO-1 antisense transduced cells. Cell cycle analysis of endothelial cell DNA distributions indicated that the TNF-α-induced decrease in the proportion of G_1-phase cells and increase in apoptotic cells in control cultures could be abrogated by transfection with HO-1 in the sense orientation. Tin mesoporphyrin (SnMP) reversed the protective effect of HO-1. These results demonstrate that overexpressing HO-1 mitigated the TNF-α-mediated changes in cell cycle progression and apoptosis, perhaps by a decrease in the levels of COX activity. J. Cell. Biochem. 87: 377–385, 2002.

Key words: Heme oxygenase; TNF; cell cycle; cyclooxygenase; cyclin

Heme oxygenase (HO) isoforms catalyze the conversion of heme to carbon monoxide (CO) and bilirubin with a concurrent release of iron (Fe), which can drive the synthesis of ferritin for Fe sequestration [Eisenstein et al., 1991; Abraham et al., 1996]. This is the sole physiological pathway of heme degradation, and consequently, plays a critical role in the regulation of endothelial cell (EC) heme levels [Abraham et al., 1996]. Heme functions as a prosthetic group in hemeprotein enzymes, e.g., nitric oxide synthase, soluble guanylate cyclase, cytochrome P450, and cyclooxygenase-1/-2 (COX). The cellular level of heme is regulated by the rate of its synthesis and degradation. Heme degradation occurs almost exclusively by oxidative cleavage of the α-meso carbon bridge of heme, eventually leading to the formation of equimolar amounts of biliverdin, iron, and CO. The HO system controls the rate-limiting step in heme degradation. To date, two HO isoforms have been shown to be active in heme catabolism, each encoded by a different gene [McCoubrey et al., 1992; Shibahara et al., 1993;

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Abraham et al., 1996]. HO-1 is catalytically very active. HO-2 shows lower activity when compared to HO-1. Under basal conditions, HO-1 is expressed at low levels in endothelial cells [Balla et al., 1993; Abraham et al., 1995; Deramaudt et al., 1998; Yachie et al., 1999] as well as in the kidney [Nath et al., 1995; da Silva et al., 2001], liver and spleen, and can be induced in these cells and in other tissues by oxidative stress causing agents, including hyperthermia [Ewing et al., 1994], oxidized lipoproteins [Ishikawa et al., 1997], inflammatory cytokines [Hibbs et al., 1992], hypoxia [Morita and Kourembanas, 1995; Minamino et al., 2001], nitric oxide (NO) and heavy metals [Abraham et al., 1996; Foresti et al., 1997]. HO-2 is constitutively expressed in blood vessels, endothelium, testis and most other tissues and its levels are relatively unaffected by the factors inducing HO-1 [Abraham et al., 1996]. All HO isoforms may be inhibited by certain synthetic heme analogs in which the central iron atom is replaced by other metals [Chernick et al., 1989].

COX catalyzes the oxygenation and peroxidation of arachidonic acid (AA) to generate prostaglandin endoperoxides, the immediate precursors of a series of biologically vasoactive and inflammatory prostaglandins. The COX reaction has an absolute requirement for heme as a cofactor in the active site. Therefore, the level of heme regulates COX activity in a concentration-dependent manner [Van der Ouderaa et al., 1979; Panara et al., 1995]. Heme binds to the COX apoenzyme with a stoichiometry of approximately one heme molecule per each subunit [Sun and Rotenberg, 1990; Smith and Marnett, 1991; Smith et al., 1996]. Two isoforms of COX have been identified as encoded by two related genes: *COX-1* is constitutively expressed and is considered to generate prostaglandins for normal physiological function, whereas *COX-2* is primarily an inducible enzyme expressed rapidly and transiently in response to a variety of stimuli, including TNF-α. Since TNF-α plays an important role during EC exposed to inflammatory conditions [Yamaoka et al., 2002], EC respond by expression of early response gene to protect against these stimuli. HO-1 is one of the early response genes, and it is considered a heat shock protein [Goldbaum and Richter-Landsberg, 2001]. Overexpression of HO-1 by gene transfer into EC protects against oxidant-induced injury [Yang et al., 1999] and promotes cell growth [Sabaawy et al., 2001; Abraham et al., 2002]. HO-1 overexpression has been shown to attenuate hypoxia induced corneal inflammation [Laniado-Schwartzman et al., 1997] and to protect transplanted organs from ischemia/perfusion injury and apoptosis [Soares et al., 1998]. Kushida et al. have shown that overexpression of HO-1 provides a protective mechanism against oxidative stress-mediated EC injury and normalizes cell cycle progression [Kushida et al., 2002].

An expanding body of information has shown that cell cycle progression, through the mammalian cell cycle is orchestrated by distinct multiple holoenzymes composed of catalytic subunits called cyclin dependent kinase (cdk) whose activities depend upon a regulatory protein called cyclin [Peter and Herskowitz, 1994; Polyak et al., 1994; Grana and Reddy, 1995] and a new class of small proteins, the so-called cyclin dependent kinase inhibitors. The latter bind to cyclin-cdk complexes and inhibit their kinase activity. Cyclin dependent kinase inhibitor proteins consist of the $p21^{cip1/waf1}$, $p27^{Kip1}$, $p57^{Kip2}$, and the INK4 family of proteins, which are specific for cdk4 and cdk6 [Darzynkiewicz et al., 1996; Juan et al., 1998]. Overexpression of these inhibitory molecules leads to cell cycle arrest in G_1 [Polyak et al., 1994; Grana and Reddy, 1995; Tooke, 1996].

The objective of this study was to examine the feasibility of utilizing the retrovirus-mediated transfer of human HO-1 sense and antisense orientation under the control of the human promoter to examine its functional expression by measurement of CO generation and prostaglandin synthesis. These cells were subsequently used to assess the significance of HO-1 in protection of cell cycle progression in presence of TNF-α. Our data demonstrate that selective delivery of the HO-1 gene in sense orientation resulted in increased CO synthesis and decreased activity of COX-2, and attenuated TNF-α-mediated cell death. In contrast, underexpression of HO-1 by delivery of HO-1 in antisense orientation exacerbated TNF-α-mediated abnormalities in cell cycle progression and increased COX-2 activity.

MATERIALS AND METHODS

Flow Cytometer and DNA Distribution

Human microvessel EC transduced with human HO-1 in sense or antisense orientation

were constructed as recently described [Quan et al., 2001], cultured under conditions of exponential growth, and treated with TNF-α (100 ng/ml) (Sigma, St. Louis, MO) for 24 h. Duplicate cultures were exposed for 24 h to either tin mesophorphorin (SnMP; 30 μM), biliverdin (10 μM) or ferrous ammonium sulfate (10 μM) for 24 h. All cells were then harvested from these cultures, washed with PBS, and stained with the DNA-specific fluorochrome, DAPI (4,6-diamidino-2-phenylindole (Molecular Probes, Inc., Eugene, OR). The cell cycle phase (DNA) distribution of the DAPI stained cells were analyzed on an EPICS flow cytometer (Beckman Coulter, Miami, FL) as previously described [Kushida et al., 2002].

Western Blot Analysis

Cells were harvested using a cell lysis buffer as previously described [Yang et al., 1999]. The lysate was collected for Western blot analysis and protein levels were visualized by immunoblotting with antibodies against human HO-1, HO-2 and/or the cyclin dependent kinase inhibitor, p27. Briefly, 30 μg of lysate supernatant was separated by SDS/polyacrylamide gel electrophoresis and transferred to a nitrocellulose membrane using a semidry transfer apparatus. The membranes were incubated with 5% milk in 10 mM Tris-HCl (pH 7.4) 150 mM NaCl, 0.05% and Tween-20 buffer (TBST) at 4°C overnight. After washing with TBST, the membranes were incubated with anti-HO-1 or anti-HO-2 antibodies (1:2,000 dilution) and anti-p27 antibodies (1:500 dilution) for 1 h at room temperature with constant shaking. The filters were then washed and subsequently probed with horseradish peroxidase-conjugated donkey anti-rabbit IgG (Amersham, city, state) at a dilution of 1:2,000 for HO-1 or HO2, and at a dilution of 1:3,000 for p27. Chemiluminescence detection was performed with the Amersham ECL detection kit according to the manufacturer's instructions.

Measurement of HO Activity

Microsomal HO activity was assayed by the method of Abraham et al. [1987] in which bilirubin, the product of HO degradation, was extracted with chloroform and its concentration determined spectrophotometrically using the difference in absorbance at wavelength from 460 to 530 nm with an absorption coefficient of 40 $mM^{-1}cm^{-1}$.

To assess CO production by EC, 24 h cell cultures were incubated for 3 h in 2 ml vials, containing 1 ml of culture media. The concentration of CO in the headspace gas was then measured. CO analyses were performed using a HP5989A mass spectrometer interfaced to a HP 5890 gas chromatograph. The separation of CO from other gases was carried out on a GS-Molesieve capillary column (30 m; 0.53 mm ID; J & W Scientific, Inc., Folsom, CA) kept at 40°C. Helium, with a linear velocity of 0.3 m/s, was used as the carrier gas. CO was eluted at 3.6 min and fully separated from N_2, O_2, H_2O, and CO_2. The mass spectrometer parameters were as follows: ion source temperature, 120°C; electron energy, 31 eV; transfer line temperature, 120°C. Using a gas-tight syringe, 100 μl aliquots of the headspace gas of either standard solutions or experimental samples were injected into a spitless injector having a temperature of 120°C. Abundance of ions at *m/z* 28, 29, and 31 corresponding to $^{12}C^{16}O$, $^{13}C^{16}O$, and $^{13}C^{18}O$, respectively, were acquired via a selected ion monitoring. The amount of CO in cell culture samples was calculated from standard curves constructed with abundance of ions *m/z* 28 and *m/z* 29 or *m/z* 31, as previously described [Zhang et al., 2001].

Measurement of PGE_2 and 6-Keto-$PGF_{1\alpha}$ Levels

The levels of PGE_2 and the stable metabolite of prostacyclin, 6-keto-$PGF_{1\alpha}$, were determined in the supernatant using an enzyme-linked immunoassay (EIA). Endothelial cells were counted and seeded in 24-well plates (1.2×10^4 cells/wells]. Cells were treated with SnMP (10 μM) and TNF-α (100 ng/ml) for 24 h, after which the media were removed and stored at −80°C. Solid-phase enzyme immunoassay was performed as suggested by the manufacturer (Cayman Chemicals, Ann Arbor, MI). 6-keto-$PGF_{1\alpha}$ and PGE_2 levels were determined using a standard curve.

Statistical Analysis

The data are presented as mean ± standard error (SE) for the number of experiments. Statistical significance ($P < 0.05$) between the experimental groups was determined by the Fisher method of multiple comparisons. For comparison between treatment groups, the null hypothesis was tested by single factor analysis of variance (ANOVA) for multiple groups or unpaired *t*-test for two groups.

RESULTS

Effect of Retroviral-Mediated Human HO-1 Sense and Antisense Transfer on Endogenous HO-1 Protein and Activity

Cells transduced with human HO-1 sense and antisense were examined for the levels of HO-1 and HO-2 proteins by Western blot analysis. The results of three representative experiments are shown in Figure 1. Western blot analysis revealed that the HO-1 protein was increased by several-fold in EC transduced with human HO-1 sense as compared with control EC. Figure 1 shows that HO-1 antisense substantially inhibited HO-1 protein expression. Transduction of HO-1 in the antisense orientation did not significantly modulate HO-2 protein in all cells types. Western blot analysis revealed that EC contain low basal levels of HO-1 protein (Fig. 1A, lane 1). The cells transduced with the human HO-1 gene markedly increased the protein levels of the HO-1 isoform (Fig. 1A, lane 3). In contrast, untreated cells expressed HO-2 protein; the levels of which were not significantly altered following retrovirus-mediated HO-1 gene transfer in the sense or antisense orientation (Fig. 1B). The addition of SnMP, a known inhibitor of HO activity and a transcriptional activator of the HO-1 gene [Chernick et al., 1989] resulted in upregulation of HO-1 but had no effect on HO-2 protein levels (Fig. 1).

The changes in HO expression brought about by retrovirus gene transfer of sense and antisense were followed with corresponding changes in HO activity. The basal level of HO activity in EC transduced with HO-1 in the sense orientation was increased by 61.08%, but this increase was inhibited by SnMP (Table I). Concomitant with the increased HO activity was a marked decrease in cellular heme content. Cellular heme content in human HO-1 cells was 65% lower than that in control cells,

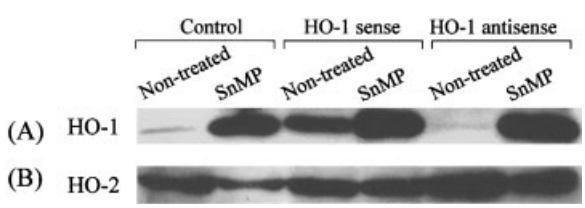

Fig. 1. Western blot analysis of control, HO-1 sense, and HO-1 antisense transduced EC treated and untreated with SnMP. Blots shown are representative of Western blot analysis from three separate experiments.

TABLE I. HO Activity in HMEC

	HO activity (nmol/bilirubin/mg protein)	
Cells	Non-treated	SnMP
Control	0.72 ± 0.01	0.29 ± 0.02**
HO-1 antisense	0.37 ± 0.06*	0.21 ± 0.01**
HO-1 sense	1.85 ± 0.10*	0.33 ± 0.08**

The control, HO-1 sense and HO-1 antisense were treated with SnMP (30 μM) and expressed as the means ± SD of three experiments, respectively. Statistical analysis were performed by *t*-test.
*$P < 0.05$, control vs. HO-1 antisense or HO-1 sense.
**$P < 0.05$, non-treated cells vs. SnMP-treated cells.

indicating higher basal HO activity in cells expressing human HO-1. In EC transduced with HO-1 antisense, heme content was increased to 265 ± 92 pmol/mg of protein as compared with 159 ± 78 pmol/mg of protein in control cells. Control cells were able to catabolize heme at a higher rate than HO-1 antisense transduced cells, reflecting the decrease in HO activity after HO-1 antisense expression. These results further indicate that the exogenously added heme was degraded primarily by HO-1, but not HO-2, since the rate of heme catabolism was diminished significantly in cells transduced with the HO-1 gene in antisense orientation without change in HO-2 protein content.

TNF-α-Mediated Abnormalities in Cell Cycle Progression

We examined DNA distributions in control EC or EC transduced with HO-1 in the sense or antisense orientation. HO-1 in the sense orientation increased the percentage of cells in S-phase compared to control EC cultures. Alternatively, the percent of cells in G_1-phase in EC transduced with HO-1 antisense increased while the proportion of S-phase cells decreased compared with the control EC ($P < 0.05$) (Fig. 2A).

To investigate the influence of TNF-α on cell cycle progression in EC transduced with HO-1 sense and antisense, these cells were treated with TNF-α. As shown in Figure 2B, control EC or EC transduced with HO-1 antisense showed significant differences in DNA distribution after exposure to TNF-α. G_2/M and apoptosis were significantly increased in control cells exposed to TNF-α compared to cells overexpressing the HO-1 gene. The DNA distribution of EC transduced with HO-1 antisense demonstrated a marked increase in apoptosis compared to both control and HO-1 sense cells. There was a

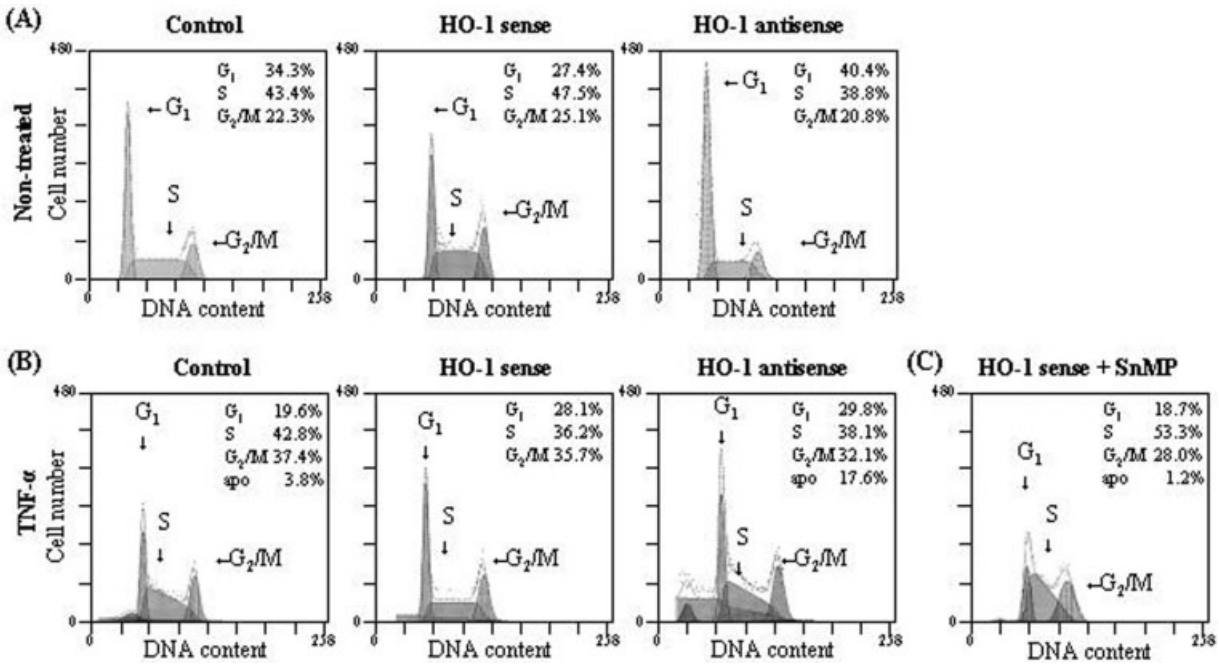

Fig. 2. Cell cycle progression distribution in control, HO-1 sense and HO-1 antisense transduced EC treated with vehicle solution (**Panel A**) or TNF-α (100 ng/ml) (**Panels B,C**). In cells transduced with HO-1 antisense, apoptosis increased after treatment compared to control. Endothelial cells transduced with HO-1 sense did not show apoptosis after treatment with TNF-α. Data are representative of three independent experiments. Representative DNA distributions are shown.

dramatic increase in TNF-α-mediated cell death in cells transduced with HO-1 antisense compared to control cells ($P < 0.01$). In contrast, the DNA distribution of EC transduced with HO-1 sense after TNF-α did not indicate that any cells were undergoing apoptosis.

To investigate the significance of selective upregulation of HO-1 gene transfer and HO activity, we evaluated the effect of SnMP on the DNA distributions of EC transduced with HO-1 sense. As a result of the addition of SnMP and TNF-α, the DNA distribution more closely resembled that of control cultures, including the small percentage of cells undergoing apoptosis (Fig. 2C). These findings suggest that overexpressing HO-1 in EC transduced with HO-1 sense resulted in increased resistance to oxidative stress and the attendant increased abnormalities in the DNA distributions accompanying that stress. Furthermore, underexpressing HO-1 enhanced TNF-α-induced abnormalities in DNA distribution and decreases in cell cycle progression.

Effect of HO Overexpression on COX Activity

The functional expression of HO-1 in EC was also assessed on the levels of COX-2. Since, we have shown previously that HO-1 inducers and inhibitors may indirectly modulate COX activities, we examined the effect of elevation of HO activity by transducing EC with the human HO-1 gene. In cells expressing the human HO-1 in the sense orientation, the basal levels of 6-keto $PGF_{1\alpha}$ and PGE_2 were decreased compared to untransduced EC (Fig. 3). Cells transduced with HO-1 in the antisense orientation expressed higher levels of PGE_2 and 6-keto-$PGF_{1\alpha}$ than

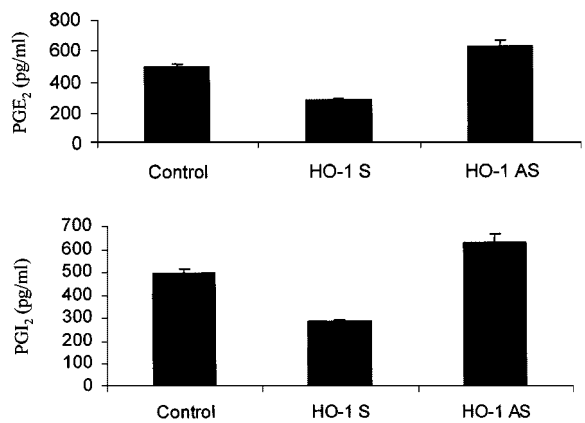

Fig. 3. PGE_2 and PGI_2 production in EC cells untransduced or transduced with the HO-1 gene.

that seen in untransduced EC. Figure 3 shows that the basal levels of PGE_2 and PGI_2 in control cells were higher than those in EC transduced with HO-1 gene in the sense orientation (497.46 ± 15.56 vs. 280.42 ± 7.36 pg/ml, $P < 0.05$ and 373.05 ± 17.63 vs. 158.82 ± 57.04 pg/ml, $P < 0.05$). Furthermore, the basal levels of PGE_2 and PGI_2 in control EC were lower than those in EC transduced with HO-1 antisense (497.46 ± 15.56 vs. 622.75 ± 40.39 pg/ml, $P < 0.05$ and 373.05 ± 17.63 vs.697.73 ± 127.87 pg/ml, $P < 0.05$). These results further support the notion that HO involves mechanisms that modulate the COX activity in EC as a result of changes in cellular heme with an increase in the heme degradation products, CO and biliverdin or ferritin synthesis which may also play a role in cell cycle progression.

Effect of HO-1 Overexpression and Underexpression on p27 Level

Our preliminary results, using cDNA microarray, to measure the effect of overexpression of HO-1 on other genes related to cell cycling revealed that overexpression of HO-1 resulted in 9.8-fold decrease in the cyclin dependent kinase inhibitor, p27 (data not shown). We measured p27 proteins in control EC transduced with HO-1 sense and HO-1 antisense and the results were evaluated by Western blot analysis. As shown in Figure 4, the level of p27 protein in EC transduced with HO-1 sense significantly decreased compared with the control EC ($P < 0.05$). On the contrary, the level of p27 protein in the cells transduced with HO-1 antisense significantly increased compared with the control EC ($P < 0.05$). These findings indicated that overexpressing HO-1 was associated with a suppression of p27 protein.

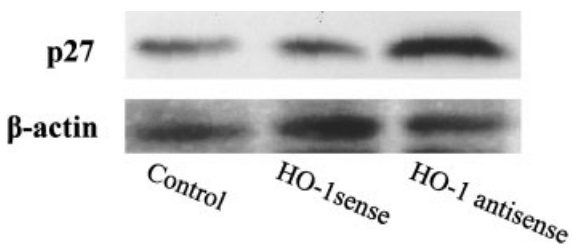

Fig. 4. Western blot analysis of control, HO-1 sense and HO-1 antisense transduced EC for p27 protein. HO-1 sense expression significantly decreased p27 expression compared with the control, untransfected cells ($P < 0.05$). Actin levels confirmed similar protein loading of cell lysates.

DISCUSSION

In the present study, we describe the physiological effect of the functional expression of human HO-1 delivery to EC via a retrovirus vector and its effects on TNF-α-mediated abnormalities in cell growth and cell cycle progression. Our results show that EC transduced with a retroviral-mediated HO-1 gene displayed enhanced CO formation in cell cultures and a decrease in COX-2 activity. Overexpression of the HO-1 gene in EC attenuated TNF-α-mediated abnormalities in cell cycle progression. TNF-α causes a rapid change in the DNA distribution of non-transduced EC. These changes in cell cycle progression following TNF-α exposure were more dramatic in exponentially growing cells when DNA synthesis was essential and less dramatic when cells were in the G_1-phase. TNF-α has been shown to cause the release of prostaglandins from various peripheral tissues [Vara et al., 1996]. There have been reports of an upregulation in COX-2 following proinflammatory stimuli including TNF-α [Jobin et al., 1998]. Reports have suggested that prostaglandins, including PGE_2, may be involved in growth regulation [MacManus and Braceland, 1976]. Prostaglandins also appear to be involved in the regulatory aspects of angiogenesis, in the early stages of pregnancy, and in intestinal crypt stem cell survival [Cohn et al., 1997] and they have been implicated in the pathogenesis of several types of cancer. Prostaglandins are also involved in the regulation of, or are regulated by, a number of cytokines and growth factors. The proinflammatory action of TNF-α is, in part, mediated by its induction of the prostaglandin-synthesizing enzyme, COX-2 [Jobin et al., 1998].

Although the mechanism by which human HO-1 gene transfer prevents TNF-α-mediated cell cycle progression abnormalities is still unclear, HO activity and its products, CO, Fe and ferritin synthesis may play an important role. SnMP, an inhibitor of HO activity, [Chernick et al., 1989] reversed the protective effect of human HO-1 gene transfer in the context of TNF-α-induced changes in cell cycle distribution. The roles of ferritin and bilirubin synthesis, which are associated with upregulation of HO activity, were also examined. The Fe release resulting from HO activity is believed to be the cause of increased ferritin synthesis, which serves to sequester Fe, thus rendering this

potential cellular oxidant inactive [Eisenstein et al., 1991]. Bilirubin and biliverdin both act as antioxidants in vitro and in vivo [Stocker et al., 1987] and their increased local concentrations, after HO induction, may be beneficial in protecting EC from injury. The upregulation of HO-1 has been shown, in our experiments with EC, to be cytoprotective. Some products of HO-1 protect the cells from TNF-α-mediated apoptosis and abnormal cell cycle progression. Overexpression of HO-1 by gene transfer or drug promotes somatic growth in vivo and in vitro [Abraham et al., 2002]. Further, overexpression protects against oxidant and inflammatory cytokine stimulated apoptosis [Laniado-Schwartzman et al., 1994] and decreases expression of adhesion molecules [Wagener et al., 1997; Wagener et al., 1999]. More recently, we suggested that the elevation of CO in EC enhances cell proliferation [Quan et al., 2001], signifying the important role of this gene in cell growth. Others have shown that when an inhibitor of HO blocks HO activity, or the action of CO is inhibited by hemoglobin, HO activity no longer prevents apoptosis [Brouard et al., 2000].

Our study defines a novel function for human HO-1 in EC proliferation and protection against TNF-α-mediated changes in DNA distribution and supports the notion that induction of HO-1 and formation of bilirubin and CO plays an important role in cell function. This is in agreement with the reports that both H_2O_2 and heme elicit cell death and that this effect can be reversed by elevation of HO-derived bilirubin levels [da-Silva et al., 1996]. In contrast, HO inhibitors enhance cell death, an effect that can be prevented by pre-elevation of endogenous bilirubin [da-Silva et al., 1996]. We hypothesize that protective effects of HO-1 on cell cycle is mediated by several signaling mechanisms involving the inhibition of p27. Alternatively, HO-1 attenuates TNF-α-mediated abnormalities in cell cycle which may be related to the physiological levels of bilirubin and CO. Figure 5 is a schematic representation of the hypothesis that overexpression of HO attenuates TNF-α-induced cell cycle arrest in G_1, as a result of inhibition of p27. HO-1 possibly induces upregulation of G_1 cyclin dependent kinases known to stimulate cell cycle progression [Polyak et al., 1994; Grana and Reddy, 1995; Tooke, 1996]. Microarray profiling of gene expression patterns in EC overexpressing the human heme oxygenase-1 demonstrated marked upregulation G_1 cyclin and downregulation of several key factors that are critical to the regulation of cell cycle progression (data not shown)

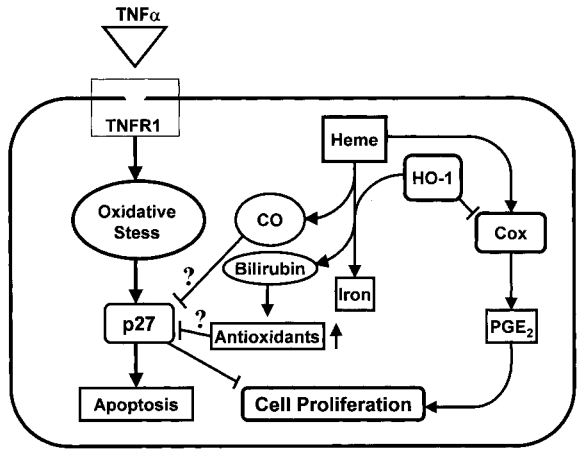

Fig. 5. Schematic representation of the hypothesis that overexpression of HO attenuates the cytostatic effects of TNF-α. We hypothesize that the anti-apoptotic effects of HO-derived CO are mediated by inhibition of p27 (cyclin kinase inhibitor) as well as bilirubin, possibly contributing to its antioxidant effect.

In conclusion, we have demonstrated a functional expression of HO-1 by increasing CO, and decreasing COX activity and p27. This important key finding highlights the biological significance of HO-1 in regulating inflammation and TNF-α-mediated abnormalities in cell cycle progression. Given the previous identification of the potent protective effects of CO in preventing inflammatory reactions such as those that leading to rejection of transplanted organs, and atherosclerosis, and autoimmune disease, our present findings may have important implications not only for the understanding of the mechanisms regulating the protective effects of HO, but also for the development of therapeutic approaches to suppress these inflammatory reactions.

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