Comparative Anti-inflammatory Activities of Curcumin and Tetrahydrocurcumin Based on the Phenolic O-H Bond Dissociation Enthalpy, Ionization Potential and Quantum Chemical Descriptor

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Abstract. *Curcumin and its reduced derivative tetrahydrocurcumin have been shown to exhibit chemopreventive activity. Cyclooxygenase-2 (COX-2) inhibition in lipopolysaccharide (LPS)- or Porphyromonas gingivalis fimbria-stimulated RAW 264.7 cells was investigated using Northern blot analysis. The fimbria-stimulated expression of the COX-2 gene was inhibited by curcumin but not by tetrahydrocurcumin. LPS-stimulated COX-2 gene expression was completely inhibited by curcumin, but an increase in the concentration of tetrahydrocurcumin did not cause complete inhibition of COX-2 expression. The inhibitory effect of curcumin on nuclear factor kappa B (NF-κB) activation in the cells was clearly observed, but that of tetrahydrocurcumin was incomplete even at a concentration of 20 μM. To explain the difference in effect between the two compounds, analysis of the frontier orbital was performed using ab initio 6-31G* wave function. The calculated chemical hardness (η) for curcumin was clearly smaller, whereas its electronegativity (χ) and electrophilicity (ω) were clearly greater than the corresponding values for the curcumin-related compounds tetrahydrocurcumin, isoeugenol and eugenol. This suggested that the anti-inflammatory activities of curcumin may be related to η-, χ- and/or ω-controlled enzymes. In addition, the bond dissociation enthalpy (BDE) of the phenolic OH was calculated using the density function theory (DFT)/B3LY. The total BDE values of curcumin and tetrahydrocurcumin were almost identical, but the BDE of one-electron oxidation and ionization potential (IP) for curcumin were lower than those for tetrahydrocurcumin, suggesting the highly pro-oxidative activity of curcumin. Curcumin has both oxidant and antioxidant properties. A causal link between the anti-inflammatory activities and molecular properties of phenolic antioxidants is suggested.*

Curcumin and its reduced derivative and major metabolite tetrahydrocurcumin (Figure 1) have received attention because of their potent antioxidant, anti-inflammatory, and anticancer activities (1). Curcumin inhibits COX-2 expression and down-regulates activation of c-Jun *N*-terminal kinase (JNK), activated protein-1 (AP-1) and nuclear factor kappa B (NF-κB) (1). Moreover, it suppresses the expression of inducible nitric oxide synthase (iNOS) (1). These properties of curcumin make it an attractive candidate for use as a therapeutic agent in the prevention of oral diseases, such as leukoplakia and destructive chronic periodontal diseases. We have previously reported that an *ortho* dimer of butylated hydroxyanisole, a curcumin-related compound, inhibited NF-κB and AP-1 activation, and gene expression of cyclooxygenase-2 (COX-2) and inflammatory cytokines, such as IL-1β and TNF-α in macrophages stimulated by fimbriae of *Porphyromonas gingivalis*, an oral anaerobic bacterium causing chronic periodontal disease (2, 3). In addition, there have been some reports of inhibitory effects of curcumin-related compounds, such as the *ortho* dimers of eugenol and isoeugenol, on lipopolysaccharide (LPS)-stimulated COX-2 expression (4-6).

Computational chemistry is one of today's most rapidly expanding and exciting areas of endeavor in the medical and medicinal sciences (7). Information available from

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Key Words: Curcumin, tetrahydrocurcumin, COX-2, NF-κB, chemical descriptors, lipopolysaccharide, *Porphyromonas gingivalis* fimbriae.

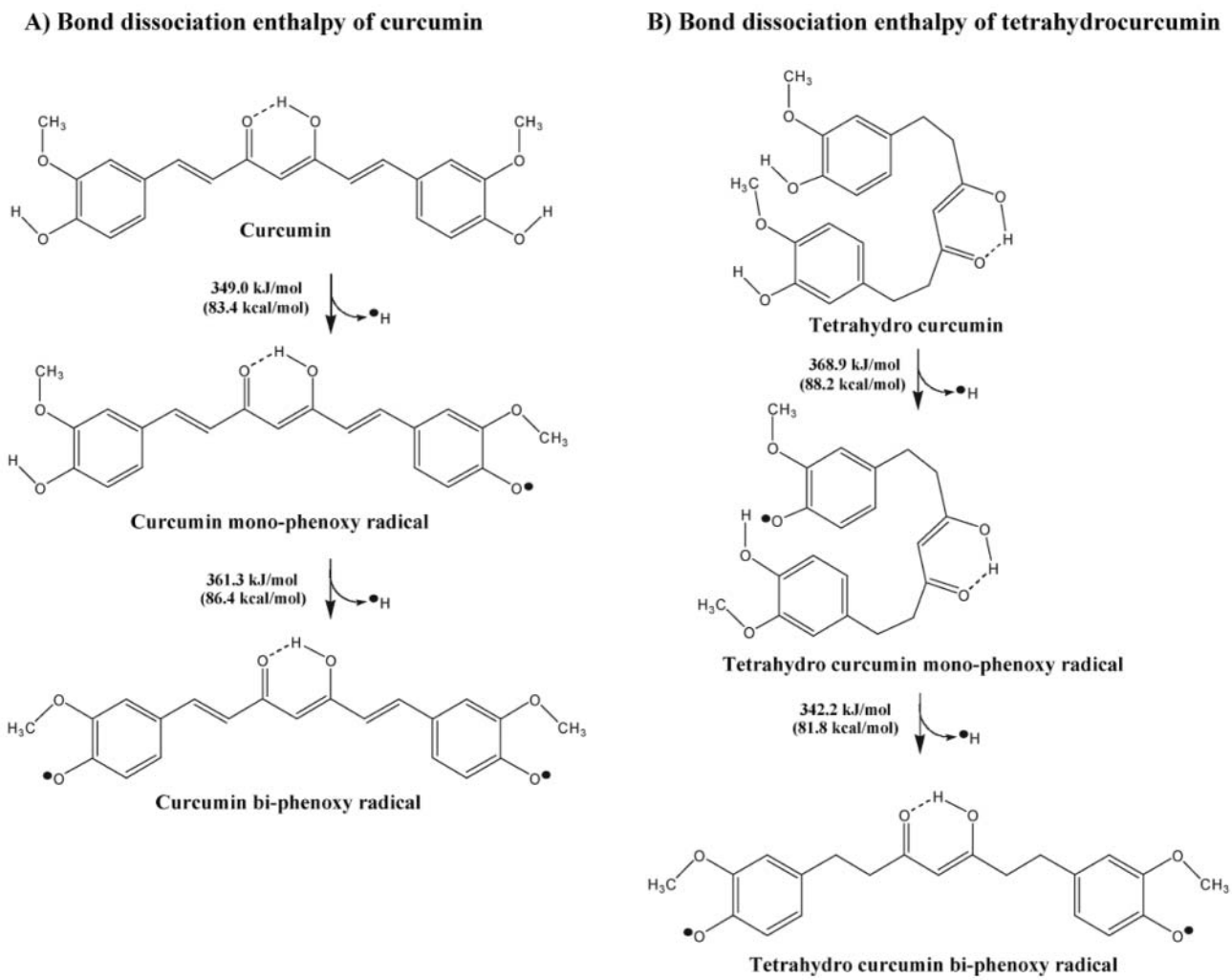

Figure 1. *A possible mechanism of oxidation for curcumin (A) and tetrahydrocurcumin (B) and their O-H bond dissociation enthalpy.*

computational methods may be useful for interpreting the molecular mechanism of non-steroidal anti-inflammatory drugs (NSAIDs). We examined the phenolic O-H bond dissociation enthalpy (BDE) derived from the density function theory (DFT)/B3LYP, and the highest molecular orbital (HOMO) energy (E_{HOMO}) and the lowest molecular orbital (LUMO) energy (E_{LUMO}) derived from the *ab initio* 6-31G* wave function calculated from the values of chemical hardness (η), electronegativity (χ) and electrophilicity (ω). In the present study, we investigated whether curcumin and tetrahydrocurcumin could inhibit fimbriae- and/or LPS-stimulated COX-2 expression and NF-κB activation in RAW264.7 cells and tried to account for differences in the activities of the two compounds in terms of the former system. The results are discussed on the basis of the chemical hardness concept.

Materials and Methods

Reagents. Curcumin was purchased from Wako Pure Chemical Industries, Ltd. (Osaka, Japan). Eugenol, isoeugenol and 1,1-diphenyl-2-picrylhydrazyl (DPPH) were purchased from Tokyo Kasei Co. (Tokyo, Japan). Tetrahydrocurcumin was kindly donated by Professor I. Yokoe at the Faculty of Pharmaceutical Science, Josai University (Saitama, Japan). The chemical structures of curcumin and tetrahydrocurcumin are shown in Figure 1. A Megaprime DNA labeling system, 5'-[α-^{32}P]dCTP, and [γ-^{32}P]ATP were purchased from Amersham Biosciences Co. (Piscataway, NJ, USA). A 5'-end labeling system was purchased from Promega Co. (Madison, WI, USA). A mouse COX-2 cDNA probe with a length of approximately 1.2 kbp was purchased from Cayman Chemical Co. (Ann Arbor, MI, USA). An 18 S rRNA cDNA probe was purchased from Maxim Biotech. Inc. (South San Francisco, CA, USA). RPMI 1640 was purchased from Invitrogen Corp. (Carlsbad, CA, USA). Fetal bovine serum

(FBS) was from HyClone (Logan, UT, USA). *Escherichia coli* O111 B4-derived LPS was from List Biological Laboratories, Inc. (Campbell, CA, USA).

Preparation of Porphyromonas gingivalis fimbriae. Porphyromonas gingivalis ATCC 33277 fimbriae were prepared and purified from cell washings by the method of Yoshimura *et al.* (8). Purified fimbria-induced biological activities were not attributable to LPS contaminants in the preparation, as documented previously (9). The protein content of the fimbriae was measured by the method of Bradford (10).

Cell culture. Cells of the murine macrophage-like cell line RAW 264.7, obtained from the Dainippon Sumitomo Pharma Biomedical Co.Ltd. (Osaka, Japan), were used. They were cultured to a subconfluent state in RPMI-1640 medium supplemented with 10% FBS at 37˚C and 5% CO_2 in air, washed and then incubated overnight in serum-free RPMI-1640. They were then washed further and treated with the test samples.

Northern blot analysis. 10^6 cells in Falcon 5 cm-diameter dishes (Becton Dickinson Labware, Franklin Lakes, NJ, USA) were pretreated for 30 min with the indicated dose of curcumin or tetrahydrocurcumin. They were then treated with or without fimbriae (4 μg/ml) or LPS (100 ng/ml), and their total RNA was prepared 3 h later by the acid guanidine phenol chloroform (AGPC) procedure (11). As described elsewhere (12), the RNA was electrophoresed in 1% agarose gels with 0.2 M sodium phosphate as a running buffer and blotted onto nylon membranes (Micron Separations, Inc., Westboro, MA, USA). The membranes were then hybridized with COX-2 and 18 S rRNA cDNA probes labeled with 5'-[alpha-^{32}P] dCTP using the Megaprime DNA labeling system (Amersham Biosciences Co.). After hybridization, the membranes were washed and dried then exposed overnight to Kodak X-ray film (Eastman Kodak Co., Rochester, NY, USA) at –70˚C. 18 S Ribosomal RNA was used as an internal standard for quantification of total RNA in each lane of the gel. Quantification of COX-2 expression was carried out by densitometry. The data are expressed as the relative signal intensity (percentage of maximum). Bars represent the mean for three independent experiments. Standard error <15%.

Preparation of nuclear extract and gel mobility shift assay. The nuclei were extracted and prepared for the gel mobility shift assay as described elsewhere (12). The cells in Falcon 15 cm-diameter dishes (10^7 cells per dish) were pretreated for 30 min with curcumin and tetrahydrocurcumin at 20 μM and then treated with LPS at 100 ng/ml for 1 h. were treated with the test samples. Thereafter, the cells were scraped into phosphate-buffered saline, pelleted and suspended in lysis buffer containing 10 mM Tris-HCl (pH 7.4), 3 mM $MgCl_2$, 10 mM NaCl, 0.5% Nonidet P-40). The nuclei were separated from the cytosol by centrifugation at 3,000 x g for 15 min. The extracted nuclei were then treated with buffer A (10 mM HEPES, [pH7.9], 1.5 mM $MgCl_2$, 10 mM KCl, 0.5 mM dithiothreitol [DTT]) and further treated by stirring for 60 min at 4˚C in buffer B (20 mM HEPES, [pH 7.9], 1.5 mM $MgCl_2$, 0.2 mM EDTA, 0.42 M NaCl, 25% glycerol, 0.5 mM DTT, 0.5 mM phenylmethylsulfonyl fluoride (PMSF)). Nuclear extracts were obtained by centrifugation for 60 min at 25,000xg and dehydrolyzed by passage through a Sephadex G-25 column equilibrated with buffer C (20 mM HEPES, [pH 7.9], 0.1 M KCl, 0.2 mM EDTA, 0.5 mM DTT, 0.5 mM PMSF). The protein concentrations were measured by the method of Smith *et al.* (13). Binding reactions were performed for 20 min at room temperature with 10 μg of the nuclear proteins in 2 mM Tris (pH7.5) containing 8 mM NaCl, 0.2 mM EDTA, 0.8% (v/v) glycerol, 0.2 mM DTT, 0.5 mM PMSF, 1 μg of poly (dI-dC) and 20,000 cpm of a 32P-labeled NF-κB oligonucleotide in a final volume of 20 μl. Poly (dI-dC) and nuclear extract were incubated at 4˚C for 10 min before the addition of the labeled oligonucleotide. The double-stranded oligonucleotide containing a tandem repeat of the consensus sequence for the binding site, -GGGGACTTTCCC-, for NF-κB was end-labeled by the T4 polynucleotide kinase-[γ-^{32}P] ATP method. DNA-protein complexes were electrophoresed in native 6% polyacrylamide gel in 0.25 x Tris borate EDTA (TBE) buffer (22 mM Tris-HCl [pH 8.0], 22 mM boric acid, 0.6 mM EDTA). The gel was dried, then exposed to Kodak X-ray film at –70˚C.

Anti-DPPH radical activity. Radical-scavenging activities were determined with 0.1 mM DPPH as a free radical. For each inhibitor, different concentrations in the range 0.001-0.5 mM were tested in ethanol. The decrease in absorbance was determined at 517 nm for 10 min at room temperature. The antiradical activity was defined as the amount of inhibitor necessary to decrease the initial DPPH radical concentration by 50% (IC_{50}, mol/l).

Computation. The BDE of the phenolic O-H was calculated as follows: First, the lowest and second-lowest energy conformers of both the phenol derivatives and their phenoxyl radical species were identified as candidates for geometry optimization using the conformer search procedure by MMFF (Merck molecular mechanics) force fields calculation. The tentative conformers were then geometrically optimized by *ab initio* molecular orbital calculation at the HF/6-31G* level for the phenols and with a UHF/6-31G* basis set for the phenoxyl radicals *in vacuo* to afford the respective energetic minimized structures. The electronic energy was further preceded by single point calculation involving density functional theory (DFT) using the B3LYP functional at the 6-31G*level. The BDE = Hr + Hh - Hp, where Hr is the enthalpy of the phenoxyl radical generated by H-abstraction, Hh is the enthalpy of the hydrogen radical and Hp is the enthalpy of the parent phenol. The absolute values of both the highest occupied molecular orbital (HOMO) and the lowest occupied molecular orbital (LUMO) energy of the fully optimized phenol derivatives were calculated at the RHF/6-31G* level. The absolute value of HOMO energy was adopted as an approximate IP value according to Koopman's theory (14).

All of the molecular modeling and calculation was performed with the Spartan 04 for Windows software package (SPARTAN' 04, Wavefunction Inc., Irvine, CA, USA).

The η, χ and ω value were calculated from Equations 1, 2 and 3, respectively.

$\eta = 1/2\ (E_{LUMO} - E_{HOMO})$ Equation 1

$\chi = -1/2\ (E_{LUMO} + E_{HOMO})$ Equation 2

$\omega = \chi^2/2\eta$ Equation 3

Results

Regulatory effect of curcumin and tetrahydrocurcumin on fimbria-induced gene expression of COX-2. Cox-2, the rate-limiting enzyme in the synthesis of prostanoids, is related to the development of inflammation and mutagenesis.

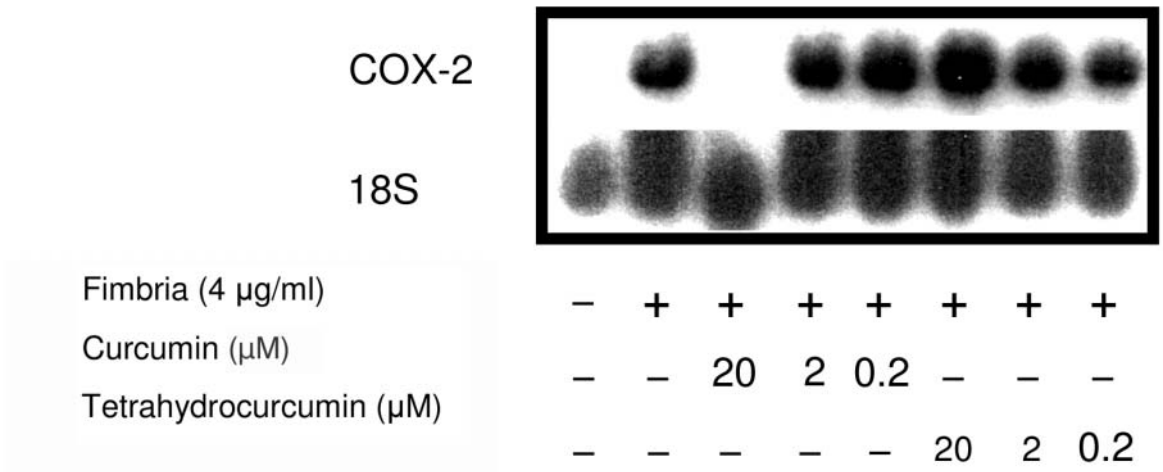

Figure 2. *Regulatory effect of curcumin and tetrahydrocurcumin on fimbria-induced expression of the COX-2 gene in RAW 264.7 cells. The cells were pretreated for 30 min with the indicated dose of curcumin or tetrahydrocurcumin. They were then treated with or without fimbriae at 4 μg/ml and their total RNA was prepared 3 h later. Northern blot analysis was performed with radio labeled COX-2 cDNA and 18 S rRNA cDNA probes. An identical experiment independently performed 3 times gave similar results.*

Effects of curcumin and tetrahydrocurcumin were examined in fimbria-induced RAW264.7 cells and the results are shown in Figure 2. Curcumin inhibited completely suppressed COX-2 expression at a relatively high dose level of >2 μM. In contrast, tetrahydrocurcumin did not inhibit COX-2 expression even at high concentrations.

Regulatory effect of curcumin and tetrahydrocurcumin on LPS-stimulated gene expression of COX-2. The effects of curcumin and tetrahydrocurcumin were examined in LPS-induced RAW264.7 cells. Curcumin markedly inhibited COX-2 expression in a concentration-dependent manner (Figure 3). This compound completely suppressed COX-2 expression at 20 μM. In contrast, tetrahydrocurcumin did not inhibit COX-2 expression effectively; complete inhibition was not achieved at the concentrations used here (Figure 3).

Regulatory effect of curcumin and tetrahydrocurcumin on LPS-stimulated NF-κB binding to its consensus sequences. Because curcumin mediates anti-inflammatory effects primarily *via* down-regulation of NF-κB, we next investigated whether tetrahydrocurcumin was able to inhibit LPS-stimulated NF-κB binding to its consensus sequence in the cells. Curcumin completely inhibited LPS-stimulated NF-κB binding to its consensus sequence, but the inhibitory effect of tetrahydrocurcumin was not complete at 20 μM (Figure 4). Neither isoeugenol nor eugenol inhibited LPS-stimulated NF-κB activation over a wide concentration range of 10-500 μM (data not shown), in accordance with previous reports (5, 6).

Anti-DPPH radical activity, BDE and IP ($IP_{Koopmans'}$). Results for curcumin-related compounds are shown in Table I. The anti-DPPH radical activity declined in the order curcumin, tetrahydrocurcumin>isoeugenol>eugenol. Their calculated BDE and IP values are also shown in Table I. The possible oxidation process is shown in Figure 1. The BDE was calculated using the B3LYP method and the $IP_{Koopman}$ was derived from the *ab initio* 6-31G* wave function. The total BDE for curcumin (168.9 kcal/mol) was similar to that of tetrahydrocurcumin (170.0 kcal/mol). The BDE derived from one-electron oxidation of curcumin (83.4 kcal/mol) was less than that of tetrahydrocurcumin (88.2 kcal/mol). The BDE values of isoeugenol and eugenol (83-84 kcal/mol) were markedly lower than that of fully oxidized curcumin or tetrahydrocurcumin. The similar anti-DPPH activities of curcumin and tetrahydrocurcumin were related to the similar BDE values for these compounds. The IP value declined in the order eugenol>tetrahydrocurcumin>curcumin> isoeugenol. No dependence of anti-DPPH radical activity on the IP value was found. In medicinal applications of antioxidants, it is important to note that when BDE or IP becomes too low, the compound can act as a pro-oxidant rather than as an antioxidant. Isoeugenol may act as a pro-oxidant, followed in order by curcumin. It was assumed from the values of BDE and IP that curcumin may possess high pro-oxidative activity.

Chemical descriptors. Chemical descriptors of curcumin and related compounds are shown in Table II. The values of the chemical descriptors η, χ and ω for curcumin were clearly different from those for tetrahydrocurcumin. In contrast, the values of χ and ω for tetrahydrocurcumin were almost the same as those for isoeugenol and eugenol.

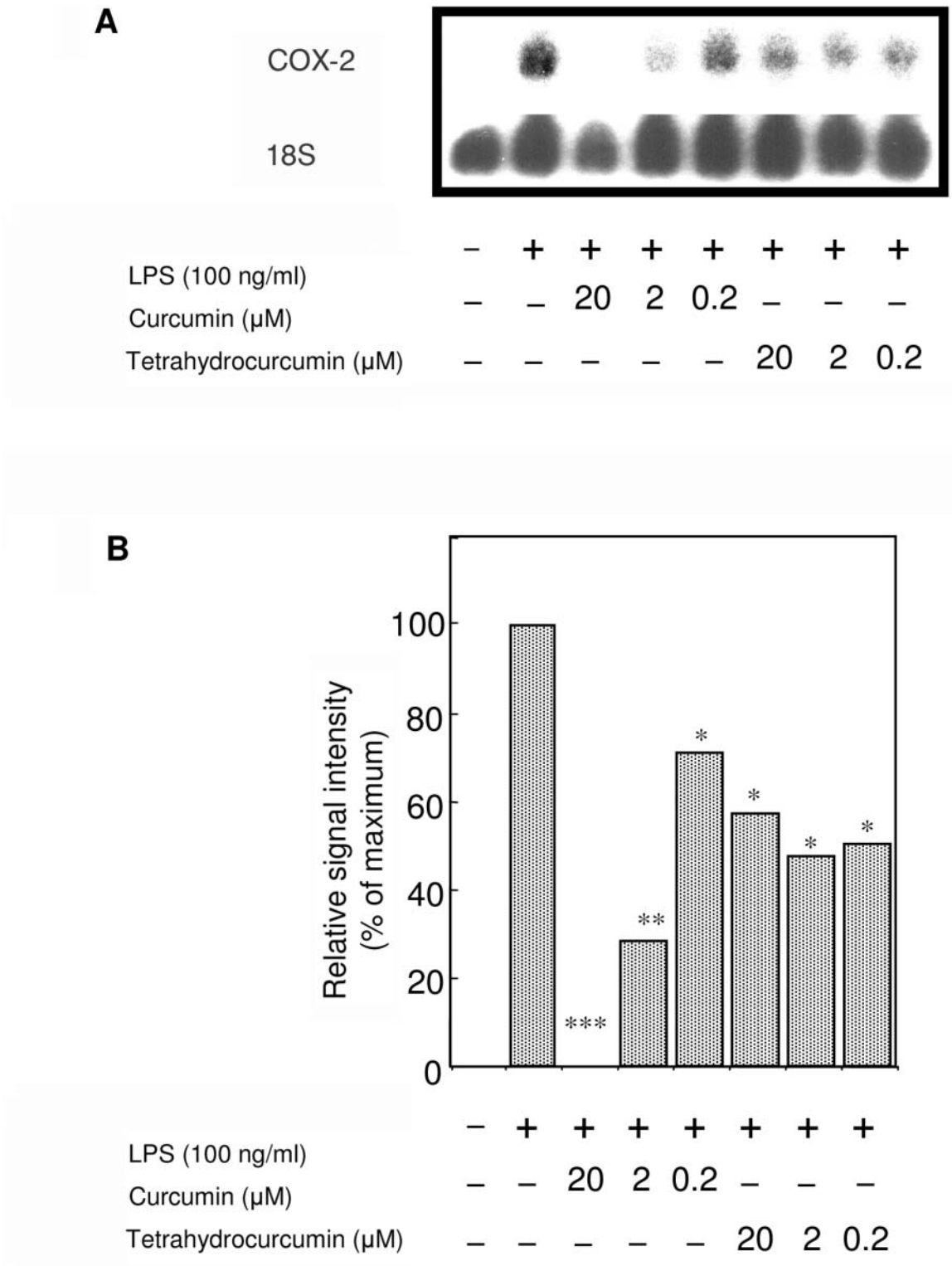

Figure 3. *Regulatory effect of curcumin and tetrahydrocurcumin on LPS-induced expression of the COX-2 gene in RAW 264.7 cells. A) The cells were pretreated for 30 min with the indicated dose of curcumin or tetrahydrocurcumin, then untreated or treated with LPS at 100 ng/ml. Their total RNA was prepared at 3 h after addition of the LPS. Northern blot analysis was performed with radio labeled COX-2 cDNA and 18 S rRNA cDNA probes. An identical experiment independently performed 3 times gave similar results. Quantification of COX-2 expression shown in (A) was carried out by densitometry. The data are expressed as the relative signal intensity (percentage of maximum). Bars represent the mean for three independent experiments. Standard error <15%. *, **, ***Significantly different from positive control (*p<0.05, **p<0.01, ***p<0.001).*

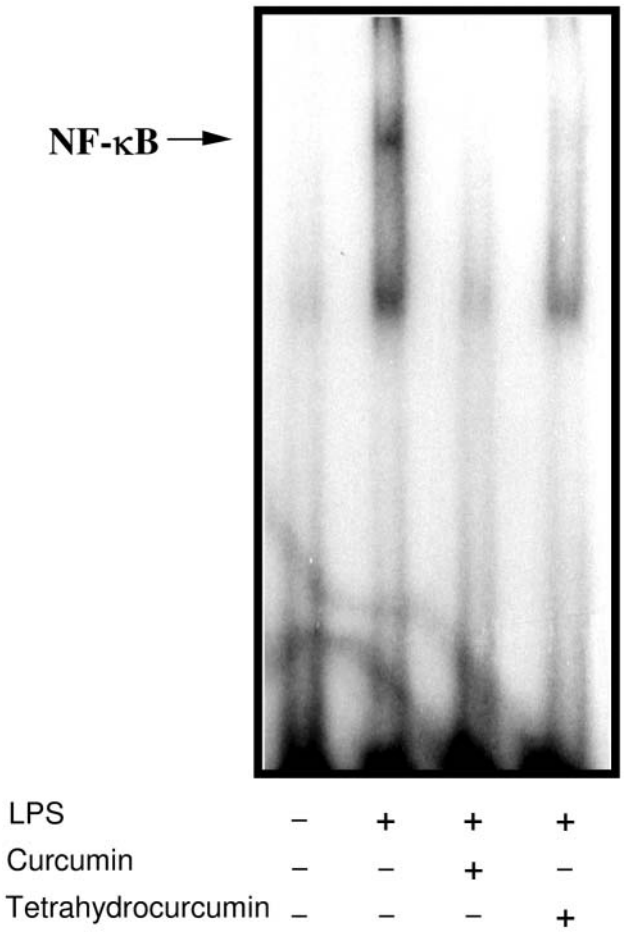

Figure 4. *Regulatory effect of curcumin and tetrahydrocurcumin on LPS-stimulated NF-κB binding to its consensus sequence in RAW 264.7 cells. The cells were pretreated for 30 min with curcumin and tetrahydrocurcumin at 20 μM and then treated with LPS at 100 ng/ml for 1 h. A gel mobility shift assay was performed with the nuclear proteins and ^{32}P-labeled oligonucleotide containing the NF-κB consensus sequence. The experiment was independently performed in triplicate and gave similar results.*

Table I. *Bond dissociation enthalpy (BDE), ionization potential $(IP)_{Koopman}$, anti-DPPH (diphenylpicrylhydrazyl) radical activity (IC_{50}) of curcumin and related compounds.*

Compound	BDE (kcal/mol)	IP (eV)	IC_{50} (mM)
Curcumin	83.4* 86.4#	7.527	0.04
Tetrahydrocurcumin	88.2* 81.8#	7.706	0.04
Eugenol	84.0	7.898	0.08
Isoeugenol	83.0	7.490	0.05

BDE and IP were calculated using the B3LYP and HF/6-31G* method, respectively. * 1st one-electron oxidation (1st); #2nd oxidation.

Table II. *Chemical descriptors of curcumin, tetrahydrocurcumin, isoeugenol and eugenol.*

Orbital	Curcumin	Tetrahydro-curcumin	Eugenol	Iso-eugenol
LUMO orbital energy (eV)	1.502	3.513	4.088	3.451
LUMO eigenvalue	0.055	0.114	0.152	0.127
HOMO eigenvalue	–0.277	–0.283	–0.291	–0.275
HOMO orbital energy (eV)	–7.527	–7.706	–7.898	–7.490
Chemical hardness (η)	4.515	5.608	5.993	5.471
Electronegativity (χ)	3.013	2.097	1.905	2.020
Electrophilicity (ω)	1.005	0.393	0.303	0.373

Discussion

Our results demonstrated that curcumin inhibited COX-2 expression in LPS- and fimbria-stimulated RAW264.7 cells. In contrast, tetrahydrocurcumin, which lacks the conjugated bonds in the central seven-carbon chain (Figure 1), did not inhibit fimbriae-stimulated expression. Moreover, an increase in the tetrahydrocurcumin concentration did not cause complete inhibition of LPS-stimulated COX-2 expression, suggesting false-positivity for COX-2 inhibition. On the other hand, tetrahydrocurcumin, in addition to curcumin, was previously reported to inhibit the release of arachidonic acid (AA) and its metabolites and also to potently inhibit the formation of prostaglandin E2 (PGE-2) in LPS-stimulated RAW cells (15). Furthermore, it was reported that curcumin inhibited COX-2 expression and increased the COX-2 level without LPS stimulation; such effects of curcumin may be responsible for the decreased PGE-2 activity rather than the catalytic inhibition of LPS stimulation. In contrast, tetrahydrocurcumin, but not curcumin, is reported to be completely ineffective for suppression of TNF-induced NF-κB activity (16). It is well known that activation of NF-κB occurs through a post-translational mechanism involving dissociation of the inhibitory protein IκB (17). The activation may be controlled by reactive oxygen species (ROS) and the intracellular redox state. It has been shown that ROS are needed for TNF-induced NF-κB activation (18). In addition, we previously demonstrated that tetrahydrocurcumin, but not curcumin, was unable to produce intracellular ROS (19). However, it is not clear how both suppression and activation of NF-κB are mediated *via* ROS generation. Isoeugenol and eugenol generate ROS but to a much lesser degree than curcumin (20). The intracellular ROS levels could mediate LPS-stimulated NF-κB activation or suppression by phenolic antioxidants. The difference in anti-inflammatory activity between curcumin and tetrahydrocurcumin may be related to differences in their ability to generate intracellular ROS.

On the other hand, LPS-dependent expression of COX-2 protein in RAW cells was recently reported to be inhibited markedly by isoeugenol, and less effectively by eugenol. However, an increase in the concentration of eugenol or isoeugenol did not cause complete inhibition of COX-2 expression; relative to the control, inhibition of COX-2 expression was approximately 50% at 400 μM eugenol and at 50 μM of isoeugenol (21). Conversely, we demonstrated that COX-2 expression was not inhibited in LPS stimulated RAW cells by either eugenol or isoeugenol (5, 6). When a high cytotoxic concentration is required for COX-2 inhibition, a false-positive result would frequently be obtained. These findings suggested that eugenol and isoeugenol are not potent COX-2 inhibitors. There is a general opinion in drug screening that if the 50% inhibitory concentration (IC_{50}) of a compound is less than 3 μM, then it can be regarded as a strong enzyme inhibitor (22). The IC_{50} threshold for candidate compounds should be less than 10 μM (23). In the present study, the IC_{50} threshold for curcumin was less than 10 μM.

COX-2 can be induced by various inflammatory stimuli, such as LPS (24), phorbol 12-myristate 13-acetate (25) and cytokines (26). The activity of the COX-2 pathway in macrophages is strong, particularly when activated by LPS. COX-2 was also induced by fimbriae, but its activity was lower than that induced by LPS. It is well known that COX-2 promotes cell growth, inhibits apoptosis and enhances cellular motility and adhesion. Curcumin induces preferentially apoptosis (27, 28). A potential cause-effect link between COX-2 expression and the induction of apoptosis in biological systems has been suggested (27). Our findings suggest that curcumin may be a potential candidate for prevention of oral diseases such as tumors and severe chronic periodontal diseases.

The physiological and pharmacological characteristics of the protective effects of tetrahydrocurcumin *in vivo* are similar to those of curcumin (29). A recent comparative study of the antioxidant activities of curcumin and tetrahydrocurcumin indicated that the scavenging activity of the latter was significantly greater than that of the former, using the DPPH radical and 2, 2'-azo*bis* (2-aminopropane) dihydrochloride (APPH)-induced red blood cell hemolytic assay (30). In the present DPPH study, the radical-scavenging activities of curcumin and tetrahydrocurcumin were almost identical.

For *in vivo* tests, tetrahydrocurcumin shows a more pronounced protective effect than curcumin against chloroquine-induced hepatotoxicity in rats (31). The antidiabetic and antioxidant effects of tetrahydrocurcumin in rats with streptozotocin-nicotinamide-induced diabetes are more potent than those of curcumin at the same dose (32). Such protective effects of tetrahydrocurcumin in rats may be due to its pronounced antioxidative activity.

Analysis of the frontier orbital allows us to explain the difference in antioxidant and biological activity between curcumin and tetrahydrocurcumin. Therefore, we examined BDE, IP and quantum chemical descriptors (Tables I and II). The relative importance of phenolic O-H and the CH-H hydrogen on the antioxidant properties of curcumin was previously reported using the density function theory (DFT), and indicated that the phenolic O-H plays a major role in this activity (33). This suggested that a phenol function controlled by phenolic NSAIDs plays a predominant role in the anti-inflammatory activity. Although the total BDE values of curcumin and tetrahydrocurcumin were similar, the 1st one-electron oxidation BDE value for curcumin was less than that of tetrahydrocurcumin, suggesting that the former is more pro-oxidative than the latter (Figure 1 and Table I). The protective effects of tetrahydrocurcumin in animal tests described above may be related to its higher BDE and higher IP value. In the medicinal application of phenolic antioxidants, it is important to note their BDE and IP values, which may have a bearing on their pro-oxidant/antioxidant activity. The higher pro-oxidant activity of phenolic antioxidants may be responsible for the generation of ROS.

We previously reported that the COX-2-inhibitory activity of curcumin-related compounds was related to quantum chemical descriptors such as η, χ and ω (34, 35). Some previous studies have evaluated biological activities based on the concept of chemical hardness (36-38). The calculated χ and ω values for curcumin with potent COX-2-inhibitory activity are greater than those for tetrahydrocurcumin, isoeugenol or eugenol, whereas the η value for curcumin is smaller. These values for tetrahydrocurcumin, isoeugenol and eugenol were almost identical to each other. These findings indicate that the COX-2- and NF-κB- inhibitory activities of curcumin are possibly related to η-, χ- and/or ω-controlled enzymes. Curcumin, with anti-inflammatory activity, is a well-known potent inhibitor of reactive oxygen-generating enzymes such as lipoxygenase/cyclooxygenase, xanthine dehydrogenase/ oxidase and inducible nitric oxide synthase. We previously reported quantitative structure-activity relationships (QSAR) for curcumin-related compounds such as vanillin, eugenol dimer, and isoeugenol dimers using η, χ and ω, and our findings indicated that those compounds with higher χ and ω, and lower η values had potent anti-inflammatory activities (35, 36). The chemical hardness concept may confirm the importance of analyzing the origin of COX inhibitors, and, in line with this concept, quantitative structure-activity relationships could be the subject of future mechanistic studies.

In conclusion, curcumin shows strong COX-2- and NF-κB-inhibitory activities, whereas tetrahydrocurcumin is less effective. The anti-inflammatory activities of these

compounds may be related to η-, χ- and/or ω-controlled enzymes. The BDE and IP values for curcumin and tetrahydrocurcumin suggest that the former may possesses pro-oxidative activity in biological systems.

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