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Comparative effects of curcuminoids on endothelial heme oxygenase-1 expression: *ortho*-methoxy groups are essential to enhance heme oxygenase activity and protection

Gil-Saeng Jeong[1,2]*, Gi-Su Oh[1]*, Hyun-Ock Pae[1], Sun-Oh Jeong[1], Youn-Chul Kim[2], Min-Kyo Shin[3], Byeong Yun Seo[4], Sang Youp Han[4], Ho Sub Lee[4], Jong-Gil Jeong[5], Jeong-Soon Koh[5] and Hun-Taeg Chung[1,6]

[1]Medicinal Resources Research Institute and Department of Microbiology and Immunology
[2]College of Pharmacy
[3]College of Oriental Medicine
[4]Professional Graduate School of Oriental Medicine Wonkwang University
Iksan 570-749, Korea
[5]College of Oriental Medicine
Dong Shin University
Naju 520-714, Korea
[6]Corresponding author: Tel, 82-63-850-6762; Fax, 82-63-851-5066; E-mail, htchung@wonkwang.ac.kr
*These authors contributed equally to this work.

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Abbreviations: BDMC, bis-demethoxycurcumin; CoPP, cobalt protoporphyrin; DMC, demethoxycurcumin; ERK1/2, extracellular signal-regulated kinase 1/2; HO, heme oxygenase; JNK, c-Jun NH_2-terminal kinase; MAPK, mitogen-activated protein kinase; SnPP, tin protoporphyrin

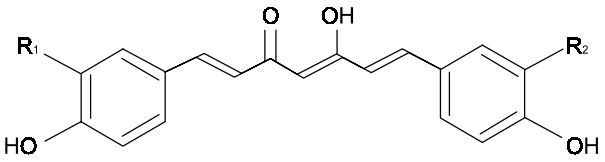
Abstract

Recently, it has been reported that curcumin, which is known as a potent antioxidant, acts as a non-stressful and non-cytotoxic inducer of the cytoprotective heme oxygenase (HO)-1. In this study, naturally occurring curcuminoids, such as pure curcumin, demethoxycurcumin (DMC) and bis-demethoxycurcumin (BDMC), were compared for their potential ability to modulate HO-1 expression and cytoprotective activity in human endothelial cells. All three curcuminoids could induce HO-1 expression and HO activity with differential levels. The rank order of HO activity was curcumin, DMC and BDMC. In comparison with endothelial protection against H_2O_2-induced cellular injury, cytoprotective capacity was found to be highest with curcumin, followed by DMC and BDMC. Interestingly, cytoprotective effects afforded by curcuminoids were considerably associated with their abilities to enhance HO activity. Considering that the main difference among the three curcuminoids is the number of methoxy groups (none for BDMC, one for DMC, and two for curcumin), the presence of methoxy groups in the *ortho* position on the aromatic ring was suggested to be essential to enhance HO-1 expression and cytoprotection in human endothelial cells. Our results may be useful in designing more efficacious HO-1 inducers which could be considered as promising pharmacological agents in the development of therapeutic approaches for the prevention or treatment of endothelial diseases caused by oxidative damages.

Keywords: antioxidants; bis-(4-hydroxycinnamoyl)methane; curcumin; demethoxycurcumin; endothelium, vascular; heme oxygenase-1

Introduction

The rhizome of *Curcuma longa* (turmeric) has been widely used as a spice and coloring agent in many foods. Consumption of turmeric has been associated with various beneficial effects on human health (Duvoix *et al.*, 2005). Turmeric has also been used as a traditional remedy for the treatment of inflammation and other diseases (Joe *et al.*, 2004). The major components of turmeric are the curcuminoids that include curcumin, demethoxycurcumin (DMC) and bis-demethoxycurcumin (BDMC) (Chainani-Wu, 2003). Their chemical structures are illustrated in Figure 1. Ongoing experimental and clinical studies suggest that curcumin and its curcuminoids exhibit unique cytoprotective (Scapagnini *et al.*, 2002), anti-inflammatory (Selvam *et al.*, 2005) and anticancer properties (Chen *et al.*, 2006). In recent years, it has also been reported that curcumin acts as a non-stressful and non-cytotoxic inducer of the cytoprotective heme oxygenase (HO)-1 and can maximize the intrinsic antioxidant potential of cells (Motterlini *et al.*, 2000). Whether other derivatives of curcumin present in turmeric, such as DMC and BDMC, would also stimulate this enzyme induction remains to be investigated.



Curcumin (R_1 = OCH_3, R_2 = OCH_3)
Demethoxycurcumin (R_1 = OCH_3, R_2 = H)
bis-Demethoxycurcumin (R_1 = H, R_2 = H)

Figure 1. Chemical structures of curcumin and its derivatives used in the present study.

HO is a cytoprotective enzyme, in response to oxidative injury, whose main function is inherently associated with the degradation of oxidative heme to biliverdin, free iron, and carbon monoxide (Kikuchi *et al.*, 2005). Subsequently, biliverdin is rapidly metabolized into bilirubin by cytosolic biliverdin reductase, and free iron is sequestered by ferritin (Kikuchi *et al.*, 2005). Three isoforms of HO have been identified as referred to HO-1, HO-2 and HO-3. Although HO-2 and HO-3 are constitutively expressed, HO-1 is inducible in many cell types, including human endothelial cells (Balla *et al.*, 2005). Numerous studies have shown that HO-1 has cytoprotective and anti-inflammatory properties (Choi *et al.*, 2003; Pae *et al.*, 2004; Pae *et al.*, 2005; Pae *et al.*, 2006) and, interestingly, its expression mediates cytoprotective and anti-inflammatory effects of naturally occurring antioxidants such as curcumin (Motterlini *et al.*, 2000) and quercetin (Chow *et al.*, 2005).

When the blood vessel is injured, in many cases, by oxidative stress, a variety of pro-inflammatory mediators and growth factors are produced mainly by injured/activated endothelial cells, and stimulate vascular smooth muscle cells to migrate into the intimal layer and expand, eventually followed by endothelial dysfunction (Yu and Lyons, 2005). Recent studies have shown that HO-1 expression protects endothelial cells from oxidative injury and reduces the production of pro-inflammatory mediators by endothelial cells (Balla *et al.*, 2005), suggesting that endothelial HO-1 has roles in cytoprotection as well as in the regulation of inflammation. In addition, human HO-1 deficiency results in severe persistent endothelial damage (Kawashima *et al.*, 2002). Thus, genetic or pharmacological induction of endothelial HO-1 would be effective for the prevention and treatment of several endothelial diseases caused by oxidative damages.

In the present study, we evaluated the comparative effects of curcumin and its two naturally occurring derivatives, DMC and BDMC, on endothelial HO-1 expression. In addition, we investigated whether the cytoprotective activities of curcuminoids could be associated with their ability to induce endothelial HO-1 expression.

Materials and Methods

Materials

3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazoliumb romide (MTT) was purchased from Sigma (St. Louis, MO), and antibodies against HO-1 and actin from Santa Cruz Biotechnology (Santa Cruz, CA). Cobalt protoporphyrin (CoPP), hemin and tin protoporphyrin (SnPP) were obtained from Porphyrin Products (Logan, UT). Other chemicals were purchased from Sigma.

Isolation and purification of curcumin and its two derivatives

Dried and pulverized rhizome of *Curcuma longa* (600 g), which was purchased from the University Oriental herbal drugstore in Korea, was extracted twice with ethyl alcohol (2 L) for 24 h at room temperature to obtain an extract (51.63 g). The extract was dissolved in 60% aqueous methanol (1 L) and partitioned with n-hexane (1 L $\times$ 3), followed by $CHCl_3$ (1 L $\times$ 3). The 60% aqueous methanol layer was then evaporated in vacuum, and the resulting residue was dissolved in H_2O and successively partitioned with normal butanol (1 L $\times$ 3). A portion (10 g) of the bioactive $CHCl_3$ soluble extract was subjected to silica gel column chromatography eluting with $CHCl_3$-CH_3OH (80:1-16:1, step gradient) to yield compound 1 (2.21 g), compound 2 (0.34 g) and compound 3 (1.30 g). The compounds were identified as curcumin, demethoxycurcumin (DMC) and bis-demethoxycurcumin (BDMC), respectively, by comparison of mass spectroscopy, ^{1}H-NMR, and ^{13}C-NMR data with those in the literature (Song *et al.*, 2001).

Endothelial cell culture

Human endothelial cells (ECV304) were cultured in 75-cm^2 culture flasks and maintained in Ham's F12K medium (Sigma). Cell medium was supplemented with 10% fetal bovine serum, 1% penicillin-streptomycin solution, 30 μg/ml of endothelial cell growth supplement and 100 μg/ml of heparin (Sigma).

MTT assay for cell viability

Endothelial cells were plated at a density of 4 $\times$ 10^4 cells/300 μl in 48-well plates and the cell viability was determined by the conventional MTT reduction assay. MTT is tetrazolium salt cleaved to formazan by the mitochondrial respiratory chain enzyme suc-

cinate dehydrogenase. After incubation, cells were treated with the MTT solution (final concentration, 1 mg/ml) for 2 h. The dark blue formazan crystals formed in intact cells were solubilized with dimethyl sulfoxide and the absorbance at 570 nm was measured with a microplate reader.

Western blotting analysis for HO-1 expression

After treatment, endothelial cells (3×10^6 cells/3 ml in 60 mm dish) were collected and washed with phosphate-buffered saline (PBS). After centrifugation, cell lysis was carried out at 4°C by vigorous shaking for 15 min in RIPA buffer [150 mM NaCl, 1% NP-40, 0.5% sodium deoxycholate, 0.1% SDS, 50 mM Tris-HCl (pH 7.4), 50 mM glycerophosphate, 20 mM NaF, 20 mM EGTA, 1 mM DTT, 1 mM Na_3VO_4 and protease inhibitors]. After centrifugation at $14,800 \times g$ for 15 min, the supernatant was separated and stored at -70°C until use. The protein concentration was determined by using the BCA protein assay kit (Pierce, Rockford, IL). After addition of sample loading buffer, protein samples were electrophoresed on a 12.5% SDS-polyacrylamide gel. Proteins were transferred to polyvinylidene difluoride blots at 300 mA for 6 h. The blots were blocked for 1 h at room temperature in fresh blocking buffer (0.1% Tween 20 in Tris-buffered saline, pH 7.4, containing 5% non-fat dried milk). Dilutions (1:1000) of primary antibodies were made in PBS with 3% non-fat dried milk. Following three washes with PBST (PBS and 0.1% Tween 20), the blots were incubated with horseradish peroxidase-conjugated secondary antibodies (Santa Cruz Biotechnology) in PBS with 3% non-fat dried milk for 1 h at room temperature. The blots were washed again three times in PBST buffer, and transferred proteins were incubated with ECL substrate solution (Amersham Pharmacia Biotech, Piscataway, NJ) for 1 min according to the manufacturer's instructions followed by visualization with X-ray film.

HO activity assay

Endothelial cells (2.5×10^6 cells) were treated with vehicle, CoPP (positive control) or curcuminoids for 6 h. Cell pellets were re-suspended in magnesium supplemented potassium phosphate solution [MgCl-PBS; 100 mM potassium phosphate and 2 mM magnesium chloride; pH 7.4] and subjected to three freeze-thaw cycles for release of cytoplasmic HO-1 proteins. HO-1 enzymatic assay used a reaction mixture containing 100 mM PBS, 2 mM $MgCl_2$, 3 mg of rat liver cytosol, 0.8 mM NADPH, 2 mM glucose-6-phosphate (Sigma), 0.2 U of glucose-6-phosphate dehydrogenase and 20 μM enzyme substrate hemin (Porphyrin Products) plus 400 μl of sample. The reaction was made up to a final volume of 1 ml for each sample and was incubated at 37°C for 1 h in the dark. Chloroform was added to terminate the reaction, and bilirubin was extracted following centrifugation, and measured by spectrophotometry reading the difference in absorbance between 464 and 530 nm ($\varepsilon = 40\ mM^{-1}cm^{-1}$). The protein concentration in each sample was determined with a Bradford protein assay, and the HO-1 activities were expressed in picomoles of bilirubin formed per milligram of protein per hour.

Statistical analysis

Data are expressed as mean ± S.E.M.. Statistical analysis in this study includes ANOVA and the *Post-Hoc* group comparisons after Bonferroni and Scheffé-procedures. The P values < 0.05 were considered as significant.

Results

Effects of curcuminoids on HO-1 expression and HO activity

Based on the fact that HO-1 is highly inducible in endothelial cells treated with curcumin (Motterlini *et al.*, 2000), we wondered whether other curcumin derivatives, DMC and BDMC, would also affect the

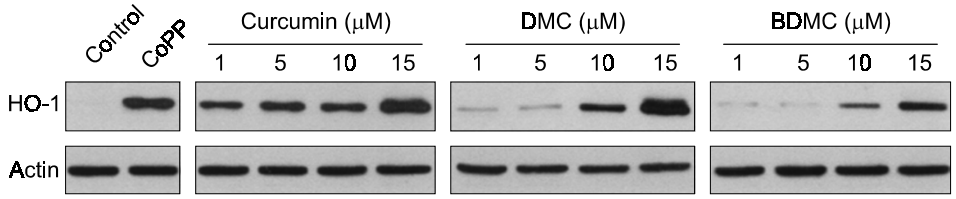


Figure 2. HO-1 expression in endothelial cells exposed to curcuminoids. Cells were incubated with various indicated constituents of curcuminoids or the HO-1 inducer CoPP (20 μM) for 6 h, and HO-1 protein expression was evaluated using anti-HO-1 antibody as described under Materials and Methods. Western blot represents three independent experiments.

expression of HO-1 in human endothelial cells. We found that pure curcumin, DMC, and BDMC all significantly increased HO-1 expression after 6 h of incubation (Figure 2). However, despite displaying a similar basic chemical structure, three compounds affected the pattern of HO-1 protein inducibility in a different fashion. For example, removal of one methoxy group from the molecule of curcumin, as in DMC, affected HO-1 expression slightly. Removal of both methoxy groups, as in BDMC, significantly decreased HO-1 expression. Consistent with these results, HO activity also differed for each curcuminoid tested in this study (Figure 3); the order being curcumin > DMC > BDMC. It has to be noted that HO activity and HO-1 expression were analyzed in non-cytotoxic concentrations of curcuminoids.

Effects of curcuminoids on endothelial cell injury caused by H_2O_2

It has been reported that curcumin protects endothelial cells against oxidative injury (Motterlini *et al.*, 2000; Scapagnini *et al.*, 2002; Foresti *et al.*, 2005). We therefore examined whether DMC and BDMC could also be cytoprotective against oxidative injury in endothelial cells. Treatment of endothelial cells with H_2O_2 resulted in marked decrease in cell viability (Figure 4). However, pre-incubation of the cells for 6 h with curcumin, DMC or BDMC significantly attenuated H_2O_2-mediated damage and restored cell viability, notably, in a different fashion. The activity at 10 μM was found to be highest with curcumin, followed by DMC and BDMC.

Role of endothelial HO-1 in curcuminoid-induced cytoprotection

Previous studies have shown that endothelial HO-1 provides cytoprotection against oxidative injury (Motterlini *et al.*, 2000; Scapagnini *et al.*, 2002; Foresti *et al.*, 2005). Thus, we tested whether curcuminoid-induced HO-1 expression could facilitate cytoprotective effect. Endothelial cells were initially pretreated with 10 μM of each curcuminoid for 6 h in

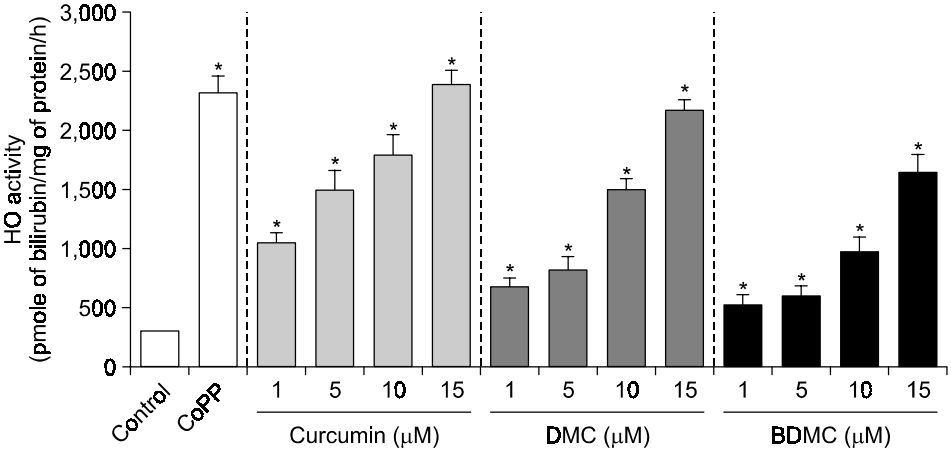


Figure 3. Effects of curcuminoids on HO activity in endothelial cells. HO activity was measured in endothelial cells 6 h after exposure to indicated concentrations of curcuminoids or the HO-1 inducer CoPP (20 μM). In the control group, cells were incubated with medium alone. Each bar represents the mean ± S.E.M. of five to six independent experiments. *, $P < 0.05$ versus control.

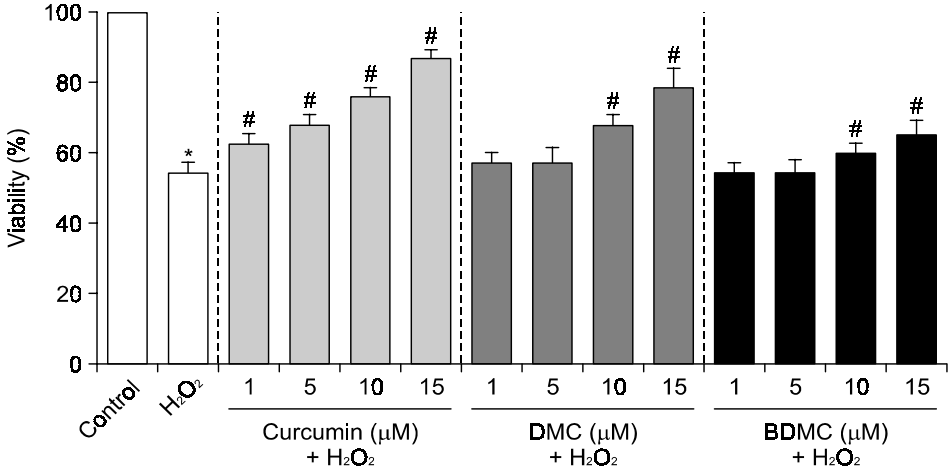


Figure 4. Protective effects of curcuminoids against oxidative stress. Endothelial cells were initially pre-incubated for 6 h in the presence of indicated concentrations of curcuminoids. Then the medium was removed, and cells were exposed to 1 mM H_2O_2 for 2 h. Cell viability was measured spectrophotometrically using MTT assay. Data are expressed as the mean ± S.E.M. of five independent experiments. *, $P < 0.05$ versus control and #, $P < 0.05$ versus H_2O_2.

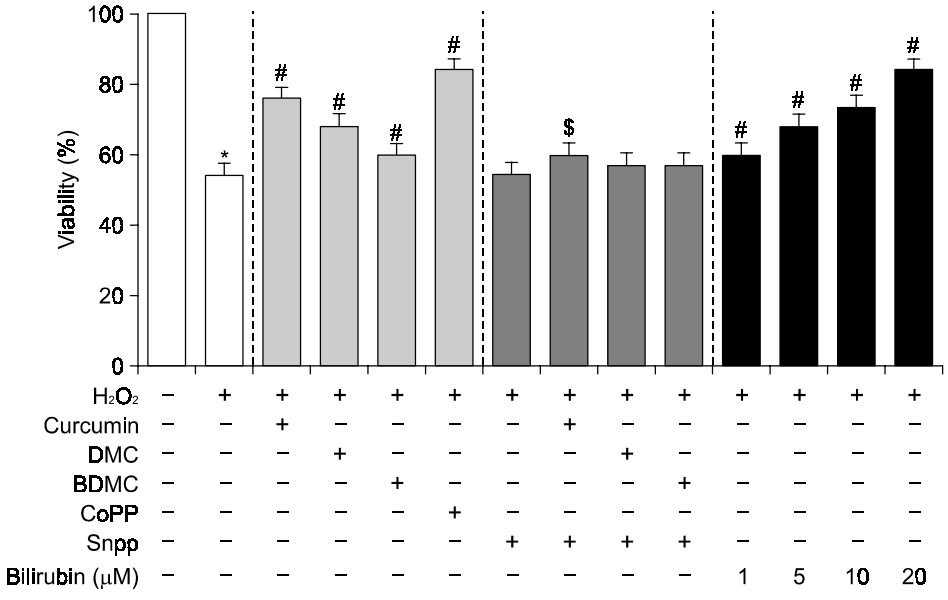


Figure 5. Effects of curcuminoid-induced HO-1 and bilirubin on H_2O_2-mediated cellular injury. Endothelial cells were exposed for 6 h to 10 μM curcuminoids, 20 μM CoPP or indicated concentrations of bilirubin in the presence or absence of 50 μM SnPP. After this pretreatment, cells were challenged for 2 h with 1mM H_2O_2. After this treatment, cells were washed and viability was assessed using MTT assay. Each bar represents the mean ± S.E.M. of 6 independent experiments. *, $P < 0.05$ versus control; #, $P < 0.05$ versus H_2O_2; $, $P < 0.05$ versus same treatment plus SnPP.

the absence or presence of SnPP, an inhibitor of HO activity. The cells were then exposed to hydrogen peroxide for 2 h followed by an assessment of cell viability. As shown in Figure 5, SnPP significantly reversed the protective effect afforded by curcuminoids. In addition, protection comparable to the potent cytoprotective curcumin was observed when the cells were pre-incubated with the HO-1 inducer CoPP or bilirubin, one of enzymatic products of HO-1 (Figure 5). Other products, carbon monoxide and Fe^{2+}, did not provide a notable cytoprotection (data not shown).

Signaling pathway(s) of curcuminoid-induced endothelial HO-1 expression

The activation of intracellular signaling pathway was important for HO-1 induction. Generally, HO-1 expression is induced by stimuli that activate the mitogen-activated protein kinases (MAPKs) (Iles *et al.*, 2005; Chen *et al.*, 2006). Three major subgroups of the MAPK family have been identified to include extracellular signal-regulated kinase 1/2 (ERK1/2), c-Jun NH_2- terminal kinase (JNK) and p38 MAPK. Depending on the stimuli specificity, contradictory results on the regulatory role of different MAPK pathways for HO-1 expression were observed (Iles *et al.*, 2005). In the case of curcumin, the activation of p38 MAPK pathway was found to be involved in HO-1 expression (Balogun *et al.*, 2003). To investigate the signal transduction pathway(s) involved in regulating HO-1 expression in response to curcuminoids, we examined the effects of three pharmacological inhibitors of signaling intermediates on HO-1 protein levels. As shown in Figure 6, we found that treatment of endothelial cells with p38 MAPK inhibitor (SB203580) reduced curcuminoid-induced HO-1 expression. Neither JNK inhibitor (SP600125) nor MAP/ERK kinase inhibitor (U0126) had a significant effect.

Discussion

The potent antioxidant curcumin has been considered as therapeutic agents for various pathologic conditions, including cancer, viral infection, inflammation, hypertension, and atherosclerosis (Duvoix *et al.*, 2005). Based on the fact that the cytoprotective HO-1 is highly inducible in endothelial cells treated with curcumin (Motterlini *et al.*, 2000), we aimed to investigate whether other curcumin derivatives present in turmeric would also affect the expression of endothelial HO-1 and to test whether HO-1 expression by these compounds would facilitate their cytoprotective effects.

Michael reaction acceptors (olefins or acetylenes conjugated to electron-withdrawing groups) constitute a major class of phase-II enzyme inducers, and their inducer potency was closely correlated with their reactivities with nucleophiles in the Michael reaction (Prestera *et al.*, 1995). Not surprisingly, curcumin and its two derivatives, which contain two Michael reaction acceptors (α, β-unsaturated carbonyl group) in their own molecules, induced the phase-II enzyme HO-1 in endothelial cells (Dinkova-Kostova and Talalay, 1999). However, our results clearly show that the pattern of HO-1 protein inducibility differed for each curcuminoid tested in this study (Figure 2), indicating that even subtle

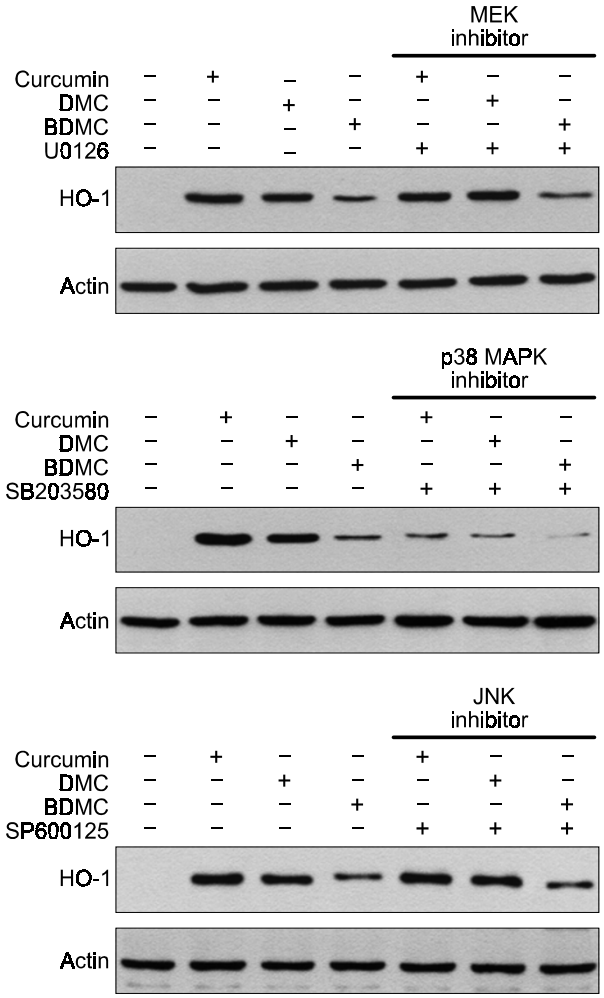


Figure 6. Cucuminoid-induced HO-1 expression mediated by p38 MAPK. Endothelial cells were pretreated with the MEK inhibitor U0126 (10 μM), a p38 MAPK inhibitor SB203580 (20 μM), and a JNK inhibitor SP600125 (25 μM) followed by 10 μM curcuminoids for 6 h. Western blot analysis for HO-1 expression was then performed. Data represent three independent experiments.

changes of the chemical structure can significantly affect the potency of curcuminoids to enhance endothelial HO-1 expression and HO activity. In fact, the main difference among the three curcuminoids is the number of methoxy groups present (none for BDMC, one for DMC, and two for curcumin; see chemical structure in Figure 1). The presence of methoxy groups in the *ortho* position on the aromatic ring is already known to noticeably enhance the bioactivities of curcuminoids (Motohashi *et al.*, 2000; Skrzypczak-Jankun *et al.*, 2003). Indeed, all three forms of curcuminoids could induce endothelial HO-1 expression; however, we found that curcumin with two *ortho* methoxy groups was the most active form among the curcuminoids present in turmeric (Figure 2). The rank order of HO activity at 10 μM is curcumin, DMC and BDMC (Figure 3). Interestingly, cytoprotective capacities of curcuminoids against oxidative injury caused by H_2O_2 exposure were in the order: curcumin > DMC > BDMC (Figure 4), which is the same as the order of HO activity. Based on these findings, it is suggested that the presence of methoxy groups in the *ortho* position on the aromatic ring of curcuminoids is essential to enhance endothelial HO-1 expression and cytoprotection in human endothelial cells.

Of interest is our finding that, in our experimental setting, the observed cytoprotective properties of curcuminoids were considerably linked to their ability to affect the levels of HO-1 expression and HO activity. Pre-incubation of human endothelial cells with curcuminoids resulted in enhanced resistance to oxidative injury; this effect was partly attributable to HO-1 expression, as the inhibitor of HO activity (SnPP) significantly reduced cytoprotective activities of curcuminoids (Figure 5). Moreover, the HO-1 inducer CoPP mimicked the cytoprotective effect of curcumin (Figure 5), which is further supporting recent reports showing that HO-1 expression confers a cytoprotection upon endothelial cells (Motterlini *et al.*, 2000).

The heme degradation enzyme, HO-1, has been proposed to have roles in cytoprotection and in the regulation of endothelial inflammation (Balla *et al.*, 2005). The molecular basis of the cytoprotective/anti-inflammatory action of endothelial HO-1 remains to be fully elucidated, but the products of heme degradation such as carbon monoxide and bilirubin seem to be responsible for the cytoprotective/anti-inflammatory effect attributed to HO-1. In our study, we showed that bilirubin, produced by reduction of biliverdin, was responsible for cytoprotective action of HO-1 (Figure 5), further supporting our conclusion that endothelial cytoprotection afforded by curcuminoids is mediated via HO-1 expression. Although we have shown that HO-1 expression by curcuminoids is cytoprotective in human endothelial cells, we do not exclude the possibility that these compounds will stimulate the expression of other defense enzymes, as is already known as inducers of other cytoprotective enzymes (Commandeur and Vermeulen, 1996), and that cytoprotective activity will be achieved via the concerted action of the multiple pathways that may be activated by curcuminoids.

The flavonoid-induced signaling mechanisms involved in HO-1 expression are poorly understood. Several studies have focused on the activation of the MAPKs that contribute to the induction of HO-1 (Iles *et al.*, 2005; Chen *et al.*, 2006), and report that the flavonoid curcumin induces HO-1 expression via the activation of p38 MAPK pathway (Balogun *et al.*,

2003). For these reasons, we used specific pharmacological inhibitors to test signaling pathways in order to elucidate the important components in curcuminoid-mediated endothelial HO-1 expression. Our results showed that DMC and BDMC, as previously reported for curcumin (Balogun *et al*., 2003), induced p38 MAPK-dependent HO-1 expression (Figure 6).

In conclusion, we report, for the first time, that the presence of methoxy groups in the *ortho* position on the aromatic ring of curcuminoids is essential to enhance endothelial HO-1 expression and that HO-1 expression by curcuminoids is cytoprotective against oxidative injury. Our results may be useful to design more efficacious HO-1 inducers which could be considered as promising pharmacological agents in the development of therapeutic approaches for the prevention or treatment of endothelial diseases caused by oxidative damages.

Acknowledgement

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References

Balla J, Vercellotti GM, Jeney V, Yachie A, Varga Z, Eaton JW, Balla G. Heme, heme oxygenase and ferritin in vascular endothelial cell injury. Mol Nutr Food Res 2005;49:1030-43

Balogun E, Hoque M, Gong P, Killeen E, Green CJ, Foresti R, Alam J, Motterlini R. Curcumin activates the heme oxygenase-1 gene via regulation of Nrf2 and the antioxidant- responsive element. Biochem J 2003;371:887-95

Chainani-Wu N. Safety and anti-inflammatory activity of curcumin: a component of tumeric (*Curcuma longa*). J Altern Complement Med 2003;9:161-8

Chen A, Xu J, Johnson AC. Curcumin inhibits human colon cancer cell growth by suppressing gene expression of epidermal growth factor receptor through reducing the activity of the transcription factor Egr-1. Oncogene 2006;25:278-87

Chen JC, Huang KC, Lin WW. HMG-CoA reductase inhibitors upregulate heme oxygenase-1 expression in murine RAW264.7 macrophages via ERK, p38 MAPK and protein kinase G pathways. Cell Signal 2006;18:32-9

Choi BM, Pae HO, Kim YM, Chung HT. Nitric oxide-mediated cytoprotection of hepatocytes from glucose deprivation-induced cytotoxicity: involvement of heme oxygenase-1. Hepatology 2003;37:810-23

Chow JM, Shen SC, Huan SK, Lin HY, Chen YC. Quercetin, but not rutin and quercitrin, prevention of H_2O_2-induced apoptosis via anti-oxidant activity and heme oxygenase 1 gene expression in macrophages. Biochem Pharmacol 2005;69: 1839-51

Commandeur JN, Vermeulen NP. Cytotoxicity and cytoprotective activities of natural compounds. The case of curcumin. Xenobiotica 1996;26:667-80

Dicato M, Diederich M. Chemopreventive and therapeutic effects of curcumin. Cancer Lett 2005;223:181-90

Dinkova-Kostova AT, Talalay P. Relation of structure of curcumin analogs to their potencies as inducers of Phase 2 detoxification enzymes. Carcinogenesis 1999;20:911-4

Duvoix A, Blasius R, Delhalle S, Schnekenburger M, Morceau F, Henry E, Dicato M, Diederich M. Chemopreventive and therapeutic effects of curcumin. Cancer Lett 2005;223:181-90

Foresti R, Hoque M, Monti D, Green CJ, Motterlini R. Differential activation of heme oxygenase-1 by chalcones and rosolic acid in endothelial cells. J Pharmacol Exp Ther 2005;312:686-93

Iles KE, Dickinson DA, Wigley AF, Welty NE, Blank V, Forman HJ. HNE increases HO-1 through activation of the ERK pathway in pulmonary epithelial cells. Free Radic Biol Med 2005;39:355-64

Joe B, Vijaykumar M, Lokesh BR. Biological properties of curcumin-cellular and molecular mechanisms of action. Crit Rev Food Sci Nutr 2004;44:97-111

Kawashima A, Oda Y, Yachie A, Koizumi S, Nakanishi I. Heme oxygenase-1 deficiency: the first autopsy case. Hum Pathol 2002;33:125-30

Kikuchi G, Yoshida T, Noguchi M. Heme oxygenase and heme degradation. Biochem Biophys Res Commun 2005;338: 558-67

Motohashi N, Yamagami C, Tokuda H, Okuda Y, Ichiishi E, Mukainaka T, Nishino H, Saito Y. Structure-activity relationship in potentially anti-tumor promoting benzalacetone derivatives, as assayed by the epstein-barr virus early antigen activation. Mutat Res 2000;464:247-54

Motterlini R, Foresti R, Bassi R, Green CJ. Curcumin, an antioxidant and anti-inflammatory agent, induces heme oxygenase-1 and protects endothelial cells against oxidative stress. Free Radic Biol Med 2000;28:1303-12

Pae HO, Choi BM, Oh GS, Lee MS, Ryu DG, Rhew HY, Kim YM, Chung HT. Roles of heme oxygenase-1 in the antiproliferative and antiapoptotic effects of nitric oxide on Jurkat T cells. Mol Pharmacol 2004;66:122-8

Pae HO, Oh GS, Choi BM, Kim YM, Chung HT. A molecular cascade showing nitric oxide-heme oxygenase-1-vascular endothelial growth factor-interleukin-8 sequence in human endothelial cells. Endocrinology 2005;146:2229-38

Pae HO, Oh GS, Jeong SO, Jeong GS, Lee BS, Choi BM, Lee HS, Chung HT. 1,2,3,4,6-penta-O-galloyl-beta-D-glucose up-regulates heme oxygenase-1 expression by stimulating Nrf2 nuclear translocation in an extracellular signal-regulated kinase-dependent manner in HepG2 cells. World J Gastroenterol 2006;12:214-21

Prestera T, Talalay P, Alam J, Ahn YI, Lee PJ, Choi AM. Parallel induction of heme oxygenase-1 and chemoprotective phase 2 enzymes by electrophiles and antioxidants: regulation by upstream antioxidant-responsive elements (ARE). Mol Med 1995;1:827-37

Scapagnini G, Foresti R, Calabrese V, Giuffrida Stella AM, Green CJ, Motterlini R. Caffeic acid phenethyl ester and curcumin: a novel class of heme oxygenase-1 inducers. Mol Pharmacol 2002;61:554-61

Selvam C, Jachak SM, Thilagavathi R, Chakraborti AK. Design, synthesis, biological evaluation and molecular docking of curcumin analogues as antioxidant, cyclooxygenase inhibitory and anti-inflammatory agents. Bioorg Med Chem Lett 2005;15:1793-7

Skrzypczak-Jankun E, Zhou K, McCabe NP, Selman SH, Jankun J. Structure of curcumin in complex with lipoxygenase and its significance in cancer. Int J Mol Med 2003;12:17-24

Song EK, Cho H, Kim JS, Kim NY, An NH, Kim JA, Lee SH, Kim YC. Diarylheptanoids with free radical scavenging and hepatoprotective activity *in vitro* from Curcuma longa. Planta Med 2001;67:876-7

Yu Y, Lyons TJ. A lethal tetrad in diabetes: hyperglycemia, dyslipidemia, oxidative stress, and endothelial dysfunction. Am J Med Sci 2005;330:227-32