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# Induction of Cytotoxicity and Apoptosis and Inhibition of Cyclooxygenase-2 Gene Expression, by Curcumin and its Analog, α-Diisoeugenol

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**Abstract.** *Cytotoxicity, induction of apoptosis, ROS generation and inhibition of cyclooxygenase-2 (COX-2) gene expression by curcumin **[1]** and α-diisoeugenol **[2]** were investigated. The cytotoxicity of curcumin and α-diisoeugenol against human promyelocytic leukemia cells (HL-60 cells) and human submandibular cancer cells (HSG cells) was similar ($CC_{50}$ 1-3 μM). However, curcumin induced much more apoptosis, particularly in HL-60 cells compared with HSG cells, as revealed by measurement of the sub-$G_1/G_0$ DNA fraction in flow cytometric histograms. Treatment with 15 μM curcumin increased the number of cells with a sub-$G_1/G_0$ DNA fraction from control levels of <5% to 55% in HL-60 cells and 30% in HSG cells. Flow cytometry, after staining with annexin V-FITC/PI (the exposure of phosphatidylserine (PS) on the surface of apoptotic cells), showed a dose-dependent induction of early apoptosis by curcumin, which reached about 65% in HL-60 cells and about 20% in HSG cells after treatment with 10 μM curcumin. In contrast, α-diisoeugenol failed to induce apoptosis in either cell type. For both cell types, the proportion of late apoptotic/necrotic cells increased rapidly at concentrations of curcumin and α-diisoeugenol greater than 10μM. The generation of intracellular reactive oxygen species (ROS) in curcumin-treated HL-60 cells was greater than that in HSG cells, as judged by CDFH-DA staining. In both cell types, ROS generation by α-diisoeugenol was at control levels. ROS generation by curcumin was suppressed by antioxidants such as N-acetyl-L-cysteine (NAC) and glutathione (GSH) and by scavengers of hydroxy radicals such as mannitol, but, conversely, was promoted by prooxidants such as the transition metal ions Cu(II) and Zn(II). ROS generation may play a part in the exposure of PS. Curcumin, but not α-diisoeugenol, at 10 μM inhibited LPS (lipopolysaccharide)-induced COX-2 gene expression in RAW 264.7 cells. Semiempirical PM 3 calculations suggested that this activity of curcumin, in which it behaves as a non-steroidal anti-inflammatory drug (NSAID)-like compound, is dependent on its phenolic function, which is more pronounced than that of α-diisoeugenol. Taken together, our results suggest that the bioactivity of curcumin is a result of its ability to act as both a prooxidant and an antioxidant.*

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*Key Words:* Curcumin, α-diisoeugenol, apoptosis, COX-2 inhibition.

Curcumin, 1,7-bis (4-hydroxy-3-methoxyphenol)-1,6-heptadiene-3,5-dione **[1]**, obtained from the rhizome of the plant *Curcumin longa*, is a naturally occurring pigment and a component of the spice called turmeric. Turmeric is used as a colorant, food preservative and dye. Currently, curcumin is of interest as a chemopreventive agent because of its potent antioxidant, anti-inflammatory and anticancer activity (1). We previously reported that curcumin and α-diisoeugenol (r-l-ethyl-5-hydroxy-t-3-(4-hydroxy-3-methoxyphenyl)-6-methoxy-c-2-methylindane) **[2]**, a curcumin analog, show similar antioxidant activity (2). Numerous earlier studies have reported the induction of apoptosis by curcumin in HL-60 cells (3) and other cancer cells (4, 5), but the effects of curcumin on oral carcinoma cells, such as the human salivary gland carcinoma cell line (HSG cells), remain unclear (6). Apoptosis is frequently accompanied by the generation of ROS (7, 8), yet a specific role for ROS in the execution or resolution of the apoptotic program has not been established. We previously reported that curcumin generates ROS and preferentially scavenges peroxide radicals (6). In the present study we compared cytotoxicity, apoptosis induction and ROS generation by curcumin and α-diisoeugenol in HL-60 and HSG cells. The induction of apoptosis was investigated by staining with propidium iodide (PI) for DNA quantification and by flow cytometry after dual staining with annexin V-FITC and PI. ROS generation was investigated with 5- (and 6-)-carboxy-2',7'-dichlorofluorescein diacetate (CDFH-DA).

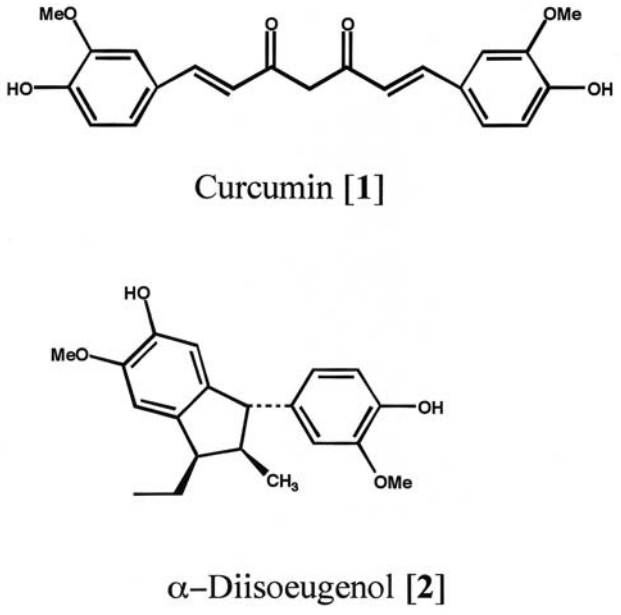


Figure 1. *Chemical structures of curcumin **[1]** and α-diisoeugenol **[2]**.*

Table I. *Cytotoxicity of curcumin and α-diisoeugenol against HL-60 cells and HSG cells.*

| Compounds | Cells | $CC_{50}$ (mM) |
|---|---|---|
| Curcumin | HL-60 | 0.0017±0.0004 |
| Curcumin | HSG | 0.0031±0.0001 |
| α-diisoeugenol | HL-60 | 0.0009±0.0001 |
| α-diisoeugenol | HSG | 0.0027±0.0002 |

HSG cells ($4x10^4$/ml of serum-free medium) were incubated with a 0.1 μM to 100 mM concentration of each compound in the wells for 24 hours and viable cell number was determined by MTT methods. HL-60 cells ($4x10^4$/ml containing 1% FBS) were incubated with each compound for 24 hours, and viable cell number was determined by trypan blue dye exclusion. The $CC_{50}$ values are determined from dose-response curves. Mean±SD are measured by 8 independent determinations. There is a significant difference in the cytotoxicity of curcumin or α-diisoeugenol between HSG and HL-60 cells.

Since recent reports have described the role of COX-2 and prostaglandins in apoptosis, particularly of cancer cells (9, 10), we also questioned whether the apoptosis-inducing activity of curcumin-related compounds is related to COX-2 inhibition. Thus, Northern blot analysis was used to investigate the regulatory effects of curcumin and α-diisoeugenol on LPS-induced COX-2 gene expression in RAW 264.7 cells. The biological activities of curcumin and α-diisoeugenol are discussed on the basis of their chemical properties, as calculated by the PM 3 semiempirical method.

## Materials and Methods

*Reagents.* Curcumin (1,7-bis(4-hydroxy-3-methoxyphenol)-1,6-heptadiene-3,5-dione) was obtained from Tokyo Kasei Chem. Co. (Tokyo, Japan) and used without further purification. α-Diisoeugenol was synthesized as previously described (11). Their chemical structures are shown in Figure 1. The following chemicals and reagents were obtained from the indicated companies: Eagle's minimum essential medium alpha modification (α-MEM) and RPMI 1640 medium (Sigma Chemical Co., St. Louis, MO, USA); fetal bovine serum (FBS) (JRH Biosciences, Lenexa, KS, USA); 5-(and -6)-carboxy-2',7'-dichlorofluorescein diacetate (CDFH-DA) (Molecular Probes Inc., Eugene, OR, USA); MTT [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide] and annexin V-FITC/PI kit (Promega Co., Madison, WI, USA); *N*-acetyl-L-cysteine (NAC), glutathione (GSH), bathocuproine and *o*-phenanthrolene (Wako Pure Industries, Ltd., Osaka, Japan); phenylmethylsulfonylfluoride (PMSF), riboflavin, nitro blue tetrazolium (NBT), catalase from bovine liver, superoxide dismutase (SOD) from bovine erythrocytes, mannitol and sodium azide ($NaN_3$) (Sigma Chemical Co.); dimethyl sulfoxide (DMSO), RNase A and proteinase K (Boehringer, Mannheim, Germany); Megaprime DNA labeling system and 5-[α-$^{32}$P]dCTP (Amersham Biosciences Co., Piscataway, NJ, USA); *Escherichia coli* O111 B4-derived LPS (List Biological Laboratories, Inc., Campbell, CA, USA); RAW 264.7 cells and HL-60 cells (Riken Cell Bank, Saitama, Japan); HSG cells (a gift from Professor Dr. M. Satoh, Tokushima University, Japan (12)); COX-2 cDNA probes (Cayman Chemical Co., Ann Arbor, MI, USA); plasmid containing β-actin cDNA (Japanese Cancer Research Bank, Tokyo, Japan).

*Cells and cell culture.* The HSG cells were cultured in MEM supplemented with 10% FBS. The HL-60 cells were cultured in RPMI 1640 medium supplemented with 10% FBS. Cells of the murine macrophage cell line RAW 264.7 were cultured to the subconfluent state in RPMI 1640 medium supplemented with 10% FBS at 37˚C under 5% $CO_2$ in air, washed and then incubated overnight in serum-free RPMI 1640. They were then washed further and treated with test samples.

*Assay for cytotoxic activity.* Near-confluent HL-60 cells were incubated for 24 hours without (control) or with various concentrations of curcumin or α-diisoeugenol. The numbers of viable HL-60 cells were determined by trypan blue exclusion. HSG cells were cultured in MEM supplemented with 10% FBS. The number of viable HSG cells was determined by the MTT colorimetric assay (13).

*Cell cycle analysis.* HSG cells were seeded at $5x10^4$ cells/2 ml into 6-well plates and cultured for 2 days to reach semiconfluence. Then, curcumin or α-diisoeugenol, at concentrations of 0, 10 or 15 μM, were added to the cells in serum-free medium and the cells were cultured for 16 hours. HL-60 cells were treated likewise except that medium containing 1% serum was used. The cells were trypsinized, harvested, washed and incubated in low tonicity solution (50 μg/ml PI, 0.25 mg/ml RNase, 0.1% sodium citrate, 0.2% NP-40) for 30 minutes at 4˚C, followed by incubation at 37˚C for 30 minutes. Fluorescence emitted from the PI-DNA

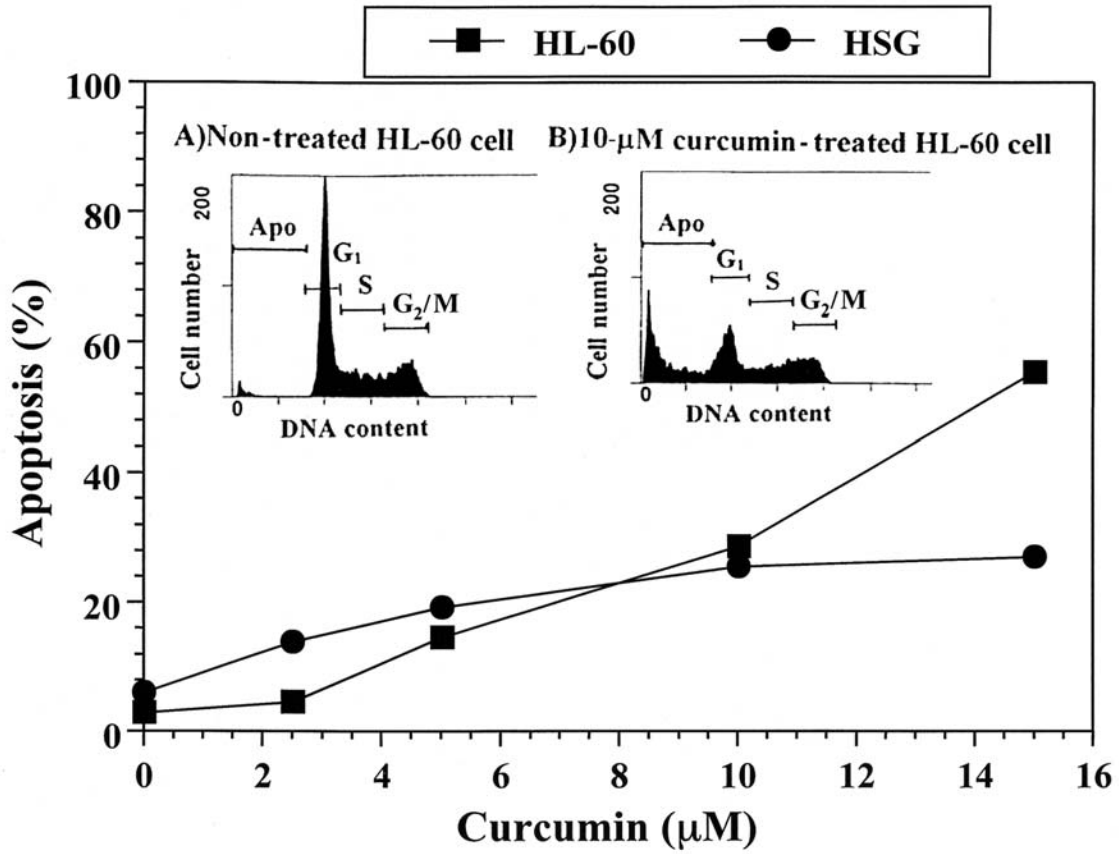


Figure 2. *Apoptotic cell number of curcumin-treated HL-60 and HSG cells estimated by DNA fragmentation. The cells were incubated with curcumin (0-15 μM) in serum-free medium, except for HL-60 cells (1% FBS), for 16 hours. Harvested cells were stained with PI as described in Materials and Methods. At least 5,000 cells were determined by use of a flow cytometer (Beckman Epics), with fluorescence intensity of PI-DNA at approximately 600 nm. A and B (inset in the figure) show DNA fluorescence histograms of curcumin-treated PI-stained HL-60 cells (A, control; B, 10 μM curcumin as an example). The bars (−) show Apo (DNA fragmentation, sub-$G_1/G_0$ peak), $G_1$, S and $G_2$/M, respectively. Apoptotic cell number (%) was calculated by FACS analysis. The figure, representing the % of apoptotic cells induced by 16-hour treatment of HL-60 (■) and HSG (●) cells with curcumin, was prepared by calculations made from the PI histograms (inset). Data are the mean of 3 independent experiments. Standard errors <15%. There is a significant difference between HL-60 cells and HSG cells at 15 μM curcumin (**p<0.01). In contrast, there is no significant difference between HL-60 cells and HSG cells at 0 or 10 μM curcumin.*

complex was quantified for 5,000 cells per sample and the data were gated on pulse-processed PI signals to exclude doublets and larger aggregates. The linear red fluorescence (approximately 600 nm) histogram with 256-channel resolution was analyzed. At least three experiments were performed. The data were analyzed with a FACScan flow cytometer (EPICS ALTRA, Beckman Coulter, Miami, FL, USA) with Multicycle WinCycle software.

*Annexin V-FITC/PI assay.* Annexin V-FITC binds with high affinity to PS residures (14), and PS is exposed during early apoptosis by a flip from the inner to the outer plasma membrane leaflet (15, 16). Furthermore, PI conjugates to necrotic cells. HSG cells ($1\text{-}2\text{x}10^5$) were cultured for 2 days in their medium to reach semiconfluence. The cells were washed with serum-free medium 60 minutes before the addition of each test compound at concentrations of 0, 2.5, 5, 10, 15, or 50 μM in serum-free medium, and incubation was continued for the desired times. Then, the cells were detached with trypsin solution and washed with FBS. The cells were stained with annexin V-FITC/PI for 10 minutes in the dark, according to the instructions in the manufacturer's kit. Stained cells were analyzed by using the flow cytometer mentioned above. The quadrant setting were based on cells in the control sample and the positive control [annexin V-FITC/PI-stained cells treated with actinomycin D (2 mg/ml) for 5 h].

*cDNA hybridization probes.* The COX-2 cDNA probe was purchased from Cayman Chemical Co. A plasmid containing β-actin cDNA was obtained from the Japanese Cancer Research Bank. The methods used for plasmid preparation were described previously (17).

*Northern blot analysis.* Cells ($10^6$) in Falcon 5-cm-diameter dishes (Becton Dickinson Labware, Franklin Lakes, NJ, USA) were treated with the test samples and total cellular RNA was extracted from them by the AGPC procedure (18). As described earlier (17), the RNA was subjected to 1% agarose electrophoresis and blotted onto nylon membranes (Micron Separations, Inc., Westboro, MA,

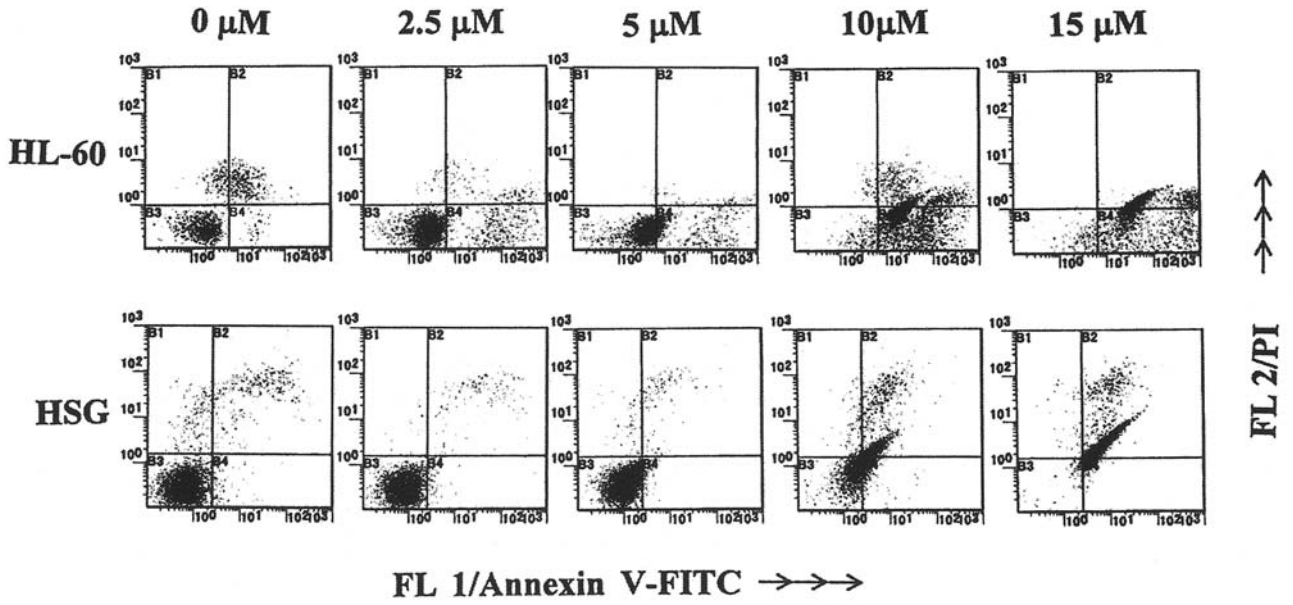


Figure 3. *Typical quadrant analysis of annexin V-FITC/PI flow cytometry in HL-60 and HSG cells treated with 0-15 μM curcumin. The procedures are described in the text. Briefly, the cells were stained with annexin V-FITC with PI to identify early and late apoptosis. At least 5,000 cells were analyzed per sample. Four distinct cell phenotypes are distinguishable: viable (lower left quadrant, B3), early apoptotic (lower right quadrant, B4), late apotic/necrotic (upper right quadrant, B2) and damaged cells (upper left quadrant, B1).*

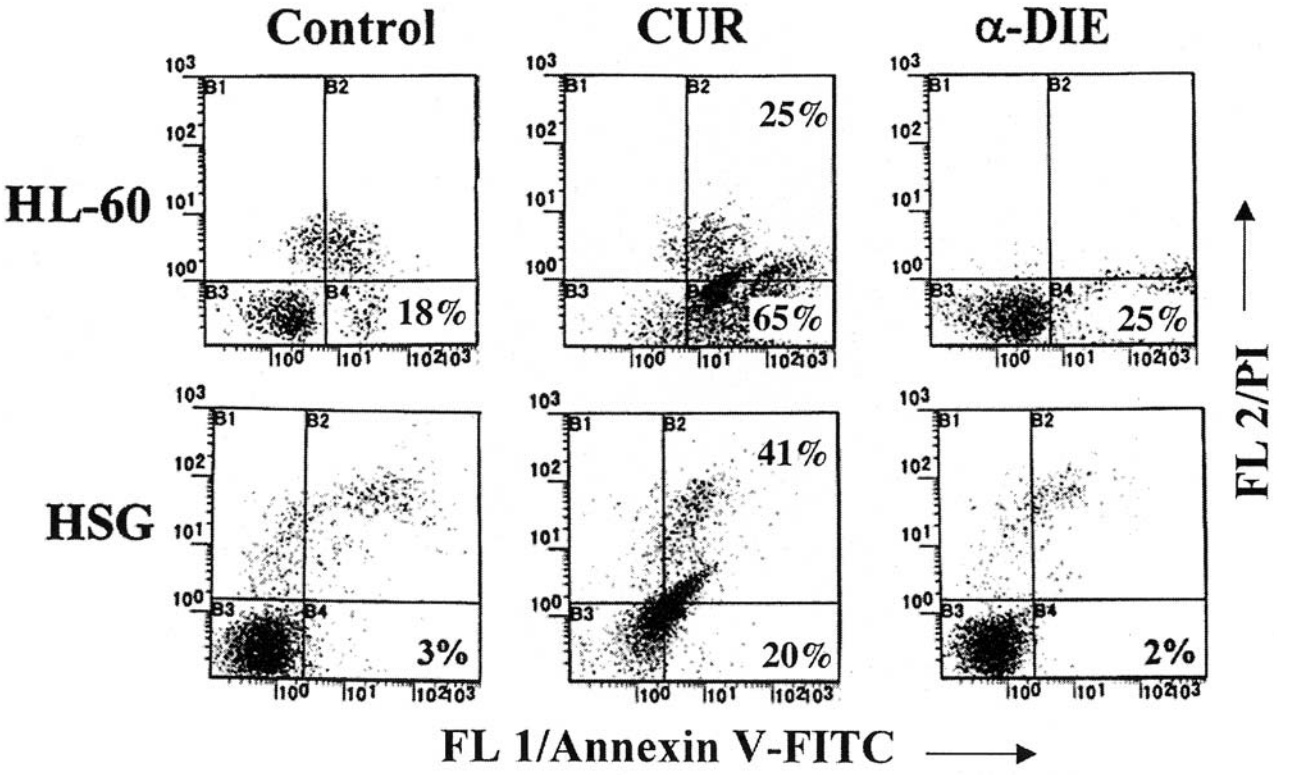


Figure 4. *Proportions of early apoptotic or late apoptotic/necrotic HL-60 and HSG cells after treatment with curcumin or α-diisoeugenol at 10 μM. Cells were stained with both annexin V-FITC and PI, as described in the text. The proportions of early apoptotic and late apoptotic/necrotic cells were calculated from quadrants B4 and B2 (the quadrant contains both late apoptotic and necrotic cells), respectively. The number inserted in the quadrant represents cell number (%).*

Table II. *ROS-generation by some additives in curcumin-treated HL-60 or HSG cells.*

| Compounds + additives | Cells | ROS generation (RFU) |
|---|---|---|
| A | | |
| Blank | HL-60 | 0.15 |
| Curcumin (10 μM) | HL-60 | 0.22 |
| Curcumin (100 μM) | HL-60 | 1.40 |
| Curcumin (1000 μM) | HL-60 | 11.1 |
| Blank | HSG | 0.20 |
| Curcumin (10 μM) | HSG | 0.30 |
| Curcumin (100 μM) | HSG | 1.45 |
| Curcumin (1000 μM) | HSG | 13.15 |
| α-Diisoeugenol (1000 μM) | HL-60 | 0.20 |
| α-Diisoeugenol (1000 μM) | HSG | 0.20 |
| B | | |
| Curcumin (200 μM) | HL-60 | 1.00 |
| + Catalase (200 U) | HL-60 | 0.97±0.12 |
| + SOD (200 U) | HL-60 | 0.98±0.13 |
| +Manitol (50 mM) | HL-60 | 0.80±0.64 |
| + $NaN_3$ (50 mM) | HL-60 | 0.71±0.62 |
| + NAC (1 mM) | HL-60 | 0.75±0.32 |
| + GSH (5 mM) | HL-60 | 0.68±0.45 |
| C | | |
| Curcumin (100 μM) | HL-60 | 1.00 |
| + $CuCl_2$ (10 μM) | HL-60 | 1.22 |
| + $CuCl_2$ (10 μM) + Bathocuproine (25 μM) | HL-60 | 0.84 |
| + $CuCl_2$ (10 μM) + Bathocuproine (50 μM) | HL-60 | 0.65 |
| + $ZnCl_2$ (10 μM) | HL-60 | 1.10 |
| + $ZnCl_2$ (100 μM) | HL-60 | 1.50 |
| + $ZuCl_2$ (100 μM) + Phenantroline (25 μM) | HL-60 | 1.28 |
| + $ZuCl_2$ (100 μM) + Phenantroline (50 μM) | HL-60 | 0.95 |
| Curcumin (100 μM) | HSG | 1.00 |
| + $CuCl_2$ (10 μM) | HSG | 1.26 |
| + $ZnCl_2$ (10 μM) | HSG | 1.10 |
| + $ZnCl_2$ (100 μM) | HSG | 1.76 |

Curcumin was added to the cells and incubation was carried out for 30 min. The cells were the stained by a 30-min incubation with 10 μM CDFH-DA. The amounts of intracellular ROS were estimated by using a flow cytometer, with the fluorescence intensity measured at 520 nm to 530 nm. The procedures are described in the text. The data are mean or mean±SD for 3 or 5 different experiments. A : The value of the ROS is expressed as relative fluorescence units (RFU) with the control as 1.0. B and C: The values are expressed as the ratio of [(the ROS in curcumin-treated cells with additives) / (the ROS in curcumin-treated cells)]. There is no significant difference between the control and catalase or SOD, whereas there is a significant difference between the control and manitol, $NaN_3$, NAC, or GSH ($p<0.01$).

USA). The membranes were then hybridized with each cDNA probe labelled with 5-[α-$^{32}$P]dCTP, by use of a Megaprime DNA labelling system. After hybridization, the membranes were washed, dried, and exposed overnight to Kodak X-ray film (Eastman Kodak Co., Rochester, NY, USA) at –70˚C. β-Actin was used as internal standard for quantification of total RNA in each lane of the gel.

*Detection of ROS generation.* The method for detection of intracellular ROS production was modified from that described in our previous report (19) in that CDFH-DA was used as a probe for peroxide or hydroperoxide. The detection of ROS production by quantitative analysis using a flow cytometer was based on Boissy *et al*. (20). HL-60 or HSG cells were seeded at 1-2x$10^5$ cells/well and cultured for 2 days to reach semiconfluence. At 60 minutes before the assay, the cells were washed twice with HBS. Each test compound was added to the wells at concentrations of 2.5 to 50 μM, and the cells were then incubated for the desired times. Next, 10 μM CDFH-DA was added to each well, after which the cells were incubated at 37˚C for 30 minutes. The cells were detached by use of trypsin solution and washed with FBS. The cells (2-4x$10^5$) were suspended in 0.5 ml of HBS, and the intensity of green fluorescence at 520-530 nm of 5,000 individual cells was measured with the flow cytometer. The amount of ROS was expressed as relative fluorescence units (RFU) with the control as 1.0. Some scavengers of ROS, such as NAC, GSH, mannitol, sodium azide, catalase and SOD, were added separately to the cells 1 hour before the addition of curcumin. Metal salts, bathocuproine or *o*-phenanthroline were added in water to the cells 10 minutes before the addition of curcumin.

*Computational details.* Theoretical calculations were performed by the restricted Hartree-Flock level MNDO-PM3 semiempirical method, as implemented in the MOPAC (molecular orbital package) program on a Tektronix CAChe work system (21).

*Data analysis.* The comparative apoptosis-inducing and ROS-producing activities of curcumin and related compounds were analyzed statistically by using the Student's *t*-test.

## Results

*Cytotoxicity.* The cytotoxicity of curcumin and α-diisoeugenol at concentrations of 0.1 μM to 100 μM was examined in the two cancer cell lines (HL-60 and HSG cells). The $CC_{50}$ values are shown in Table I. The cytotoxicity of curcumin and and α-diisoeugenol against HL-60 cells was two-fold greater than that against HSG cells. The cytotoxicity of curcumin was similar to that of α-diisoeugenol.

*Apoptosis induction.* Flow cytometric analysis of the cell cycle was performed to determine the dose-response for the induction of DNA fragmentation by curcumin, which is the final stage of apoptosis, and characterized by nuclear condensation and oligonucleosomal DNA fragmentation. The results of DNA quantification by PI stain are shown in Figure 2. Curcumin treatment of HL-60 cells led to a dose-dependent increase in the number of cells with hypodiploid/fragmented DNA (sub-$G_1$/$G_0$). It was also clear from the data in Figure 2 that curcumin induces growth arrest in the $G_2$/M-phase of the cell cycle in HL-60 cells. In particular, treatment of HL-60 cells with 15 μM curcumin increased the proportion of apoptotic cells to 55%, whereas

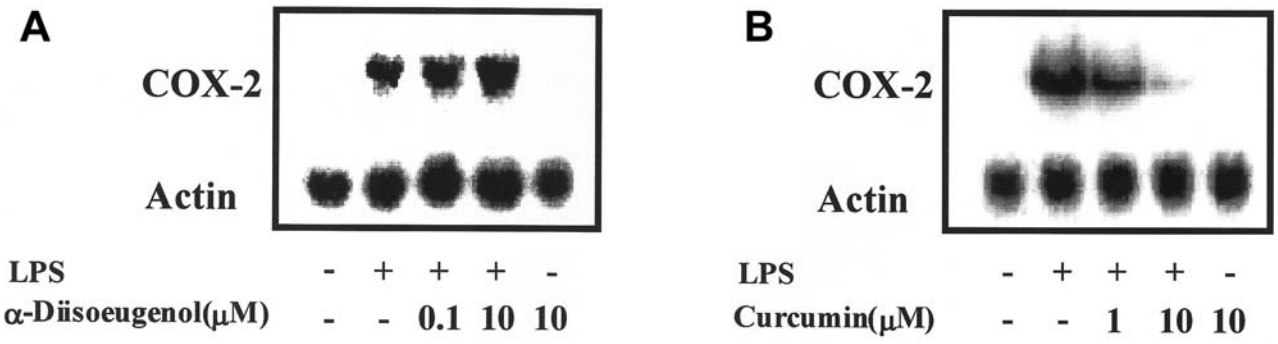


Figure 5. *Inhibition of LPS-induced COX-2 gene expression by curcumin and α-diisoeugenol in RAW 264.7 cells. Northern blot assay was performed, as described in Materials and Methods. Two independent experiments were conducted and similar results were observed.*

similar treatment of HSG cells did not increase the proportion of apoptotic cells beyond that observed at 10 μM curcumin (30%). The proportion of apoptotic cells in controls was less than about 5%. These results show that induction of apoptosis by curcumin was greater in HL-60 cells than in HSG cells.

Next, early apoptosis induced by curcumin was evaluated by externalization of PS in both HL-60 and HSG cells. The results are shown in Figure 3. After treatment of HL-60 cells with 2.5 μM curcumin for 5 hours, there was a significant increase in the proportion of early apoptotic cells (B4) compared with controls, whereas 10 μM curcumin for 5 hours was required for significant induction of apoptosis in HSG cells. The proportions of early apoptotic and late apoptotic/necrotic cells in HL-60 or HSG cells treated with 10 μM curcumin or α-diisoeugenol are shown in Figure 4. The proportion of early apoptotic cells in curcumin-treated HL-60 and HSG cells was about 65% and about 20%, respectively, whereas for both cell types the proportion of early apoptotic cells after treatment with α-diisoeugenol was similar to that of the controls (Figure 4). These observations confirm that HL-60 cells are particularly sensitive to induction of apoptosis by curcumin. The proportion of late apoptotic/necrotic cells of both types increased at concentrations of curcumin and α-diisoeugenol exceeding 15 μM, and at 50 μM the majority of cells were necrotic (data not shown).

*ROS generation.* The results of measurement of intracellular ROS generation are shown in Table II. Curcumin generated ROS dose-dependently in both HL-60 cells and HSG cells. ROS generation in HL-60 cells was strongly enhanced by Cu(II) or Zn(II). ROS generation in HL-60 cells was suppressed by bathocuproine, a Cu(I)-specific sequestering agent, and also by *o*-phenanthroline, a chelating agent. ROS generation by curcumin was also suppressed by mannitol (a specific inhibitor of hydroxy radicals), sodium azide (a specific inhibitor of singlet oxygen) and antioxidants (NAC, GSH), whereas it was not suppressed by SOD or catalase.

*COX-2 inhibition.* Since we found a marked difference in the abilities of curcumin and α-diisoeugenol to induce apoptosis, we next used a Northern blot assay to investigate whether these compounds could inhibit LPS-induced expression of the COX-2 gene in RAW cells. LPS-induced gene expression of COX-2 was clearly inhibited by curcumin at a concentration of 10 μM, whereas α-diisoeugenol was not inhibitory (Figure 5).

## Discussion

In this study, we attempted to determine whether the cytotoxic activity of α-diisoeugenol, which is a similar cytotoxic activity to that of curcumin, was due to apoptosis by examining some of the most accepted signs (biomarkers) of cell death, such as externalization of PS, DNA fragmentation and ROS-generation. These biomarkers occur in sequence beginning with depolarization of the mitochondria membranes, through caspase-3 activation and externalization of PS, to the final DNA fragmentation (sub-$G_1/G_0$ accumulation of DNA). Curcumin induced apoptosis but α-diisoeugenol failed to induce apoptosis. Previous studies have reported ROS generation and induction of apoptosis by curcumin. Notably, the prooxidative activity of curcumin is involved in the inhibition of mitochondrial respiration (22), and curcumin in the presence of $Cu^{2+}$ causes strand cleavage in DNA through the generation of ROS (23). Curcumin treatment results in the hyperproduction of ROS, loss of mitochondrial membrane potential and cytochrome c release to the cytosol, with concomitant exposure of PS (24). These findings suggest that the formation of early apoptosis markers ("eat-me signals"), such as PS externalization, may be accelerated by oxidative stress derived from the prooxidative activity of curcumin in cancer cells. In the present study, curcumin at concentrations of 10 μM and above caused

marked generation of ROS. Curcumin has a bimodal action, and whether it acts as an antioxidant or a prooxidant is dependent on factors such as concentration, enzymes, light or pH. ROS are generated endogenously during execution of apoptosis programs, suggesting that they may act as secondary messengers in apoptotic pathways. The present study demonstrated that curcumin generated ROS in both cells. The addition of Cu(II) or Zn(II) to curcumin markedly enhanced ROS generation. Curcumin, but not α-diisoeugenol, undergoes autooxidation catalyzed by transition metals to produce ROS. Also, intracellular curcumin phenoxyl radicals (redoxing-cycling phenols), formed by myeloperoxidase, induce lipid peroxidation and cooxidize GSH to form thiyl radicals with concomitant oxygen activation in HL-60 cells (25). Curcumin is much more prooxidant than α-diisoeugenol. Oxidant (an anticancer drug)-induced apoptosis leads to rapid oxidation of different classes of phospholipids with substantial oxidation of PS in cell membranes (26). The oxidation of PS precedes its externalization during apoptosis (27, 28) and is blocked by the antiapoptotic protein Bcl-2 (28).

Expression of various cell survival and cell proliferative genes, including Bcl-2, IL-6 and COX-2, can be suppressed by curcumin (1). Curcumin is a well-known inhibitor of the transcription factor activator protein-1 (AP-1), which activates various stimuli such as growth factors, cytokines and LPS (1). We investigated the effects of curcumin and α-diisoeugenol on LPS-induced COX-2 expression in RAW cells. As expected, curcumin inhibited COX-2 expression at the relatively low concentration of 10 μM, whereas α-diisoeugenol did not (Figure 5).

Why the biological activity of curcumin is different from that of α-diisoeugenol needs to be explained. The chemical properties of curcumin, a redox-cycling phenol, have attracted considerable attention. Phenoxyl radical generation by curcumin and α-diisoeugenol would be determined primarily by the homolytic bond dissociation energy (BDE) of the phenolic O-H bond, which is determined in part by resonance stabilization of the steric hindrance to abstraction of the phenolic hydrogen by free radicals (6). BDEs (enthalpy, kcal/mol), calculated by a PM3 semiempirical method (29), are 83.87 kcal/mol and 71.24 kcal/mol for curcumin and α-diisoeugenol, respectively (data not shown). This suggests that the ability of curcumin to inhibit COX-2 gene expression is dependent on its phenolic function. Dimerization of curcumin has been proposed previously, suggesting that curcumin functions as an antioxidant *via* its ability to scavenge free radicals (6). This antioxidant property may explain the pharmacological activity of curcumin against COX-2, in which it appears to act as an NSAID-like compound.

In summary, the present findings suggest that ROS are crucial for triggering the curcumin-induced early apoptotic program, including PS externalization, and that this process may be dependent on the prooxidative activity of curcumin. On the other hand, curcumin as an antioxidant may suppress LPS-induced gene expression partly by reducing the oxidative stress caused by LPS treatment in RAW cells, and this mechanism may also be responsible for inhibition of AP-1, NFÎ B DNA-binding activity and COX-2 expression (1, 30, 31). The causal link between the anticancer and anti-inflammatory activity for curcumin-related compounds remains to be investigated.

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