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Fast Track Paper

CURCUMIN, AN ANTIOXIDANT AND ANTI-INFLAMMATORY AGENT, INDUCES HEME OXYGENASE-1 AND PROTECTS ENDOTHELIAL CELLS AGAINST OXIDATIVE STRESS

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Abstract—Curcumin, a widely used spice and coloring agent in food, has been shown to possess potent antioxidant, antitumor promoting and anti-inflammatory properties in vitro and in vivo. The mechanism(s) of such pleiotropic action by this yellow pigment is unknown; whether induction of distinct antioxidant genes contributes to the beneficial activities mediated by curcumin remains to be investigated. In the present study we examined the effect of curcumin on endothelial heme oxygenase-1 (HO-1 or HSP32), an inducible stress protein that degrades heme to the vasoactive molecule carbon monoxide and the antioxidant biliverdin. Exposure of bovine aortic endothelial cells to curcumin (5–15 μM) resulted in both a concentration- and time-dependent increase in HO-1 mRNA, protein expression and heme oxygenase activity. Hypoxia (18 h) also caused a significant ($P < 0.05$) increase in heme oxygenase activity which was markedly potentiated by the presence of low concentrations of curcumin (5 μM). Interestingly, prolonged incubation (18 h) with curcumin in normoxic or hypoxic conditions resulted in enhanced cellular resistance to oxidative damage; this cytoprotective effect was considerably attenuated by tin protoporphyrin IX, an inhibitor of heme oxygenase activity. In contrast, exposure of cells to curcumin for a period of time insufficient to up-regulate HO-1 (1.5 h) did not prevent oxidant-mediated injury. These data indicate that curcumin is a potent inducer of HO-1 in vascular endothelial cells and that increased heme oxygenase activity is an important component in curcumin-mediated cytoprotection against oxidative stress.

Keywords—Antioxidant, heme oxygenase-1, cytoprotection, bilirubin, carbon monoxide, hypoxia, pharmacological preconditioning, Free radicals

INTRODUCTION

Curcumin is a major active component of the food flavor turmeric. It is extracted from the powdered dry rhizome of *Curcuma longa Linn* (Zingiberacee), a perennial herb widely cultivated in tropical regions of Asia. It has been used for centuries in indigenous medicine for the treatment of a variety of inflammatory conditions and other diseases [1]. Several studies in recent years have shown that curcumin is a potent inhibitor of tumor initiation in vivo [2,3] and possesses antiproliferative activities against tumor cells in vitro [4,5]. Besides its anticarcinogenic properties, relatively low concentrations of curcumin exhibit remarkable anti-inflammatory and antioxidant effects [1,6–8]. Although the exact mechanism(s) by which curcumin promotes these effects remains to be elucidated, the antioxidant properties of this yellow pigment appear to be an essential component underlying its pleiotropic biological activities. In fact, curcumin has been reported to inhibit lipid peroxidation and to effectively scavenge superoxide anion and hydroxyl radicals [7]. In addition to its inherent ability to attenuate the reactivity of oxygen free radical species, curcumin has been shown in vivo to enhance the activities of detoxifying enzymes such as glutathione-S-transferase [9]. Whether induction of distinct antioxidant genes in mammalian tissues contributes to the variety of pharmacological actions mediated by curcumin has yet to be examined.

Among the various genes encoding for proteins that possess antioxidant characteristics, heme oxygenase-1

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(HO-1) has attracted particular interest as it is finely up-regulated by stress conditions and generates products that might have important biological activities [10]. Heme oxygenase is a widely distributed enzyme in mammalian tissues and its main function is associated with the degradation of heme to iron, carbon monoxide (CO), and biliverdin [11], the latter being converted to bilirubin by the cytosolic enzyme biliverdin reductase. Both biliverdin and bilirubin possess antioxidant properties [12] and HO-1-derived CO has been implicated in vasoregulation and signal transduction mechanisms [13,14]. Physiological concentrations of bilirubin have been shown to protect endothelial cells against hydrogen peroxide-mediated injury and prevent protein oxidation in human blood plasma [15,16]. We have recently reported that increased bilirubin production following HO-1 stimulation is associated with decreased peroxynitrite-mediated cytotoxicity [17] and reduction of postischemic myocardial dysfunction in an isolated rat heart preparation [18]. Otterbein and coworkers have also demonstrated that exposure of rats to low concentration of CO increases tolerance to hyperoxic lung injury [19]. Collectively, the HO-1 pathway appears to: play a key role in the preservation of tissue integrity against oxidative stress [17,20, 21]; contribute to the modulation of inflammatory responses in vivo [22,23]; and act in synchrony with other crucial enzymatic systems in the maintenance of cellular homeostasis [21].

In the present study we report the effect of curcumin on HO-1 gene regulation, protein expression, and heme oxygenase activity in vascular endothelial cells. In addition, we have examined the interrelation between curcumin and HO-1 in protection against oxidant-mediated injury under normoxic and hypoxic conditions. The biological relevance of these findings will be discussed.

MATERIALS AND METHODS

Materials

Tin protoporphyrin IX (SnPPIX), an inhibitor of heme oxygenase activity, was obtained from Porphyrin Products Inc. (Logan, UT, USA). Curcumin and all other chemicals were purchased from Sigma-Aldrich (Poole, UK) unless otherwise specified. Stock solutions of curcumin (5 mM) were prepared freshly on the day of the experiment by dissolving the compound in ethanol. A cDNA probe for rat HO-1 (kindly provided by Prof. Shibahara, Japan) was used to detect HO-1 mRNA expression [24].

Experimental protocol

Bovine aortic endothelial cells (European Collection of Animal Cell Culture, Salisbury, UK) were used in all studies. Cells were cultured in 75 cm^2 flasks and grown in Iscove's modified Dulbecco's medium supplemented with 8% fetal bovine serum, 2 mM L-glutamine, penicillin (100 U/ml) and streptomycin (0.1 mg/ml). Flasks were kept at 37°C in a humidified atmosphere of air and 5% CO_2. Confluent cells were exposed to various concentrations of curcumin (0–30 μM) and heme oxygenase activity, HO-1 mRNA and protein expression were determined at different times after treatment. N-acetylcysteine (1 mM), a precursor of glutathione synthesis and a sulphydril donor with potent antioxidant properties, was also used to examine whether changes in heme oxygenase activity by curcumin are mediated by pro-oxidant mechanisms. Experiments under hypoxic conditions were performed by transferring endothelial cells to an air-tight chamber (Billups-Rothenberg Inc., Del Mar, CA, USA) and flushed with a mixture of 95% N_2-5% CO_2 for 18 h [25]. The gas was infused continuously into the air-tight chamber at a flow rate of 5 l/min for the first 2 h and at 1 l/min for the following hours of incubation. In preliminary experiments conducted under these conditions, it was found that the pO_2 measured in the media by an oxygen electrode was 2 mmHg at 2 h incubation and did not fluctuate from this lowest level throughout the remaining incubation period. Within the hypoxia chamber, untreated cells or cells exposed to 5 μM curcumin were maintained in a humidified atmosphere at 37°C for the times indicated. After treatment, cells were harvested and HO-1 mRNA expression and heme oxygenase activity were determined as reported below. To assess the susceptibility to oxidative stress, cell viability was determined in cells treated with 10 μM curcumin (1.5 or 18 h) or exposed to hypoxia for 18 h followed by incubation for 2 h in the presence of glucose oxidase (100 mU/ml); this oxidant system generates hydrogen peroxide at constant rate and is known to promote cellular injury in vitro [26]. After treatment with glucose oxidase, cells were washed and exposed to complete medium containing Alamar blue for 5 h in order to assess cell viability (see below). To investigate a possible contribution of heme oxygenase activity in protection against oxidative stress, cells were exposed to curcumin or hypoxia in the presence of SnPPIX (10 μM) and cell viability was determined after glucose oxidase-mediated challenge.

Assay for endothelial heme oxygenase activity

Heme oxygenase activity assay was performed as previously described [27]. Briefly, microsomes from harvested cells were added to a reaction mixture containing NADPH, rat liver cytosol as a source of biliverdin reductase and the substrate hemin. The reaction was conducted at 37°C in the dark for 1 h, terminated by the

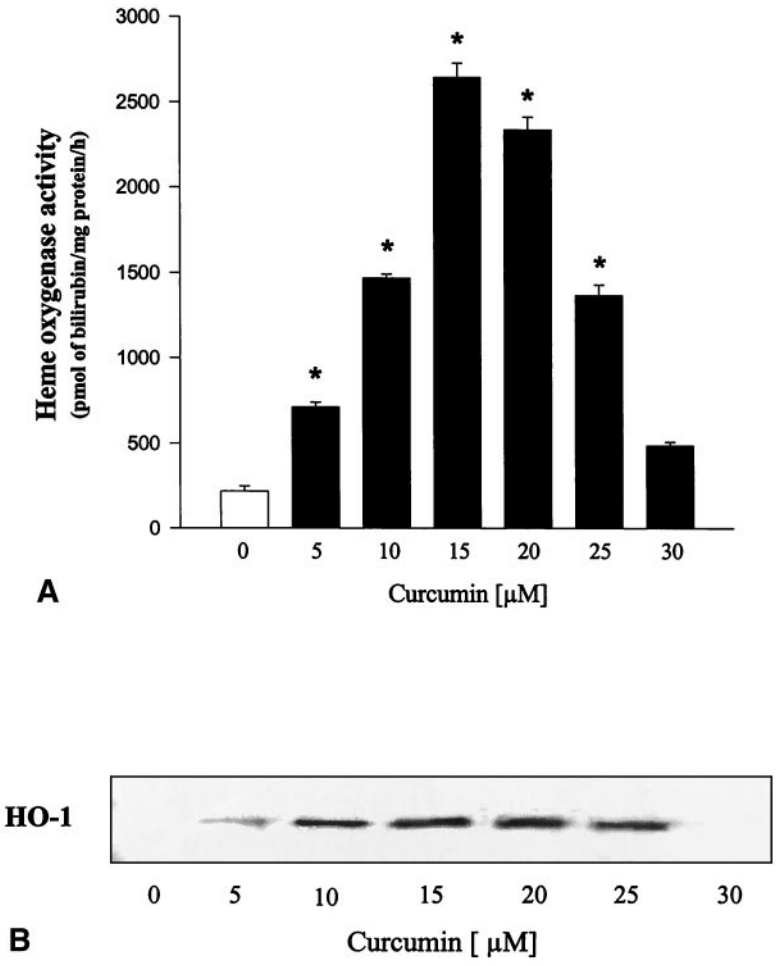

Fig. 1. Effect of curcumin on heme oxygenase activity and HO-1 protein expression in vascular endothelial cells. (A) Heme oxygenase activity was measured in endothelial cells 18 h after exposure to various concentrations of curcumin (0–30 μM). In control group cells were incubated to medium alone. Each bar represents the mean ± SEM of five independent experiments. *$P < 0.05$ vs. curcumin 0 μM. (B) HO-1 protein expression was analyzed by Western immunoblot technique as described in Material and Methods in samples of cells treated as above.

addition of 1 ml of chloroform and the extracted bilirubin was calculated by the difference in absorbance between 464 and 530 nm ($\epsilon = 40\ mM^{-1}\ cm^{-1}$).

Western blot analysis for heme oxygenase-1 (HO-1)

Samples of endothelial cells treated for the heme oxygenase activity assay were also analyzed by Western immunoblot technique as previously described [28]. Cells were lysed in cold phosphate-buffered saline containing 1% Triton X-100. An equal amount of protein (30 μg/well) from each sample was boiled for 5 min in Laemmli buffer and protein separation was carried out by SDS-polyacrylamide gel electrophoresis using a 12% acrylamide resolving gel (Mini Protean II System, Bio-Rad, Herts, UK). Separated proteins were then transferred overnight to nitrocellulose membranes and the nonspecific binding of antibodies was blocked with 3% nonfat dried milk in PBS, pH 7.4 for 2 h at room temperature. Membranes were then probed with a polyclonal rabbit anti-HO-1 antibody (Stressgen, Victoria, Canada) (1:1000 dilution in Tris-Buffered saline, pH 7.4) for 2 h at room temperature. After three washes with PBS containing 0.05% (v/v) Tween-20, blots were visualized using an amplified alkaline phosphatase kit from Sigma (Extra-3A) and the relative density of bands analyzed by an imaging densitometer (Model GS-700, Bio-Rad, Herts, UK).

RNA extraction and Northern blot analysis

These methods were performed as previously described by our group [14,29]. Briefly, total RNA was isolated by phenol-chloroform using the method described by Chomczynski and Sacchi [30]. Total RNA was run on a 1.3% denaturing agarose gel containing 2.2 M formaldehyde and transferred onto a nylon membrane overnight. The membrane was hybridized using [α-^{32}P]dCTP-labelled cDNA probes to rat HO-1 gene and staining of the 18S RNA band was used to confirm integrity and equal loading of RNA. The hybridized membrane was exposed to radiographic film and bands analyzed using a densitometer.

Cell viability assay

To determine cytotoxicity, cells grown in 24 wells were used and cell viability was assessed by an Alamar Blue assay according to manufacturers' instructions (Serotec, UK). After each treatment, cells were washed twice, complete medium containing 1% Alamar blue solution was added and wells were returned to the incubator for 5 h. After the incubation period, optical density in the medium of each well was measured using a plate reader (Molecular Devices, Crawley, UK). The assay is based on detection of metabolic activity of living cells using a redox indicator which changes from oxidized (blue) to reduced (red) form. The intensity of the red colour is proportional to the viability of cells which is calculated as difference in absorbance between 570 nm and 600 nm and expressed as percentage of control.

Statistical analysis

Differences in the data among the groups were analyzed by using one-way ANOVA combined with the Bonferroni test. Values were expressed as a mean ± SEM and differences between groups were considered to be significant at $P < 0.05$.

RESULTS

Effect of curcumin on heme oxygenase activity and HO-1 protein expression in vascular endothelial cells

The effect of various concentrations of curcumin (0–30 μM) on endothelial heme oxygenase activity and HO-1 protein expression is shown in Fig. 1. Exposure of endothelial cells to curcumin (1–15 μM) for 18 h resulted in a concentration-dependent increase in heme oxygenase activity (Fig. 1A). The increase was significantly different from control (untreated cells, $P < 0.05$), with a maximal enzymatic activity (12-fold increase) at 15 μM curcumin. The antioxidant N-acetylcysteine did not significantly affect the increase in heme oxygenase mediated by curcumin (data not shown) indicating that the activation of this pathway is not mediated by prooxidant mechanisms. Western blot analysis revealed that enhanced heme oxygenase activity by curcumin treatment directly correlated with HO-1 protein levels (Fig. 1B). At concentrations of 20 μM curcumin and above, heme oxygenase activity and HO-1 protein levels gradually decreased to control values. Ethanol, the vehicle in which curcumin was solubilized, did not produce any change in heme oxygenase activity and HO-1 protein (data not shown).

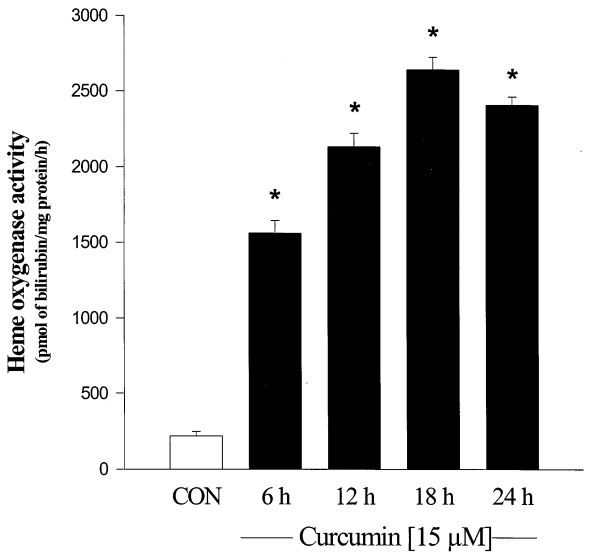

Fig. 2. Time course of heme oxygenase activity in vascular endothelial cells exposed to curcumin. Endothelial cells were exposed to 15 μM curcumin and heme oxygenase activity was measured at various time points as reported in Materials and Methods. Data are expressed as the mean ± SEM of 5 independent experiments. *$P < 0.05$ vs. control group (CON).

Time course of heme oxygenase activity, HO-1 mRNA and protein expression in endothelial cells exposed to curcumin

The concentration of curcumin that produced maximal increase in endothelial heme oxygenase activity (15 μM) was chosen to determine the effect of this yellow pigment on activity and HO-1 gene transcription over time. As shown in Fig. 2, treatment of endothelial cells with curcumin resulted in a time-dependent increase in heme oxygenase activity. After 18 h exposure to curcumin, the activity reached a maximum and rose from 218 ± 29 pmol bilirubin/mg protein/h (time 0) to 2647 ± 83 pmol/bilirubin/mg protein/h ($P < 0.05$). This effect correlated with a time-dependent increase in HO-1 mRNA and protein expression, as shown by Northern and Western blot, respectively (Fig. 3A and 3B).

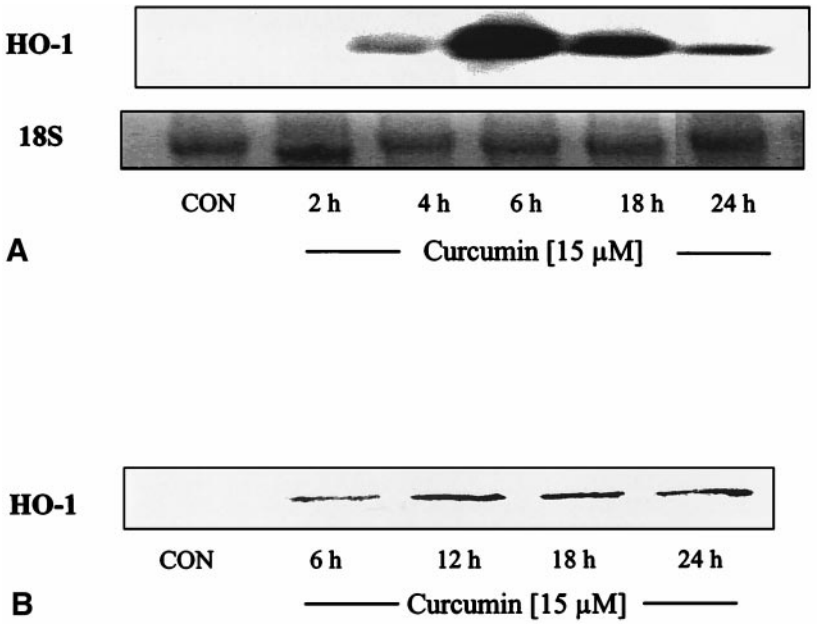

Fig. 3. Time course of HO-1 mRNA and protein expression in vascular endothelial cells exposed to curcumin. (A) Endothelial cells were exposed to 15 μM curcumin and total RNA was extracted at the times indicated. HO-1 mRNA expression was analysed by Northern blot as reported in Materials and Methods. The 18S band is shown to confirm integrity and equal loading of RNA. (B) Total cellular proteins were isolated from cells treated as above and Western blot analysis was performed using specific antibodies for HO-1.

Effect of hypoxia and curcumin treatment on endothelial HO-1 gene expression and heme oxygenase activity

We analyzed the expression of endothelial HO-1 in response to hypoxia and we examined the modulatory role of low concentrations of curcumin on this effect. As shown in Fig. 4A, hypoxia stimulated HO-1 mRNA transcript which was evident at 6 h and gradually increased after 8 h hypoxia. The increase in HO-1 mRNA was followed by a considerable elevation of heme oxygenase activity at 18 h hypoxia. Interestingly, exposure of cells to hypoxia in the presence of curcumin (5 μM) significantly potentiated the increase in HO-1 mRNA levels which was already visible at 4 h and was markedly intensified at 6 and 8 h (Fig. 4B). As shown in Fig. 5, incubation of cells for 18 h with 5 μM curcumin in normoxic conditions increased endothelial heme oxygenase activity from 218 $\pm$ 29 to 1470 $\pm$ 24 pmol bilirubin/mg protein/h ($P < 0.05$). Hypoxia enhanced heme oxygenase activity to 2231 $\pm$ 140 pmol bilirubin/mg protein/h and the presence of curcumin further increased protein activity by 79% (3992 $\pm$ 251 pmol bilirubin/mg protein/h, $P < 0.05$ vs. hypoxia alone). These data suggest that low concentrations of curcumin are effective in inducing the HO-1 gene and increasing heme oxygenase activity even in conditions of limited oxygen availability.

Effect of curcumin on cell viability

Figure 6 shows endothelial cell viability measured 18 h after exposure to various concentrations of curcumin using a redox indicator. Concentrations of 5 and 20 μM curcumin, which markedly increase heme oxygenase activity, did not change cell viability compared to untreated cells. However, higher concentrations of the drug produced a small (5%) but significant decrease in cell viability ($P < 0.05$ vs. control). As exposure of cells to 25–30 μM curcumin did not result in activation of the heme oxygenase pathway, these data suggest that when cells are damaged the induction of certain antioxidant genes is impaired.

Heme oxygenase activation by curcumin and hypoxia protects endothelial cells against oxidant-mediated injury

Treatment of cells for 2 h to glucose oxidase, which generates hydrogen peroxide in the culture medium, resulted in 78% decrease in cell viability ($P < 0.05$, Fig. 7). However, exposure of cells for 18 h to curcumin (10 μM) or hypoxia reduced glucose oxidase-mediated damage and restored cell viability to 57 and 39% of control, respectively ($P < 0.05$). Incubation with SnPPIX (50 μM), an inhibitor of heme oxygenase activity, completely reversed the protective effect afforded by pretreatment of cells with curcumin or low oxygen tension.

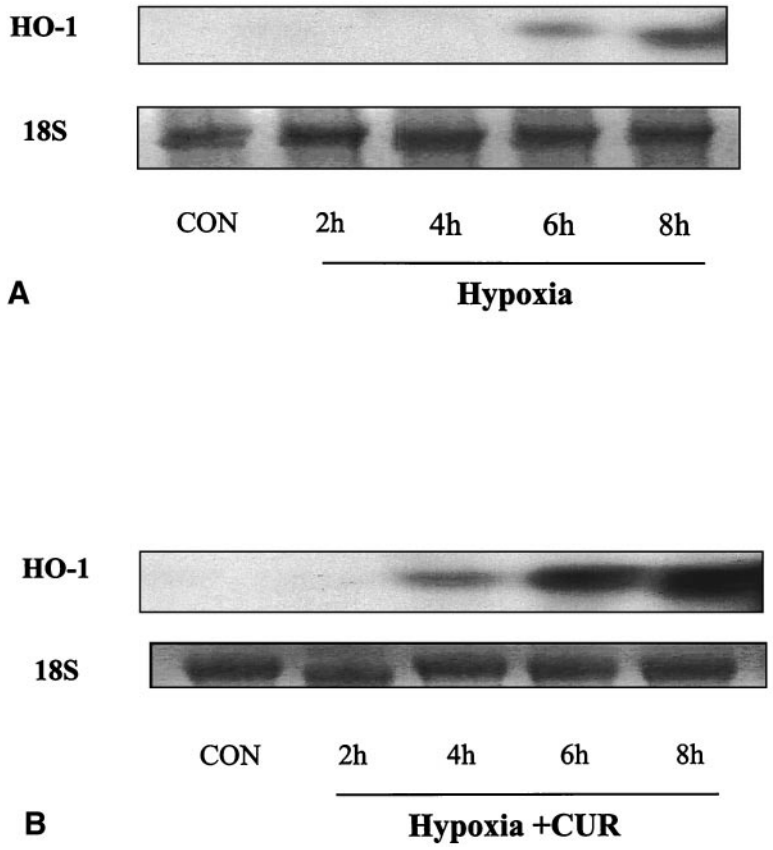

Fig. 4. Effect of curcumin on HO-1 mRNA expression in vascular endothelial cells exposed to hypoxia. Cells were placed in an hypoxic chamber for the times indicated in the presence of complete medium alone (A) or medium supplemented with 5 μM curcumin (B). After treatment, total RNA was isolated and analyzed for HO-1 mRNA expression by Northern blot analysis. The 18S band is shown to confirm integrity and equal loading of RNA. CUR = curcumin 5 μM.

Interestingly, the presence of curcumin (5 μM) under hypoxic conditions, which was shown to potentiate HO-1 induction (see Fig. 5), markedly increased the resistance to oxidative stress leading to 77% of viable cells after treatment with glucose oxidase. This effect was significantly attenuated by the presence of SnPPIX suggesting that the heme oxygenase pathway actively contributes to protection against hydrogen peroxide-mediated damage. Cells were also incubated for 1.5 h with 10 μM curcumin prior to challenge with glucose oxidase. This short period of exposure to the drug does not increase HO-1 expression (see Fig. 3) and this protocol was used to investigate whether the cytoprotective action of curcumin is related to its intrinsic antioxidant properties. Interestingly, cell viability was still considerably reduced by 75% after this treatment suggesting that (i) curcumin per se is not effective in preventing the damage caused by glucose oxidase and (ii) the cytoprotection observed after prolonged (18 h) incubation with the drug is, at least in part, attributable to induction of the cellular stress response by curcumin.

DISCUSSION

In the present study we report that low concentrations of curcumin, the active component of the food flavor turmeric, up-regulate endothelial HO-1 gene, protein expression and heme oxygenase activity in a concentration- and time-dependent manner. Interestingly, this effect was greatly potentiated when endothelial cells were exposed to curcumin under conditions of reduced oxygen tension (hypoxia). Prolonged treatment with curcumin resulted in high resistance to oxidant-mediated cell injury and induction of the HO-1 pathway contributed to the observed cytoprotection.

In recent years, several studies highlighted the ability of curcumin to promote a variety of pharmacological and biological activities [1]. For instance, by virtue of its flavonoid chemical structure, this yellow pigment appears to possess antioxidant and free radical-scavenging characteristics. Curcumin, in fact, neutralizes active oxygen species including superoxide, hydroxyl radical and nitric oxide [31,32]. In renal epithelial cells, curcumin has been reported to inhibit lipid peroxidation resulting

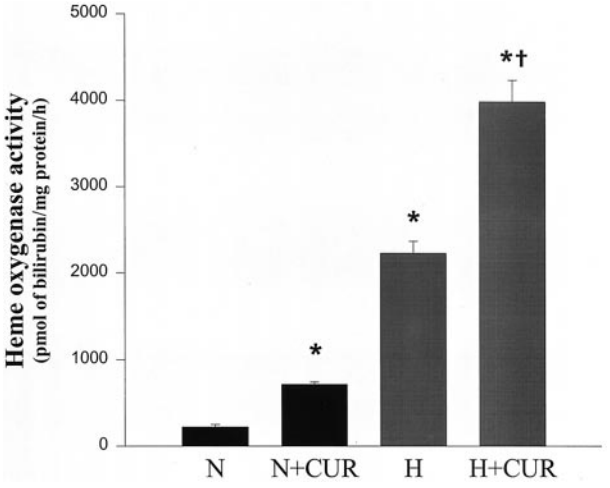

Fig. 5. Effect of curcumin on heme oxygenase activity in vascular endothelial cells incubated under normoxic or hypoxic conditions. Cells were exposed to medium or medium supplemented with curcumin (5 μM) and incubated for 18 h either in normoxia or hypoxia chamber as reported in Materials and Methods. Data are expressed as the mean ± SEM of 5 independent experiments. *$P < 0.05$ vs. normoxia (N); †$P < 0.05$ vs. hypoxia (H). CUR = curcumin 5 μM.

in protection against the cytotoxic action of hydrogen peroxide [33]. In this study, we wanted to investigate whether the antioxidant properties of curcumin could also be related to its ability to affect the levels of HO-1, a heat shock protein implicated in protection against various stress stimuli. Indeed, curcumin in the range of 5–15 μM markedly up-regulated endothelial HO-1 levels, whereas at higher concentrations a gradual decline in

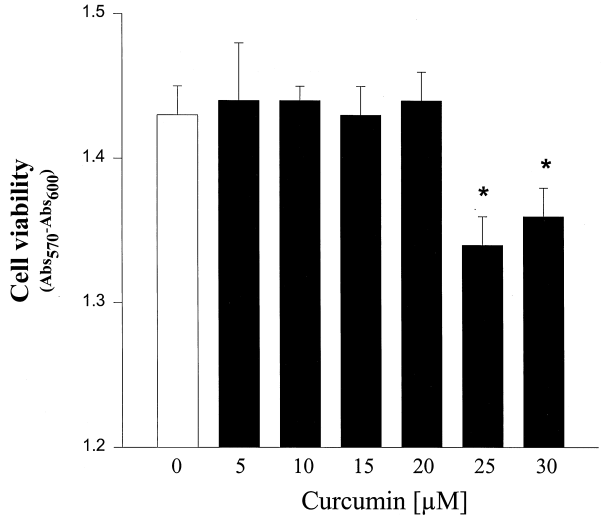

Fig. 6. Effect of curcumin on endothelial cell viability. Endothelial cells were exposed for 18 h to various concentrations of curcumin (0–30 μM) and cell viability was measured spectrophotometrically using an AlamarBlue assay as reported in Materials and Methods. Data are expressed as the mean ± SEM of 5 independent experiments. *$P <$ 0.05 vs. 0 μM curcumin.

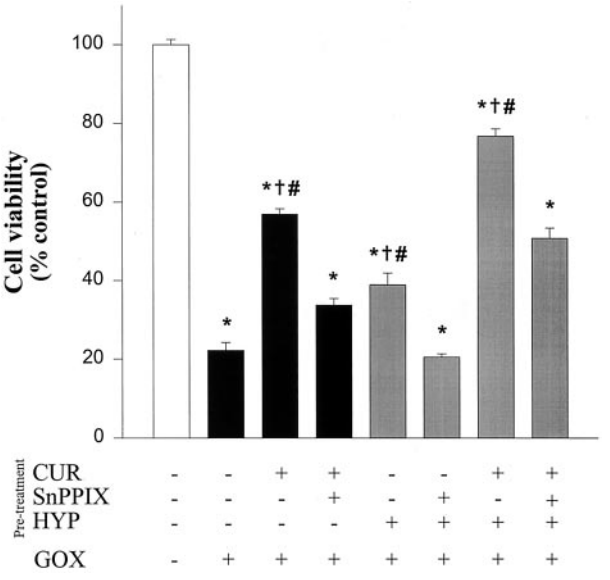

Fig. 7. Effect of curcumin and hypoxia on glucose oxidase-mediated cellular injury. Cells were exposed for 18 h to 10 μM curcumin (CUR), hypoxia (HYP) or HYP + 5 μM CUR in the presence or absence of 50 μM tin protoporphyrin IX (SnPPIX). After this pretreatment, cells were challenged for 2 h with glucose oxidase (GOX, 100 mU/ml), an oxidant system that generates hydrogen peroxide. After this treatment, cells were washed and viability was assessed using the Alamar Blue assay. Each bar represents the mean ± SEM of 6 independent experiments. *$P < 0.05$ vs. control (white bar); †$P < 0.05$ vs. GOX alone; #$P <$ 0.05 vs. same treatment plus SnPPIX.

heme oxygenase activity was observed. Experiments performed to assess cell viability showed no apparent damage after incubation with 5–20 μM curcumin; however, 30 μM curcumin produced a reduction in viability which could explain the inability of high concentrations of the drug to increase heme oxygenase activity. Our data are in agreement with previous reports showing that 1 to 15 μM curcumin do not cause cytotoxicity in vascular smooth muscle cells [34], but concentrations of 30–100 μM appear to mediate pro-oxidant effects and, to some extent, also apoptosis in various cell types [34,35].

Of major interest are our findings on the cellular response to glucose oxidase-induced injury in relation to prolonged or short exposure to curcumin. Incubation of cells for 18 h with the drug resulted in enhanced resistance to oxidative damage; this effect was partly attributable to induction of HO-1 as the inhibitor of heme oxygenase activity (SnPPIX) markedly reduced cytoprotection afforded by curcumin treatment. In contrast, exposure of cells to curcumin for only 1.5 h, a period of time insufficient to up-regulate HO-1 expression (see Fig. 3), did not produce any evident protective effect. These data strongly indicate that, in our experimental setting, the antioxidant properties of this yellow pigment are essentially linked to its ability to stimulate a defensive intracellular enzymatic pathway, namely HO-1. Our

results do not exclude the participation of other inducible systems in curcumin-mediated cytoprotection; in fact, a previous report showed that curcumin increases the expression of the 70-kDa heat shock protein (HSP70) in colorectal carcinoma cells [36].

The induction of HO-1 and other antioxidant genes in various stress conditions is being regarded as a refined stratagem adopted by tissues to counteract a variety of pathophysiological states such as oxidative stress [20], ischemia-reperfusion injury [18], inflammation [22], cell growth [37] and apoptosis [17,23]. The majority of these conditions are characterized, within the challenged tissue, by a reduced oxygen tension which results in brief or sustained periods of hypoxia. As curcumin has also been shown to possess anti-inflammatory, antioxidant and anticarcinogenic activities, it was of interest to investigate whether the marked effect of this yellow pigment on HO-1 expression and heme oxygenase activity observed at physiological oxygen levels could also be reproduced in hypoxic conditions. We found that cells exposed to severe hypoxia displayed higher levels of heme oxygenase activity compared to control, and curcumin greatly potentiated this effect. The enhancement occurred at transcriptional level since hypoxia stimulated HO-1 mRNA expression more rapidly and strongly in the presence of curcumin. Although NF-κB and AP-1 are known to regulate HO-1 gene expression [38], it is unlikely that these transcriptional factors are involved in the mechanism of HO-1 induction by curcumin since this drug is a potent inhibitor of NF-κB and AP-1 activation [39]. We have previously demonstrated that, under hypoxic conditions, both oxidative and nitrosative stress contribute to increased HO-1 expression in endothelial cells, an effect that is significantly attenuated by the antioxidant and thiol donor, N-acetylcysteine [25]. In this study, we found that N-acetylcysteine does not affect the increase in heme oxygenase activity caused by curcumin indicating that this effect is unlikely mediated by pro-oxidant mechanisms.

We aimed to verify whether, similarly to the effect observed with curcumin, induction of HO-1 by hypoxia prior to challenge with oxidants resulted in a better maintenance of cell viability. Indeed, the results presented here demonstrate that, after prolonged hypoxia, cells were less susceptible to glucose oxidase-mediated damage. Remarkably, the additive effect on endothelial HO-1 up-regulation elicited by low oxygen tension plus curcumin correlated with a greater cytoprotection to oxidative stress compared to either hypoxia or curcumin alone. Once again, an inhibitor of heme oxygenase activity (SnPPIX) considerably suppressed the acquired resistance. These findings substantiate the notion that the HO-1 pathway is a fundamental and very effective inducible antioxidant system. Our data also seem to indicate that the higher the expression of HO-1 is at the time of exposure to glucose oxidase, the stronger the ability of cells to counteract oxidant challenge. CO and bilirubin, generated in high amounts as a consequence of HO-1 induction, are most likely the effector molecules responsible for cytoprotection. These end products of heme breakdown by heme oxygenase have been recently shown to reduce cell death caused by hydrogen peroxide and peroxynitrite-induced apoptosis [15,17], increase tolerance to hyperoxic lung injury [19], inhibits monocyte transmigration induced by oxidized LDL [40] and improve postmyocardial dysfunction in isolated rat hearts [18].

The use of curcumin as a therapeutic agent to mitigate cardiovascular disease and other vascular dysfunctions is currently being investigated [41,42]. Although it is unlikely that micromolar concentrations of curcumin can be achieved in human plasma at doses normally used in the diet, pharmacokinetic studies in rats reveal that tissue absorption of curcumin is feasible [43]. Interestingly, concentrations in the range of 1–15 μM curcumin has been detected in rat blood, liver, and kidney between 15 min and 24 h after oral administration of 400 mg of the pigment. Thus, it is conceivable that curcumin could be used as a pharmacological "preconditioning" agent to modulate the expression of intracellular pathways in organs or tissues requiring increased protection against oxidant-mediated injury; in vivo experiments are now necessary to address and verify this hypothesis.

In summary, our data show that curcumin is a potent inducer of HO-1 in vascular endothelial cells both in normoxic and hypoxic conditions, and that increased heme oxygenase activity is an important element in curcumin-mediated cytoprotection against oxidative stress. It is interesting that many of the biological actions of curcumin including inhibition of cell proliferation [44], antioxidant capacities [6], and modulation of inflammatory processes [1] have also been ascribed to overexpression of HO-1 [20,22,37]. In view of the results presented here, it is tempting to speculate that some beneficial effects of curcumin might take place because of the intrinsic ability of this yellow pigment to increase HO-1 and possibly other intracellular protective pathways. This study also raises the hypothesis that similar mechanisms involving induction of the tissue stress response might apply to other antioxidant and anti-inflammatory drugs.

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ABBREVIATIONS

HO-1—heme oxygenase-1
SnPPIX—tin protoporphyrin IX
CO—carbon monoxide