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# Overexpression of heme oxygenase-1 in human pulmonary epithelial cells results in cell growth arrest and increased resistance to hyperoxia

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ABSTRACT **Heme oxygenase (HO) catalyzes the rate-limiting step in the degradation of heme to biliverdin, which is reduced by biliverdin reductase to bilirubin. Heme oxygenase-1 (HO-1) is inducible not only by its heme substrate, but also by a variety of agents causing oxidative stress. Although much is known about the regulation of HO-1 expression, the functional significance of HO-1 induction after oxidant insult is still poorly understood. We hypothesize and provide evidence that HO-1 induction serves to protect cells against oxidant stress. Human pulmonary epithelial cells (A549 cells) stably transfected with the rat HO-1 cDNA exhibit marked increases of HO-1 mRNA levels which were correlated with increased HO enzyme activity. Cells that overexpress HO-1 (A549-A4) exhibited a marked decrease in cell growth compared with wild-type A549 (A549-WT) cells or A549 cells transfected with control DNA (A549-neo). This slowing of cell growth was associated with an increased number of cells in $G_0/G_1$ phase during the exponential growth phase and decreased entry into the S phase, as determined by flow cytometric analysis of propidium iodide-stained cells and pulse experiments with bromodeoxyuridine. Furthermore, the A549-A4 cells accumulated at the $G_2/M$ phase and failed to progress through the cell cycle when stimulated with serum, whereas the A549-neo control cells exhibited normal cell cycle progression. Interestingly, the A549-A4 cells also exhibited marked resistance to hyperoxic oxidant insult. Tin protoporphyrin, a selective inhibitor of HO, reversed the growth arrest and ablated the increased survival against hyperoxia observed in the A549-A4 cells overexpressing HO-1. Taken together, our data suggest that overexpression of HO-1 results in cell growth arrest, which may facilitate cellular protection against non-heme-mediated oxidant insult such as hyperoxia.**

Heme oxygenase (HO), found in the smooth endoplasmic reticulum, catalyzes the first and rate-limiting step in the oxidative degradation of heme to biliverdin (1), which is reduced enzymatically to bilirubin by biliverdin reductase (2). Two isoforms of HO, HO-1 and HO-2, exist and are the products of separate genes (3, 4). HO-2 is a constitutive enzyme found primarily in the central nervous system while HO-1 is highly induced by heme, its major substrate, as well as by heavy metals, cytokines, endotoxin, and hormones (5–9). Since its initial identification, the major focus of the studies on HO-1 has been on the regulation and function of HO-1 in heme metabolism. However, much further interest in HO-1 has been generated recently by accumulating evidence that HO-1 is also induced by agents causing oxidative stress, including glutathione depletors, hydrogen peroxide, electrophiles, and hyperoxia (10–14). Although extensive investigations have been conducted to improve our understanding of the regulation of HO-1 induction, the functional significance of HO-1 induction following oxidative stress has not been well studied and is poorly understood.

Recent observations have led to the speculation that increased HO-1 production provides cellular protection against heme-mediated oxidant injury (15, 16). In a rat model of rhabdomyolysis, in which high levels of heme are released from myoglobin, Nath *et al.* (15) have observed that prior induction of HO-1 with infusion of hemoglobin prevented kidney failure and reduced mortality. Conversely, inhibition of HO activity by a competitive inhibitor of HO tin protoporphyrin exacerbated kidney dysfunction. Otterbein *et al.* (16) have reported similar protective effects of HO-1 induction in a rat model of endotoxic shock and lung injury, which is characterized by extensive lung hemorrhage. Furthermore, Abraham *et al.* (17) have demonstrated that overexpression of HO-1 in cultured endothelial cells provided protection against heme and hemoglobin toxicity. Although the mechanism(s) underlying these phenomena are not clearly understood, these data support the hypothesis that HO-1 plays an important role in providing protection against heme-mediated oxidant injury.

We hypothesize that HO-1 induction also provides cellular protection against oxidant injury mediated by agents other than heme. The general strategy to test this hypothesis was to generate cell lines overexpressing HO-1 and to determine whether these cells exhibit increased tolerance to oxidant injury. We obtained human pulmonary epithelial cells, a major target cell of hyperoxic insult, that overexpress HO-1. These cells that overexpress HO-1 exhibit marked decreases in cell growth and DNA synthesis, and increased survival to hyperoxic oxidant injury. Tin protoporphyrin, a selective inhibitor of HO, relieved the growth arrest and increased the susceptibility of the HO-1-overexpressing cells to hyperoxic cell injury. These observations suggest that HO-1 may play an important role in mediating cell growth arrest, which may facilitate cellular protection against hyperoxic oxidant cell injury.

## MATERIALS AND METHODS

**Cell Culture.** Human pulmonary epithelial cells (A549), derived from a lung carcinoma, were obtained from the American Type Culture Collection. Cultures were maintained in Ham-F12 medium (GIBCO/BRL) supplemented with 10% fetal bovine serum (FBS) (HyClone) and gentamicin (50 μg/ml) at 37°C in a humidified atmosphere of 5% $CO_2$/95% air. Cells were exposed to hyperoxia (95% $O_2$/5% $CO_2$) in a tightly sealed modular chamber (Billups-Rothenberg, Del

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Abbreviations: HO, heme oxygenase; FBS, fetal bovine serum; SOD, superoxide dimutase; WT, wild type.

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Mar, CA) at 37°C. Since hyperoxia inhibits cell growth, all experiments were conducted in confluent quiescent cells in medium containing 0.5% FBS to avoid cell density variability between control cells and those exposed to hyperoxia during the course of the experiment.

**RNA Extraction and Northern Blot Analyses.** Total RNA was isolated by the STAT-60 RNAzol method with direct lysis of cells in RNAzol lysis buffer followed by chloroform extraction (Tel-Test, Friendswood, TX). Northern blot analyses were performed as described (9). Ten micrograms of total RNA was electrophoresed in a 1% agarose gel, then transferred to GeneScreenPlus nylon membranes (DuPont) by capillary action and cross linked with a UV Stratalinker (Stratagene). The nylon membranes were treated for 2 h at 65°C in hybridization buffer (1% BSA/7% SDS/1.0 mM EDTA/0.5 M phosphate buffer, pH 7.0), followed by hybridization for 24 h at 65°C in the same buffer containing $^{32}$P-labeled rat HO-1 cDNA. Nylon membranes were then washed twice in buffer A (0.5% BSA/5% SDS/40 mM phosphate buffer, pH 7.0/1 mM EDTA) for 15 min at 65°C followed by four washes in buffer B (1% SDS/1.0 mM EDTA/40 mM phosphate buffer, pH 7.0) each for 15 min at 65°C. Ethidium bromide staining of the gel was used to confirm RNA integrity. To control for variation in either the amount of RNA in different samples or loading variability, blots were hybridized with an oligonucleotide probe complementary to 18S rRNA after stripping of the HO-1 probe. Autoradiographic signals were quantified by densitometric scanning (Molecular Dynamics). All densitometric values obtained for the HO-1 mRNA transcript (1.8 kb) were normalized to values for 18S rRNA obtained on the same blot. The HO-1 mRNA level in treated cells was expressed in densitometric absorbance units, normalized to control untreated samples, and expressed as fold induction compared with controls.

**cDNA and Oligonucleotide Probes.** A full-length rat HO-1 cDNA, generously provided by S. Shibahara (Tohoku University, Sendai, Japan; ref. 18), was subcloned into pBluescript vector and *Hind*III/*Eco*RI digestion was performed to isolate a 0.9-kb HO-1 cDNA subfragment. Rat manganese superoxide dimutase (MnSOD) (pJL4) and CuZnSOD (pCu/ZnSOD) cDNAs were provided by H. Nick (University of Florida, Gainesville). The rat liver catalase cDNA (PMJ1010) was provided by S. Furuta (Shinshu University School of Medicine, Nagano, Japan). The rat liver glutathione peroxidase cDNA (pGPX1211) was provided by S. Reddy (Pennsylvania State University, University Park). A 24-bp oligonucleotide (5′-ACGGTATCTGATCGTCTTCGAACC-3′) complementary to 18S rRNA was synthesized using a DNA synthesizer (Applied Biosystems). cDNAs were labeled with [$\alpha$-$^{32}$P]dCTP using a random primer kit (Boehringer Mannheim). The 18S rRNA oligonucleotide was labeled with [$\alpha$-$^{32}$P]ATP at the 3′ end with terminal deoxynucleotidyltransferase (BRL).

**Transfections.** An HO-1 mammalian expression plasmid, p$\beta$HO-1, was constructed with the following sequences: (*i*) a 4.3-kbp *Eco*RI/*Alu*I human $\beta$-actin gene fragment that contains the promoter, 5′-untranslated region and intron (19); (*ii*) a 1-kbp *Xho*I/*Hin*dIII fragment that contains the coding region of the rat HO-1 cDNA (18); and (*iii*) a 1-kbp *Hin*dIII/*Kpn*I, mouse HO-1 genomic fragment that includes part of the 3′-untranslated region and provides the polyadenylylation sequence (20). The plasmid (10 $\mu$g) was then stably cotransfected into A549 cells with pcDNA 3-neo (1 $\mu$g), a plasmid containing neomycin selection marker, using Lipofectin reagent (GIBCO/BRL) according to the manufacturer's protocol. The cells were transfected for 24 h, after which time the plates were washed twice with serum-free medium and then incubated in Ham-F12 medium containing 10% FBS, gentamicin (50 $\mu$g/ml), and Geneticin (100 $\mu$g/ml) (GIBCO/BRL). Approximately every 3 days the concentration of Geneticin in the medium was increased up to a maximum of 400 $\mu$g/ml. The surviving neomycin-resistant colonies on each plate were then subcloned and grown to establish the sublines (designated as A549-A1 to A549-A8 for the eight isolated sublines). As controls, in addition to the A549-WT cells, we also isolated A549 cells stably transfected with pcDNA3-neo vector alone using the protocol described above. The A549-neo and A549-WT cells exhibited similar cell growth characteristics and susceptibility to hyperoxia.

**HO Activity.** Cells were scraped with phosphate-buffered saline (PBS) and resuspended in 0.25 M Tris·HCl (pH 7.40) prior to microsomal HO activity determination (1). One unit of HO activity was defined as the amount of bilirubin (nmol) produced per mg of protein per h.

**Cell Growth Curve.** Equal numbers ($5 \times 10^4$) of A549-A4, A549-neo, or A549-WT cells were plated into 6-well tissue culture dishes and cell number was determined by hemocytometer counting at 0, 1, 2, 3, 4, and 5 days of cell plating. Duplicate plates were used for each time point.

**Cell Morphology and Survival After Hyperoxia.** A549-A4 and A549-neo cells were grown to confluence in medium containing 10% FBS prior to exposure to hyperoxia in medium containing 0.5% serum. On each of the following 7 days, cells were removed from the hyperoxic chamber, washed, and counted by hemocytometer. The medium was changed on day 4 for all cells. Survival curves were generated by calculating the percentage of cells surviving after each day of hyperoxia. Photomicrographs of cell morphology were obtained on day 6 of exposure to hyperoxia, immediately after removal from the oxygen chamber, with a Nikon HFX-DX camera.

**Fluorescence-Activated Cell Sorter Analysis.** The relative number of cells occupying a particular state of the cell cycle at a specific time was obtained by staining cells with the DNA intercalator propidium iodide (5 $\mu$g/ml). Stoichiometric binding results when cells are fixed in cold 70% ethanol for at least 1 h, followed by washes and incubation with 100 $\mu$g/ml RNase. This incubation continued for 30 min at 37°C followed by two washes with Hanks' balanced salt solution. Finally the cells were resuspended in 5 $\mu$g/ml propidium iodide in Hanks' balanced salt solution. Single parameter histograms were generated by analyzing both cell preparations on a FACStar$^{PLUS}$ flow cytometer (Becton Dickinson).

Cytostasis was confirmed by pulsing cells with BrdUrd (10 $\mu$M) for 30 min (21). The cells were then harvested and fixed in 70% ethanol and stored at −20°C until analysis. Prior to analysis, samples were acid hydrolyzed (4 M HCl for 30 min), washed, and resuspended in 2 ml of 0.1 M $Na_2B_4O_7$. The cells were washed again, pelleted by centrifugation, and resuspended in 50 $\mu$l of 0.5% Tween 20 in PBS. Twenty microliters of fluorescein isothiocyanate-conjugated antibody to BrdUrd (Becton Dickinson) was reacted with each sample to quantify the number of cells entering S phase during a fixed time period. Two-parameter contour plots were generated by analyzing cell preparations on the flow cytometer.

## RESULTS

**Isolation of Cells Overexpressing HO-1.** A549 cells were stably transfected with a full-length rat HO-1 cDNA subcloned into a mammalian expression vector driven by the $\beta$-actin promoter. Eight G418 resistant clones were selected for analysis. These clones were examined for increased HO-1 mRNA expression by Northern blot anlayses. We confirmed increased levels of HO-1 mRNA in these cloned cell sublines, especially in sublines A549-A4 and A549-A5 (5-fold), as compared with cells transfected with control DNA (A549-neo) (Fig. 1*A*). A549-WT cells exhibited only basal level of HO-1 mRNA similar to the A549-neo cells (data not shown). The cell subline (A549-A4) was chosen for all subsequent studies.

To confirm increased HO enzyme activity in the A549-A4 subline, we isolated microsomes from A549-A4 cells and

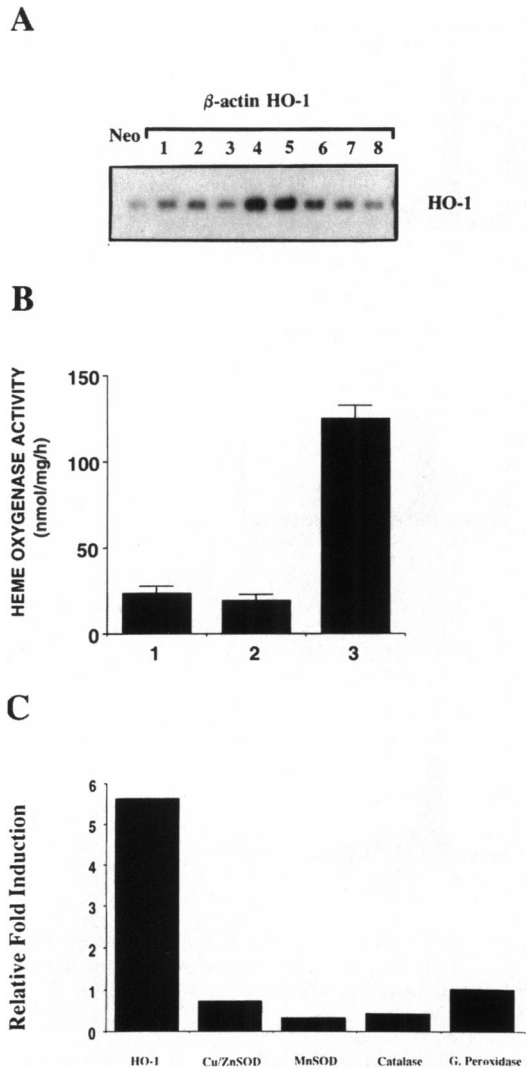


FIG. 1. Determination of HO-1 expression in overexpressing A549 cells. (*A*) Northern blot analysis of HO-1 transfectants. Total RNA was isolated from confluent cultured cells of each of the cloned A549 HO-1 transfectants (eight sublines) and control A549-neo cells and were analyzed for HO-1 mRNA expression. Neo, A549-neo control cells. β-actin HO-1 (1–8) represents eight different A549 HO-1 transfectants (A549-A1 to A549-A8). (*B*) HO enzyme activity in HO-1-overexpressing A549-A4 cells and controls. HO enzyme activity in confluent cultured cells of A549-WT control cells (bar 1), A549-neo control cells (bar 2), and A549-A4 cells (bar 3) determined by bilirubin generation. Enzyme activity represents mean value ± SEM of measurements from three independent experiments. (*C*) Levels of antioxidant enzyme mRNA expression in HO-1-overexpressing A549-A4 cells. Total RNA was isolated from confluent cultured cells of HO-1-overexpressing A549-A4 cells and A549-neo control cells and was analyzed for mRNA expression of the indicated antioxidant enzymes. 18S rRNA hybridization was used as a normalization control. Densitometric values obtained for the mRNA transcript of each of the antioxidant enzymes, were normalized to values for 18S rRNA obtained on the same blot. The steady-state mRNA levels of each of the antioxidant enzymes in HO-1-overexpressing A549-A4 cells were measured in densitometric absorbance units and expressed as fold induction compared with A549-neo control cells.

assayed for HO activity by bilirubin determination. Fig. 1*B* shows increased basal level of HO activity in A549-A4 cells (5-fold) compared with A549-WT and A549-neo cells ($P < 0.001$). No difference in mRNA expression of antioxidant enzymes such as MnSOD, CuZnSOD, glutathione peroxidase, and catalase were observed between transfected A549-A4 HO-1 and A549-neo control cells (Fig. 1*C*).

**Cell Growth Arrest in Cells Overexpressing HO-1.** Fig. 2 compares the growth curves of A549-A4, A549-A5, and A549-neo control cells. A marked inhibition of cell growth was observed in both the A549-A4 and A549-A5 clone sublines compared with A549-neo control cells. Growth curves of A549-WT cells were similar to the A549-neo control cells (data not shown). We further determined the percentage of cells in the $G_1$ phase of the cell cycle by flow cytometric analysis of propidium iodide-stained cells. During the proliferative exponential phase (50% confluency), a higher percentage of A549-A4 cells were in the $G_1$ phase compared with the A549-neo control cells. The majority of the A549-A4 cells (78%) remained in the $G_1$ phase, with decreased entry into the S phase. Pulse experiments with BrdUrd further confirmed this observation as a smaller percentage of A549-A4 cells arrested stationed in the S phase (13%) compared with A549-neo control cells (31%). We extended these studies to determine the progression of the cell cycle in both A549-neo cells and A549-A4 cells during serum stimulation. After 48 h of serum starvation, cells were stimulated with serum for 2 h, stained with propidium iodide, and then subjected to flow cytometric analysis. Fig. 3 demonstrates the normal progression of A549-neo control cells from the $G_0/G_1$ phase to S phase upon serum stimulation (Fig. 3 *D* and *E*). In contrast, this normal progression from the $G_0/G_1$ phase to S phase was not observed when A549-A4 cells were stimulated with serum. The A549-A4 cells accumulated at the $G_2/M$ phase and were not able to progress through the cell cycle (Fig. 3 *A* and *B*). Interestingly, when the HO-1 overexpressing A549-A4 cells were stimulated with serum in the presence of tin protoporphyrin, this $G_2/M$ phase block (Fig. 3*B*) was reversed and cells reverted to normal cell cycle progression (Fig. 3*C*). The release of $G_2/M$ phase block by tin protoporphyrin observed in A549-A4 cells was accompanied by an increase in cell number (32% increase) (data not shown).

**HO-1 Overexpressors Exhibit Increased Tolerance to Hyperoxic Stress.** To test the hypothesis that HO-1 induction has a protective function against hyperoxic injury, we then examined whether the HO-1 overexpressors exhibited increased resistance to hyperoxia. Evidence of cell toxicity manifested by cytoplasmic vacuolization and ruffled cell membranes was observed in the A549-neo control cells after three days of continuous exposure to hyperoxia. Increased cytoplasmic vacuolization was noted after four days of hyperoxia, and cells became detached from the plate by the fifth day of hyperoxia. Fig. 4 shows these morphological changes with marked increased survival of the A549-A4 cells compared with A549-neo

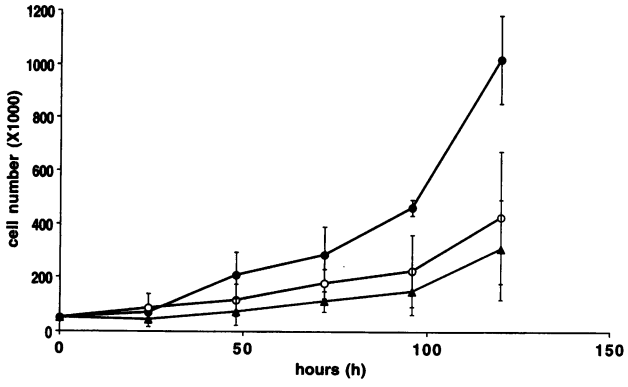


FIG. 2. Growth curve of cells overexpressing HO-1. Equal numbers of cells ($5 \times 10^4$) of A549-A4, A549-A5, or A549-neo cells were plated into 6-well tissue culture dishes and incubated in DMEM containing 10% FBS. Cell numbers were determined by hemocytometer counting at 0, 1, 2, 3, 4, and 5 days of cell harvest. Duplicate plates were used for each time point. Each data point is an average value from four independent experiments. ●, A549-neo; ○, A549-A4; ▲, A549-A5.

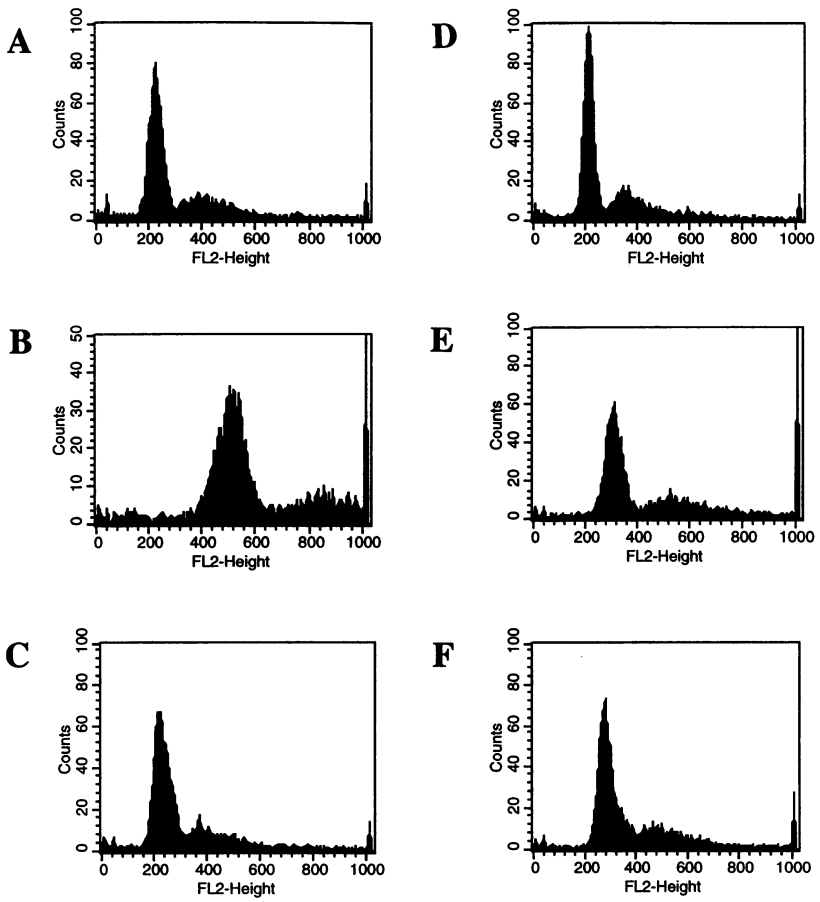


FIG. 3. Cell cycle analysis by flow cytometer. Confluent A549-A4 and A549-neo cells were serum starved for 48 h prior to serum stimulation (15% FBS) for 2 h in the absence or presence of tin protoporphyrin (50 μM) (SnPP) at which time cells were stained with propidium iodide for flow cytometric analysis of the cell cycle. Single-parameter histogram contour plots were generated. (*Left*) A549-A4 cells: *A*, − serum; *B*, + serum; *C*, + serum/SnPP. (*Right*) A549-neo cells: *A*, − serum; *B*, + serum; *C*, + serum/SnPP.

control cells after 6 days of continuous exposure to hyperoxia. Significant differences in cell survival on continuous exposure to hyperoxia were observed between the HO-1 overexpressing A549-A4 cells and A549-neo control cells (Fig. 5). Thus, the HO-1 overexpressing A549-A4 cells exhibited a 4-fold increase (60% versus 15%) in survival at day 3, 10-fold increase (65% versus 6%) in survival at day 4, and >30-fold increase (35% versus 1%) in survival at day 5, as compared with A549-neo control cells (Fig. 5*A*). Based on this increased survival by the HO-1 overexpressing cells against hyperoxia, we inferred that HO-1 induction plays an important role in providing protection against hyperoxic oxidant injury. If our hypothesis is correct, inhibiting HO activity should increase the susceptibility of the HO-1-overexpressing cells to hyperoxic cell injury. As illustrated in Fig. 5*B*, the HO-1-overexpressing A549-A4 cells exhibited decreased survival against hyperoxia when subjected to hyperoxia exposure in the presence of tin protoporphyrin, as compared with A549-A4 cells exposed to hyperoxia alone.

## DISCUSSION

The deleterious effects of hyperoxia are thought to be mediated by reactive oxygen species such as superoxide and hydroxyl radicals, and hydrogen peroxide (22). The lung responds to hyperoxia by upregulating expression of antioxidant enzymes such as SOD, glutathione peroxidase, and catalase, whose primary function is to detoxify toxic reactive oxygen species (23, 24). Increasing evidence has accumulated in recent years to support the hypothesis that these enzymes play a critical role in providing protection against hyperoxic stress. For instance, transgenic mice overexpressing MnSOD and CuZnSOD exhibit increased resistance to toxic effects of

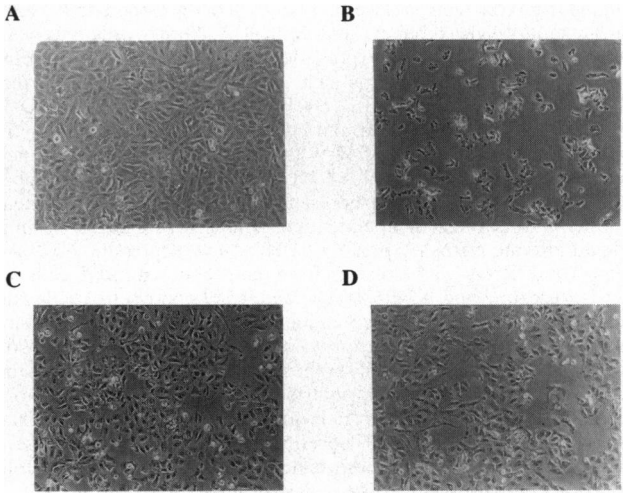


FIG. 4. Cell morphology of HO-1-overexpressing cells after exposure to hyperoxia. Confluent A549-A4 and A549-neo cells were exposed to hyperoxia for 7 days in medium containing 0.5% serum. Medium was changed on day 4 for all cells. Illustration of cell morphology of cells at day 6 of exposure to hyperoxia. (*A*) A549-neo control cells grown under normal oxygen tension. (*B*) A549-neo control cells exposed to hyperoxia. (*C*) A549-A4 cells grown under normal oxygen tension. (*D*) A549-A4 cells exposed to hyperoxia. (×26.)

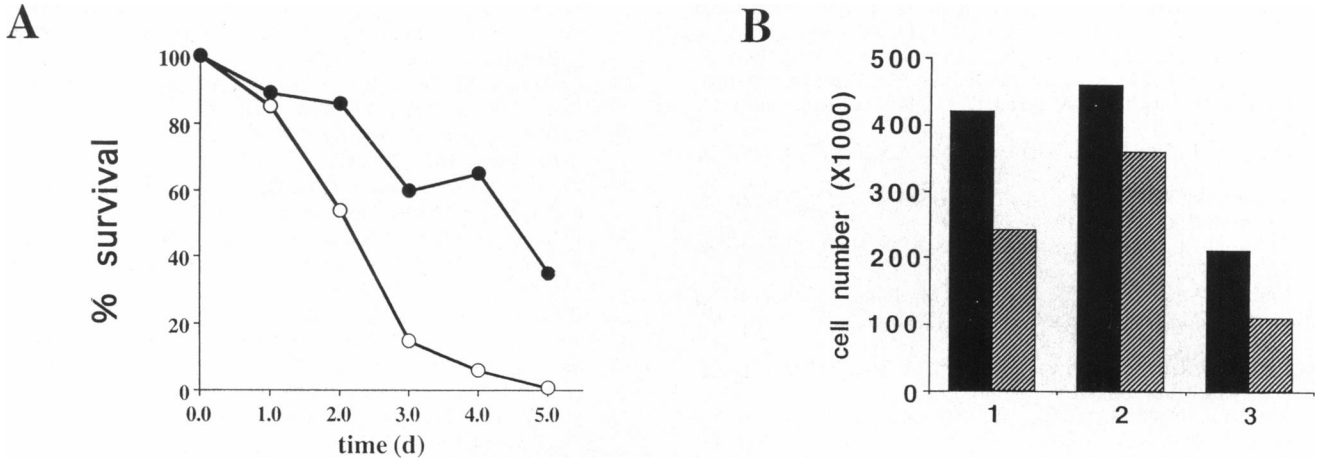


FIG. 5. Survival of cells overexpressing HO-1 after exposure to hyperoxia. (*A*) Confluent A549-A4 and A549-neo cells were exposed to hyperoxia for 5 days in 0.5% serum-containing medium. Survival curves were generated by calculating the percentage of cells left after each day of hyperoxia. Duplicate plates were used for each time point. Each data point is an average value from four independent experiments. ●, A549-A4; ○, A549-neo. (*B*) Confluent A549-A4 cells were exposed to hyperoxia in the absence (■) or presence of SnPP (50 μM) (▨) for up to 8 days in 0.5% serum-containing medium. Medium was changed every 2 days. Survival curves were generated by calculating the percentage of cells left after each day of hyperoxia. Times: 1, day 5 of hyperoxia; 2, day 6 of hyperoxia; 3, day 7 of hyperoxia.

hyperoxia, and mice lacking extracellular SOD are more sensitive to hyperoxia (25–27). Overexpression of MnSOD in cultured endothelial cells has also been shown to confer resistance to hyperoxia (28). Our laboratory has previously shown that in addition to the above-mentioned antioxidant enzymes, HO-1 was also highly induced after hyperoxic stress (10, 29). Although we have further characterized the molecular regulation of HO-1 expression *in vivo* and *in vitro* (10), the functional significance of HO-1 induction in response to hyperoxia remains poorly understood. Here we report that overexpression of HO-1 in pulmonary epithelial cells resulted in increased survival against hyperoxic oxidant injury.

Although the mechanism(s) by which HO-1 confers protection against oxidant stress is still unclear, the products of reactions catalyzed by HO-1 may provide some insight. For example, the induction of ferritin as a result of iron removal from the degradation of heme by HO may serve to restrict iron from participation in the Fenton reaction, thereby reducing the oxidant burden of the cell (30). For instance, in cultured endothelial cells, methemoglobin increases not only HO-1 expression, but also increases ferritin production. This accumulation of ferritin is associated with iron sequestration and protection against oxidative damage (31). Bilirubin, another byproduct of heme degradation by HO-1, has been shown to exhibit potent antioxidant properties (32). Bilirubin can scavenge peroxyl radicals *in vitro* (especially at low oxygen tensions that prevail in tissues) as efficiently as α-tocopherol, which is regarded as the most potent antioxidant of lipid peroxidation (33).

The propensity of the HO-1-overexpressing cells to lag in $G_1$ phase with subsequent decreased entry into the S phase of the cell cycle is intriguing in view of recent observations that cell growth arrest is associated with induction of other stress response gene products such as gadd153, gadd45, and heat shock protein (34, 35). In particular, induction of these genes including HO-1 have been reported by agents causing growth arrest including cyclopentenone prostaglandins, oxidative and DNA damage (10, 29, 34). Transforming growth factor type β, a cytokine that inhibits cell growth and promotes differentiation in epithelial cells, induces high levels of HO-1 in epithelial cells (36). Although the significance of cell growth arrest in response to cellular stress is not clearly understood, it has been speculated that in one particular cellular stress, oxidative DNA damage, cells are genetically programmed to lag behind in $G_1$ phase to allow time for cells to repair the damaged DNA prior to entry into S phase (37, 38). Recently, Smith *et al.* (39) have identified a gene product, gadd45, that mediates not only cell cycle arrest but also is involved directly in the repair of damaged DNA after oxidant injury such as UV irradiation. Given this association of cellular stress with growth arrest and DNA damage repair, it is tempting to speculate that the growth arrest observed in the HO-1 overexpressors plays an important role in allowing the cells to cope with hyperoxic stress. Although the cellular mechanisms of cell growth arrest by HO-1 induction in epithelial cells are not revealed by our studies, increased levels of the product carbon monoxide resulting from HO-1 induction could mediate growth arrest by stimulating cGMP production via guanylyl cyclase, in analogy to nitric oxide-induced cell growth arrest by cGMP (40).

In conclusion, increased HO-1 expression induces growth arrest in A549 epithelial cells and confers protection against hyperoxia. The antiproliferative effect associated with HO-1 induction in lung epithelial cells resulting in growth arrest may represent a mechanism by which cells can survive hyperoxic oxidant stress. Further molecular approaches, such as transgenic animal models may represent effective means to delineate the physiologic role of HO-1 *in vivo* in oxidant-induced lung diseases.

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