

Curcumin induces heme oxygenase 1 through generation of reactive oxygen species, p38 activation and phosphatase inhibition

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Abstract. Curcumin is a naturally occurring compound which is known to induce heme oxygenase 1 (HO-1), although the underlying mechanism has not been fully elucidated. This study investigates in detail the mechanism of HO-1 induction by curcumin in human hepatoma cells. There was increasing toxicity of curcumin at concentrations higher than 10 μ M. Curcumin was found to induce HO-1 at doses of 10 to 25 μ M. At both non-toxic and toxic doses, HO-1 induction was found to correlate with production of reactive oxygen species (ROS), suggesting a causative relationship. This was reinforced by the finding that pretreatment with the antioxidants N-acetylcysteine, vitamin E and catalase prevented HO-1 induction by curcumin. ROS production appeared to be mitochondrial in origin, and curcumin treatment resulted in depolarisation of the mitochondrial membrane potential. Nrf2 was induced by curcumin treatment, which was also partly ROS dependent. Using siRNA, Nrf2 was demonstrated to contribute to HO-1 induction. A panel of kinase inhibitors was used to examine the contribution of MAP kinases to the induction of HO-1 by curcumin. PKC and p38 MAPK activity are required for full induction of HO-1. Furthermore, curcumin also inhibited protein phosphatase activity. In conclusion, curcumin treatment results in ROS generation, activation of Nrf2 and MAP kinases and the inhibition of phosphatase activity in hepatocytes, and when curcumin is not administered in toxic doses, these multiple pathways converge to induce HO-1.

Introduction

Curcumin is a natural extract of turmeric, the spice produced from *Curcuma longa* L (Zingiberaceae), part of the ginger

family (1). Curcumin has been shown in recent years to have cytoprotective properties by inducing the protective protein heme oxygenase 1 (HO-1) (2,3). HO-1 is a stress responsive enzyme and part of the cell's natural defence mechanisms. It catalyses the degradation of heme, generating the end-products biliverdin, carbon monoxide and iron. Increased HO-1 expression prolongs cardiac xenograft survival (4), allows prolonged cold preservation of liver transplants (5), and limits the severity of ischaemia reperfusion injury and development of chronic allograft nephropathy in kidney transplants (6). For this reason there has been great interest in understanding the cellular mechanisms through which curcumin acts, both to improve the understanding of the molecular regulation of HO-1 and to facilitate the design of safe clinical agents which can harness the HO-1 inducing properties of curcumin.

HO-1 induction is a complex multi-regulated process involving a variety of transcription factors (7). It is readily upregulated in response to oxidative stress, although it can also be induced by many other stimuli. Most 'classical' inducers depend on their prooxidant activity to induce HO-1. However, curcumin has both prooxidant and antioxidant properties (8,9). Indeed, curcumin has been found to have potent chemopreventive properties, as a result of its ability to induce apoptosis of tumour cells through generation of reactive oxygen species (8,10). Hence it is unclear whether the HO-1 inducing effect of curcumin represents a general response to cell injury or activation of a more specific signal transduction pathway.

Many of the classical HO-1 inducers such as cadmium have been shown to activate the transcription factor Nrf2 (11). Curcumin has been shown to induce HO-1 through Nrf2 binding to the antioxidant response element in the HO-1 promoter (12). However, the signal transduction mechanism through which curcumin triggers Nrf2 activation has not been examined. Furthermore there are significant interspecies differences in the HO-1 promoter (7), the result of which is that certain stimuli such as heat and hypoxia, which induce HO-1 in rodent models, do not induce HO-1 in human cells (13,14).

MAP kinases also have a role in the regulation of HO-1 induction (15), and curcumin differentially activates certain MAP kinases including p38, JNK and ERK, depending on cell line and treatment conditions (12,16,17).

Curcumin has many potential mechanisms through which it may induce HO-1, including ROS generation, Nrf2 activation and kinase activation. Therefore in this paper we examined in

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Abbreviations: HO-1, heme oxygenase 1; Nrf2, nuclear factor E2 related factor 2; ROS, reactive oxygen species; p38 MAPK, p38 mitogen activated protein kinase; PKC, protein kinase C; PP2A, protein phosphatase 2A; PP2C, protein phosphatase 2C

Key words: curcumin, heme oxygenase 1, hepatocyte

detail the potential pathways through which curcumin may induce HO-1 in a human hepatocyte cell line.

Materials and methods

Cell culture and reagents. All reagents were purchased from Sigma-Aldrich Ltd. (Poole, Dorset, UK) unless otherwise stated. Curcumin was diluted in dimethyl sulfoxide (DMSO) to a stock concentration of 100 mM. HUH7 human hepatoma cells were obtained from the European Tissue Culture Collection (Porton Down, UK), and maintained in Dulbecco's modified Eagle's medium containing 10% fetal calf serum, 50 u/ml penicillin G and 50 µg/ml streptomycin. Whole cell extracts were obtained using RIPA buffer with protease inhibitors.

Western blotting. Western blotting was performed using a standard protocol with a Bio-Rad Miniprotein II system. Protein concentrations of samples were determined using a Bio-Rad DC protein assay kit. Antibody to HO-1 was purchased from Stressgen Biotechnologies (Victoria, BC, Canada). Secondary HRP-labelled antibodies were purchased from Santa Cruz Biotechnology (Santa Cruz, CA, USA) and Upstate (Waltham, MA, USA). Equality of protein loading was tested by ponceau staining and by probing membranes with an antibody to β actin (Santa Cruz). Chemiluminescence was performed using the Amersham ECL system (Amersham Biosciences Ltd., Buckinghamshire, UK).

Cytotoxicity assay. Cell viability assays were performed on HUH7 cells using an MTT (methyltetrazolium) dye technique. Cells were cultured in 96-well plates at 3×10^4 cells per well in the presence or absence of curcumin or vehicle for the indicated times. The medium was changed and 10 µl of MTT solution (5 mg/ml in PBS) was added. After 4-h incubation at 37°C, 150 µl extraction buffer (10% SDS, pH 3.0) was added and incubated overnight, following which the optical densities were measured at 570 nm using a plate reader (Dynex MRX II, Dynatech). All treatments were performed in sextuplicate on each occasion. Results are expressed as a percentage of medium only controls.

Measurement of caspase 3/7 activity. Caspase activity was measured using a proprietary kit, Apo-ONE homogenous caspase 3/7 assay (Promega), as per the manufacturer's instructions.

Measurement of the mitochondrial membrane potential. Mitochondrial membrane potential was measured by two methods; 3,3'-dihexyloxycarbocyanine (DiOC6) and MitoTracker green and red. For DiOC6, cells were treated with curcumin or vehicle for 1 h, trypsinised and then suspended in PBS at 10^6 cells/ml. They were incubated in DiOC6 (50 nM final concentration) for 15 min at room temperature. Propidium iodide (5 µg/ml final concentration) was added and the cells were read immediately on an Epics XL-MCL flow cytometer (Coulter). However, this method does not allow for variations in total mitochondrial mass. For this reason, a normalised mitochondrial membrane potential was measured using MitoTracker green and red. Cells were treated as above, and

then suspended in PBS. The 2 dyes were added at a final concentration of 100 nM, and the cells were incubated at 37°C in the dark for 30 min. The cells were then placed on ice and read immediately on the flow cytometer. The ratio of red:green fluorescence represents the normalised mitochondrial membrane potential.

Measurement of reactive oxygen species. ROS generation was measured using two methods; dichlorodihydrofluorescein diacetate (H₂DCFDA) and dihydroethidium. For the H₂DCFDA, cells were plated in 96-well plates at a density of 2×10^4 cells per well. The next day the cells were washed three times with HBSS and then treated as required, in the presence of H₂DCFDA at a final concentration of 10 µM. The plate was placed in a preheated fluorometer (at 37°C) and continuous measurement performed for 1 h, at excitation 480 nm and emission 530 nm. Following this, the supernatant was removed and a protein assay was performed on the cells. The plate was snap frozen, and the protein retrieved by applying hot (95°C) Laemmli buffer. The protein concentration was measured using a proprietary kit (Bio-Rad). The final results were corrected for variations in the protein concentration between wells and are expressed as a percentage with activity in untreated cells being 100%. Dihydroethidium was used with cells prepared as above. The required treatments were applied in the presence of 100 µM dihydroethidium (final concentration). The cells were then placed back into normal conditions for 4 h. The wells were washed 3 times with HBSS and ethidium fluorescence was measured with an excitation of 480 nm and an emission of 560 nm.

RNA interference. Transfections with siRNA encoding specific sequences for Nrf2 and HIF-1α were used as per the manufacturer's instructions (Santa Cruz). Control siRNA was transfected to exclude non-specific effects.

Transfections. Transient transfections were performed using Fugene reagent (Roche) as per the manufacturer's instructions, at a ratio of 6 µl Fugene/µg plasmid DNA. HUH7 cells were plated at 4×10^5 cells per 35-mm dish, and transfected the day after plating. The pHOGL3/11.6 reporter construct containing 11.6 kb of the human HO-1 promoter was a kind gift from Dr Agarwal (University of Alabama, USA). The HIF-1α reporter construct was a kind gift from Professor Esumi (National Cancer Research Institute East, Japan). PSV-β-galactosidase vector (0.5 µg; Promega) was co-transfected with the reporter constructs, to control for transfection efficiency. The cells were treated 24 h later and the β-galactosidase or luciferase activity assayed using a proprietary kit as per the manufacturer's instructions (Promega). Results were calculated as units of luciferase activity per units of β-galactosidase activity.

Phosphatase activity. Cells were treated with curcumin for the required time and then phosphatase activity was assayed using a non-radioactive molybdate dye based proprietary kit as per the manufacturer's instructions (Promega).

Data analysis. All values are expressed as means ± standard error (SEM). SPSS software was used for statistical analysis (SPSS, IL, USA) Analysis of variance and Student's t-test

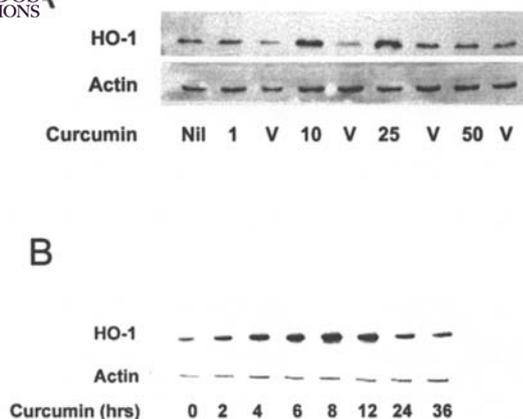


Figure 1. Curcumin induces HO-1 in human hepatocytes. (A) Representative Western blot of lysates from cells treated with curcumin (1-50 μM) for 8 h run in parallel with matched vehicle controls (V) (n=3). (B) Representative Western blot of lysates from cells treated with 10 μM curcumin for increasing periods of time (n=3).

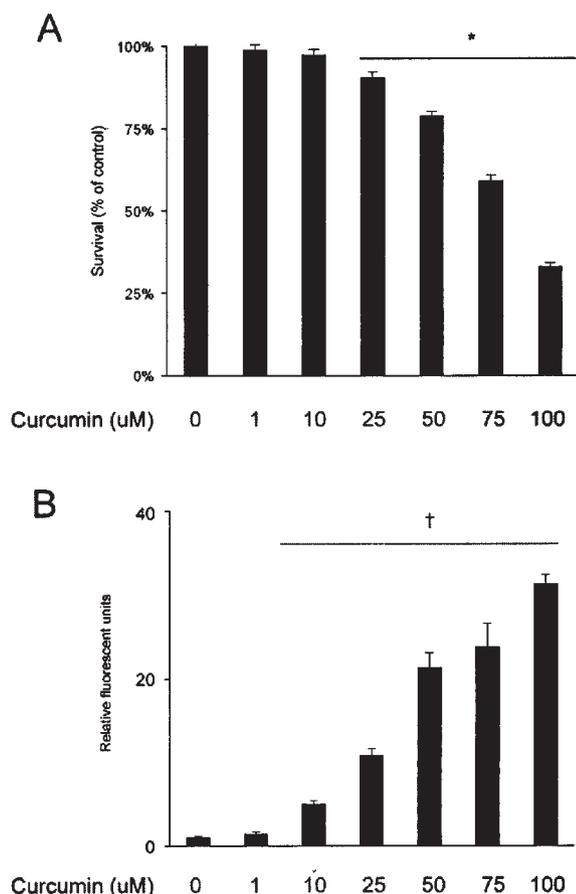


Figure 2. Curcumin is toxic in high doses. (A) Cells were treated with curcumin as indicated for 24 h, and survival assessed. Curcumin produced a dose-dependent reduction in survival at doses of $\geq 25 \mu\text{M}$ ($^*p < 0.01$ vs nil control). (B) Cells were treated as above with curcumin for 24 h and caspase 3/7 activity was measured. Caspase activity was increased at doses of $\geq 50 \mu\text{M}$ ($^*p < 0.05$ vs nil control). Each bar represents the mean \pm SEM of six repeats from one experiment. Each experiment was performed 3 times with similar results.

were used for statistical analysis, and the differences between groups were considered to be significant at $P < 0.05$.

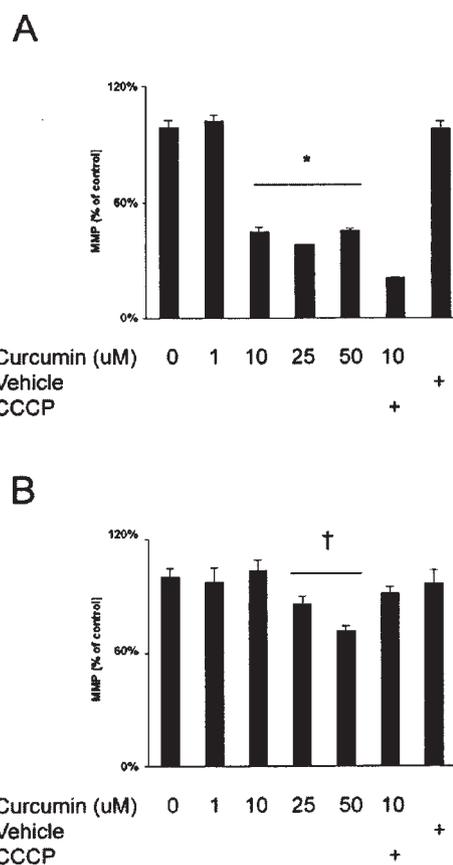


Figure 3. Curcumin induces loss of the mitochondrial membrane potential. (A) MitoTracker red and green were used to measure the normalised mitochondrial membrane potential. Cells were treated with increasing doses of curcumin for 1 h and the mitochondrial membrane potential was measured as described. Treatment with 10-50 μM curcumin lead to reduced mitochondrial membrane potential ($^*p < 0.01$ vs nil control). Vehicle (10 μM equivalent) had no effect. (B) Cells were treated as above, and the mitochondrial membrane potential measured with DiOC6. Treatment with 25-50 μM curcumin lead to reduction in the mitochondrial membrane potential ($^*p < 0.05$ vs nil control). Vehicle had no effect. Each bar represents the mean \pm SEM of six independent experiments.

Results

Curcumin induces HO-1 in a dose- and time-dependent manner. Treatment with curcumin induced HO-1 in hepatocytes at doses of 10-25 μM . This induction was seen from 2 to 12 h, but was maximal at 6 to 8 h after administration (Fig. 1).

Curcumin toxicity is associated with caspase activation. Curcumin treatment reduces cell viability in a dose-dependent manner. This effect is seen after treatment with doses $\geq 25 \mu\text{M}$. Exposure to 25 μM curcumin for 24 h reduces survival by 10% ($p < 0.01$, Fig. 2A). The reduction in survival is associated with caspase activation. Caspase 3/7 activity was increased after treatment with curcumin at $\geq 50 \mu\text{M}$ ($p < 0.05$, Fig. 2B). The same induction of caspase activity was seen when measured after 6 h of curcumin treatment (data not shown).

Curcumin induces ROS production. Reactive oxygen species production was measured as a possible mechanism for curcumin toxicity. ROS production was significantly increased with curcumin treatment ($p < 0.05$, Fig. 3). Using H_2DCFDA the ROS generation was measured continuously for 2 h. ROS

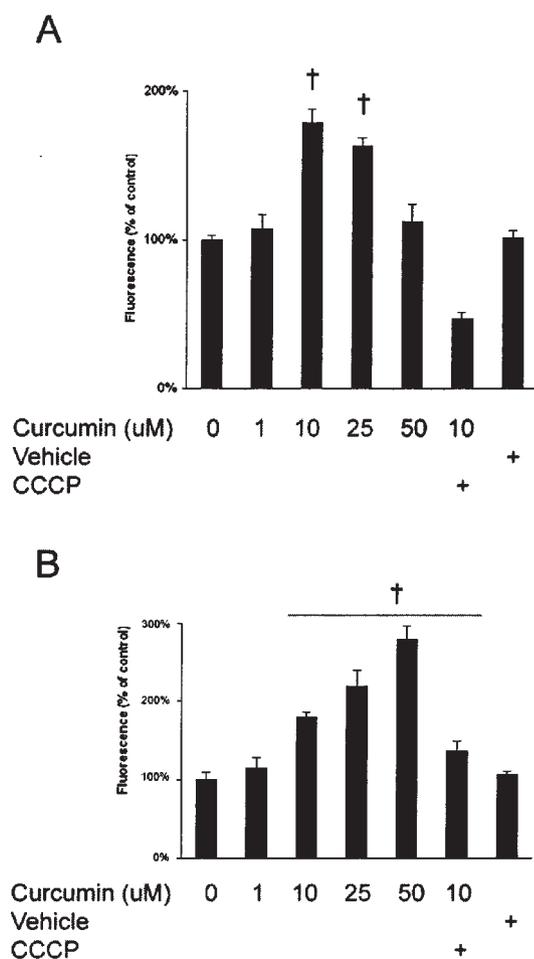


Figure 4. Curcumin induces ROS production. (A) Cells were treated with increasing doses of curcumin and ROS production measured continuously for 2 h using H₂DCFDA. Data shown represents total ROS production after 1 h of treatment, with 10–25 μM curcumin inducing ROS production. (B) Cells were treated as above and ROS production at 4 h was measured with dihydroethidium. Curcumin induced ROS production in doses from 10–50 μM. Each bar represents the mean ± SEM of six repeats from one experiment. Each experiment was performed 3 times with similar results. †p<0.05 vs nil control.

were generated at a high rate within a minute of commencing curcumin treatment, and continued to be produced for the period studied. There was a clear dose dependency between the concentration of curcumin and ROS production when measured with dihydroethidium. However, when measured with H₂DCFDA, ROS production was found to be maximal after 10 and 25 μM curcumin, but near basal levels after treatment with 50 μM. The protonophoric uncoupler CCCP prevented ROS production, suggesting it had a mitochondrial origin.

Curcumin treatment results in partial mitochondrial depolarisation. In view of a possible mitochondrial origin for the ROS production, attention was then focused on curcumin induced changes in mitochondria. Curcumin treatment with 10–50 μM curcumin resulted in loss of the mitochondrial membrane potential as measured by MitoTracker red and green dye (p<0.01, Fig. 4A). Use of DiOC6 similarly found depolarisation of the mitochondria at 25–50 μM, although 10 μM had no effect (Fig. 4B).

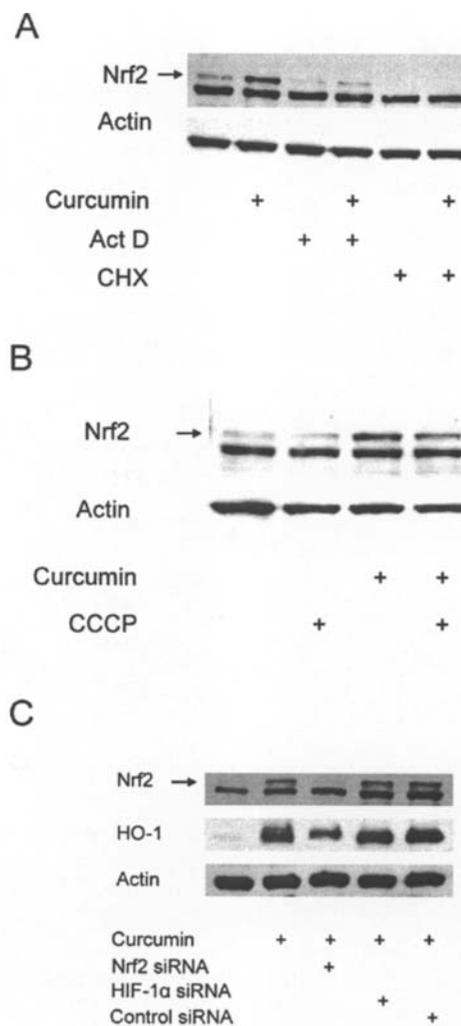


Figure 5. Curcumin induces HO-1 through Nrf2. (A) Nrf2 was measured in whole cell lysates from cells treated with 10 μM curcumin (upper band, arrowed). Nrf2 was strongly induced by curcumin treatment, and this was inhibited by treatment with actinomycin D or cyclohexamide. (B) Nrf2 induction (upper band, arrowed) by 10 μM curcumin was partially inhibited by CCCP, which prevents ROS production. (C) The induction of HO-1 by 10 μM curcumin was inhibited by siRNA for Nrf2 (upper band, arrowed). siRNA for HIF-1α or a control sequence had no effect on HO-1 induction. Blots shown are representative of three individual experiments.

Induction of HO-1 by curcumin is mediated through Nrf2. Nrf2 is an oxidant-responsive transcription factor that has previously been shown to be involved in curcumin-mediated induction of HO-1. Nrf2 was induced following curcumin treatment, as a result of *de novo* synthesis which could be prevented by inhibiting transcription or translation with actinomycin D and cyclohexamide respectively (Fig. 5A). The induction of Nrf2 in response to curcumin appears partially dependent on ROS production (Fig. 5B). To demonstrate a definite effect of Nrf2 in mediating HO-1 induction by curcumin, siRNA to Nrf2 was used. This prevented the upregulation of Nrf2 by curcumin treatment and partially abrogated the induction of HO-1 by curcumin. Control siRNA and siRNA for an irrelevant transcription factor had no effect (Fig. 5C).

Antioxidants inhibit HO-1 induction by curcumin. To confirm the role of oxidative stress in induction of HO-1 by curcumin,

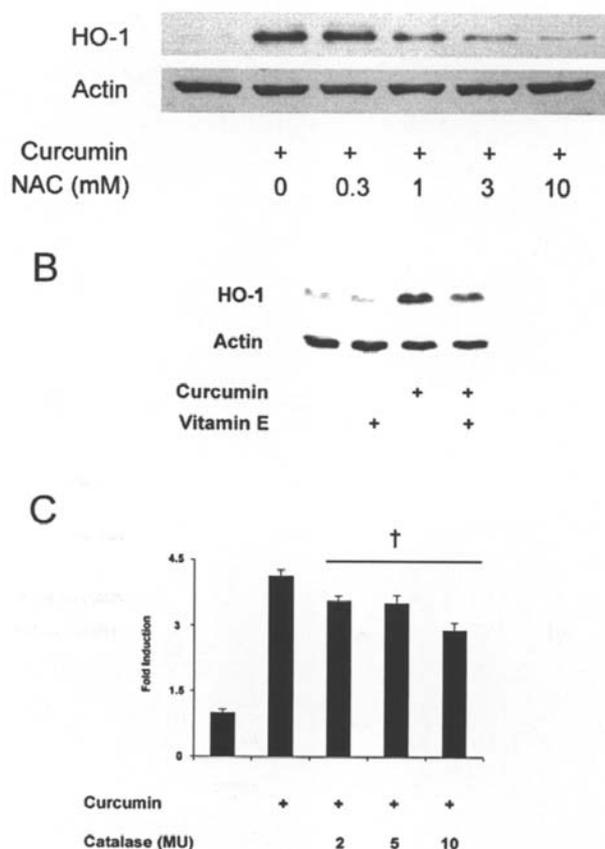


Figure 6. Antioxidants inhibit HO-1 induction by curcumin. (A) Cells were pretreated with increasing doses of N-acetylcysteine for 30 min prior to treatment with 10 μ M curcumin. There was a dose-dependent reduction in HO-1 expression with increasing doses of N-acetylcysteine (0-10 mM). (B) Pretreatment with 50 μ M vitamin E for 30 min reduced the HO-1 induction seen with 10 μ M curcumin. Blots shown are representative of three individual experiments. (C) Cells transfected with the HO-1 reporter were simultaneously treated with increasing doses of catalase and 10 μ M curcumin for 6 h and reporter activity was measured. Increasing doses led to a significant reduction in HO-1 induction († p<0.05 for doses of 2 to 10 MU). Doses of catalase are given in 1000 units (MU).

antioxidants were used. Cells pretreated with the antioxidants N-acetylcysteine (Fig. 6A) or vitamin E (Fig. 6B) demonstrated a reduction in HO-1 levels following curcumin treatment. Treatment with catalase also reduced the induction of HO-1 in a dose-responsive manner, suggesting hydrogen peroxide as an important contributing ROS (Fig. 6C). Co-treatment of cells with 10,000 units of catalase with 10 μ M curcumin led to a reduction in HO-1 reporter activity of 411% of normal controls to 287% (p<0.05).

PKC and p38 contribute to the induction of HO-1 by curcumin.

A panel of kinase inhibitors was used to examine the contribution of MAP kinase activity to the induction of HO-1 by curcumin. Inhibition of PKC and p38 significantly reduced HO-1 induction (from 280% to 168% and 184%, p<0.01 and p<0.05 respectively, Fig. 7A). Inhibition of PI3 kinase also reduced the HO-1 induction, although this did not reach significance (280% to 231%, p=0.181), and inhibition of ERK had no effect. The reporter results were confirmed by Western

blotting (Fig. 7B). Activation of p38 by curcumin was also confirmed by Western blotting (Fig. 7C).

Curcumin inhibits phosphatase activity. Phosphatase activity was examined after curcumin administration. PP2A activity was significantly inhibited within 5 min of curcumin treatment and remained reduced for 1 h (80% of basal activity, p<0.05; Fig. 8). PP1 activity was unchanged by curcumin treatment. Tyrosine phosphatase activity was reduced only after 1 h of curcumin treatment (77% of basal activity, p<0.05).

Discussion

This study examines in detail for the first time the mechanisms through which curcumin acts to induce HO-1. We have previously shown that curcumin induces HO-1 in hepatocytes and that this is cytoprotective (3). In this paper we have demonstrated that curcumin is toxic in high doses. This is reflected by caspase activation, which suggests that curcumin induces apoptotic cell death. Whilst curcumin has predominantly been described as inducing apoptosis through a classical mitochondrial pathway, associated with caspase activation (18,19), atypical variants have been described where there is no change in caspase activity and no mitochondrial depolarisation (20,21). It appears that in hepatocytes the classical caspase-mediated pathway is activated by high-dose curcumin treatment.

Curcumin induced ROS production. These ROS may originate from the mitochondria, as they could be inhibited by the uncoupler CCCP, and curcumin treatment also led to mitochondrial depolarisation, which can occur as the result of mitochondrial ROS production. Curcumin has been found to generate superoxide in cell lines that are sensitive to its pro-apoptotic properties (22) and, in isolated rat mitochondria, curcumin induces mitochondrial depolarisation in an ROS-dependent manner (23).

The two methods used for ROS measurement produced slightly different results. This probably reflects differences in sensitivity to the different ROS species. H2DCFDA is more sensitive for hydrogen peroxide and hydroxyl radicals, whilst dihydroethidium is more sensitive for superoxide although neither is species-specific (24,25).

The ROS generating properties of curcumin reflect its chemical structure, and certain other flavonoids can also generate ROS (26). Ligeret examined the effects of curcumin derivatives on rat liver mitochondria, and found that the phenolic group was essential to effect depolarisation of the mitochondria (23). This allows curcumin to reduce Fe³⁺ to Fe²⁺, which subsequently allows generation of hydroxyl radicals by the Fenton reaction. Ligeret proposed that it is the reductive 'antioxidant' properties of curcumin which results in increased ROS production. That the Fenton reaction is involved with HO-1 induction by curcumin is in keeping with our finding that this was prevented by catalase, suggesting that hydrogen peroxide contributes to the rise in HO-1.

In this study we have found that the transcription factor Nrf2 is induced by curcumin treatment. Nrf2 is rapidly induced by oxidative stress, and binds to consensus sequences in the HO-1 promoter to mediate its induction. Nrf2 has previously been shown to be involved in the HO-1 induction by curcumin

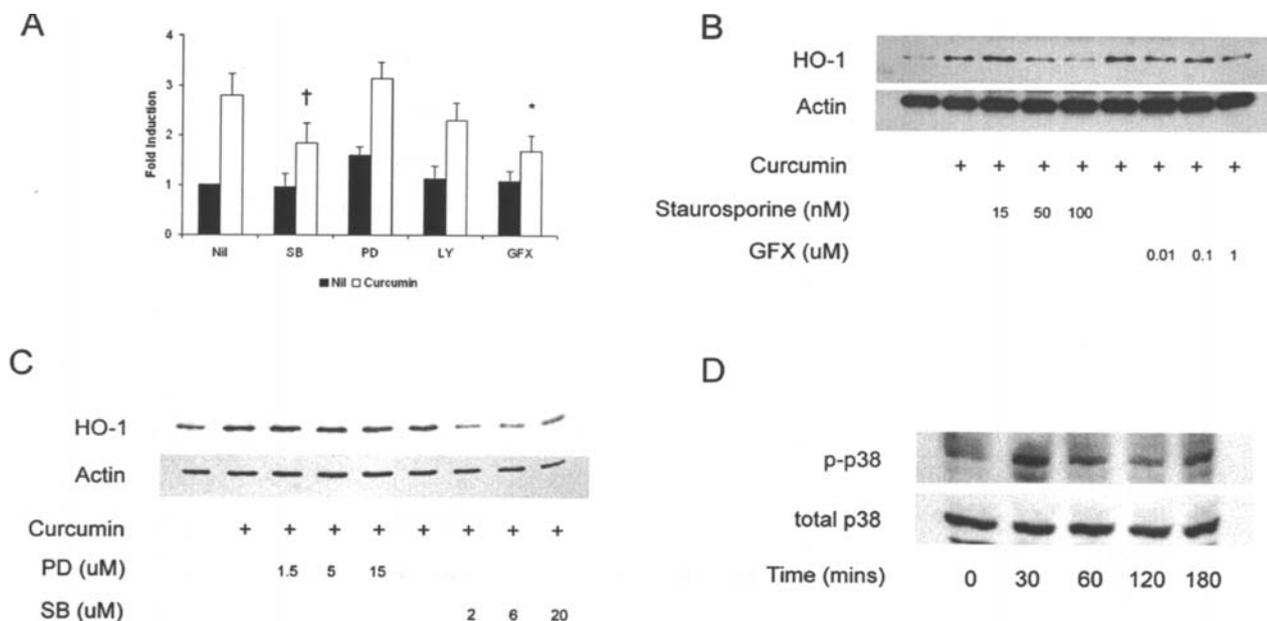


Figure 7. PKC and p38 contribute to HO-1 induction by curcumin. (A) HUH7 cells were transfected with pHOGL3/11.6 and the response to 10 μ M curcumin was measured in the presence of a panel of kinase inhibitors. Nil, no inhibitor; SB, 20 μ M SB203580, a p38 inhibitor; PD, 5 μ M PD98059, an ERK inhibitor; LY, 2 μ M LY294002, a PI3K inhibitor; GFX, 5 μ M GF109203X, a PKC inhibitor. SB and GFX inhibited HO-1 induction by curcumin ([†]p<0.01 and ^{*}p<0.05). (B) Dose response of two different PKC inhibitors, staurosporine and GFX (GF109203X) showing inhibition of HO-1 induction by 10 μ M curcumin. (C) Dose response of an ERK inhibitor (PD, PD98059) and a p38 inhibitor (SB, SB203580) showing inhibitory effect of p38 inhibition on HO-1 induction by 10 μ M curcumin. (D) Western blot showing activation of p38 following treatment with 10 μ M curcumin. p38 was activated after 30 min of curcumin treatment. p-p38, phosphorylated (active) p38 MAPK.

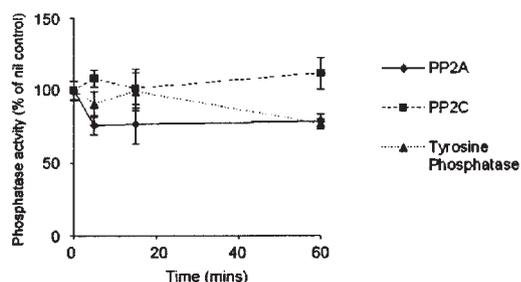


Figure 8. Curcumin inhibits protein phosphatase activity. HUH7 cells were treated with 10 μ M curcumin and the activity of protein phosphatases 2A (PP2A), 2C (PP2C) and tyrosine phosphatase were measured at 5, 15 and 60 min. Data shown represent the mean \pm SEM of 8 independent repeats.

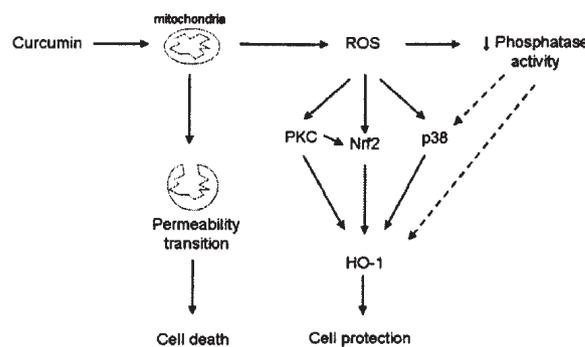


Figure 9. Proposed mechanisms through which curcumin induces HO-1. Curcumin penetrates the cell and leads to ROS generation from mitochondria. If it is given in high doses (>25 μ M) the mitochondria undergoes the permeability transition pore and cell death ensues. If given at non-toxic doses (\leq 25 μ M), the ROS generated triggers Nrf2 accumulation and activation, activation of PKC and p38, and inhibition of protein phosphatases, all of which contribute to the induction of HO-1 and the protection of the cell from injury.

(12). There are significant interspecies differences in the HO-1 promoter (7), and we have confirmed this finding for the first time in human cells, and for the first time using siRNA and HO-1 protein. We also examined the transcription factors HIF-1 α and HSF-1, which were found not to contribute to the HO-1 induction (unpublished data).

We have demonstrated for the first time that HO-1 induction by curcumin can be inhibited by the use of the antioxidants N-acetylcysteine, vitamin E and catalase. We propose that curcumin generates mitochondrial ROS and that this is the trigger for Nrf2 induction and subsequent upregulation of HO-1 (Fig. 9). Curcumin treatment results in dose-dependent mitochondrial generation of ROS. If a high dose of curcumin is used, the levels of ROS generated are overwhelmingly toxic, leading to the mitochondrial permeability transition pore, and subsequent caspase-mediated

apoptosis. If lower doses of curcumin are administered, the mitochondria generates levels of ROS which are survivable. This results in diverse effects, activating Nrf2 and the protein kinases p38 and PKC, and inhibiting protein phosphatase activity, the net result of which is induction of HO-1 and cellular protection from further stress. This proposed mechanism is consistent with experiments performed using the garlic extract DAS, another HO-1 inducer which has recently been found to stimulate ROS production (27).

In addition to the effects on Nrf2, PKC and p38 activity are important in the induction of HO-1 by curcumin. Protein



SPANDIDOS PUBLICATIONS (28) is involved in activation of Nrf2, by phosphorylation, allow dissociation from the cytoplasmic inhibitor Keap1. This allows nuclear migration of Nrf2 where it binds to the requisite promoters (28). Whilst this would explain the contribution of PKC activity to HO-1 induction by curcumin, a direct effect on other transcription factors cannot be excluded. p38 has previously been found to contribute to HO-1 induction by cadmium and curcumin, although the mechanism is unclear (11,12). In rat hepatocytes, only the p38 γ isoform promotes HO-1 induction, whilst p38 α , β and δ isoforms inhibit HO-1 (15). The contribution of different p38 isoforms to the induction of HO-1 by curcumin has not been assessed in this study.

Control of cellular processes is regulated through a delicate balance of protein kinase and protein phosphatase activity. After having demonstrated the involvement of kinase activation in curcumin's induction of HO-1, we examined the cellular phosphatase activity. We found that PP2A was rapidly inhibited by curcumin and that tyrosine phosphatase activity was inhibited after one hour of treatment. It is likely that this plays a role in the HO-1 induction seen with curcumin, as the PP2A inhibitor okadaic acid is a potent HO-1 inducer (29). PP2A can be inhibited by ROS (Kim 2003), and although not directly tested, this would be consistent with the rapid decrease in its activity seen in our experiments, as ROS were generated constantly at high levels from the beginning of curcumin treatment. Furthermore, PP2A acts directly on both p38 and caspase 3 resulting in their inactivation (30). This may represent part of the link between curcumin treatment and p38 and caspase 3 activation.

In conclusion, we report that curcumin treatment results in ROS generation, activation of Nrf2 and MAP kinases and the inhibition of phosphatase activity in hepatocytes, and when curcumin is not administered in toxic doses, these multiple pathways converge to induce HO-1. Thus curcumin can be used at low doses to pharmacologically induce HO-1, although its induction is through generation of non-lethal levels of reactive oxygen species.

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