Comparative Antioxidant Activities of Curcumin and Its Demethoxy and Hydrogenated Derivatives

Poorichaya SOMPARN,[a] Chada PHISALAPHONG,[b] Somjai NAKORNCHAI,[a] Supeenun UNCHERN,[c] and Noppawan Phumala MORALES*,[c]

[a] *Department of Pharmacology, Faculty of Pharmacy, Mahidol University; Bangkok 10400, Thailand:* [b] *Government Pharmaceutical Organization; Rama 6 Rd, Rajatevee, Bangkok 10400, Thailand: and* [c] *Department of Pharmacology, Faculty of Science, Mahidol University; Rama 6 Rd, Rajatevee, Bangkok 10400, Thailand.*
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The antioxidant activities of curcumin, its natural demethoxy derivatives (demethoxycurcumin, Dmc and bisdemethoxycurcumin, Bdmc) and metabolite hydrogenated derivatives (tetrahydrocurcumin, THC; hexahydrocurcumin, HHC; octahydrocurcumin; OHC) were comparatively studied using 2,2-diphenyl-1-picrylhydrazyl (DDPH) radical, 2,2′-azobis(2-amidinopropane)dihydrochloride (AAPH) induced linoleic oxidation and AAPH induced red blood cell hemolysis assays. Hydrogenated derivatives of curcumin exhibited stronger DPPH scavenging activity compared to curcumin and a reference antioxidant, trolox. The scavenging activity significantly decreased in the order THC>HHC=OHC>trolox>curcumin>Dmc>>>Bdmc. Stronger antioxidant activities toward lipid peroxidation and red blood cell hemolysis were also demonstrated in the hydrogenated derivatives. By the model of AAPH induced linoleic oxidation, the stoichiometric number of peroxyl radical that can be trapped per molecule (*n*) of hydrogenated derivatives were 3.4, 3.8 and 3.1 for THC, HHC and OHC, respectively. The number (*n*) of curcumin and Dmc were 2.7 and 2.0, respectively, which are comparable to trolox, while it was 1.4 for Bdmc. The inhibition of AAPH induced red blood cell hemolysis significantly decreased in the order OHC>THC=HHC>trolox>curcumin=Dmc. Results in all models demonstrated the lower antioxidant activity of the demethoxy derivatives, suggesting the *ortho*-methoxyphenolic groups of curcumin are involved in antioxidant activities. On the other hand, hydrogenation at conjugated double bonds of the central seven carbon chain and β diketone of curcumin to THC, HHC and OHC remarkably enhance antioxidant activity.

Key words antioxidant activity; curcumin; hexahydrocurcumin; octahydrocurcumin; tetrahydrocurcumin

Oxidative stress plays a major role in the pathogenesis of various diseases including neurodegenerative diseases, myocardial ischemia-reperfusion injury and cancer. Curcumin [1,7-bis(4-hydroxy-3-methoxyphenyl)-1,6-heptadiene-3,5,-dione], the principal yellow pigment isolated from turmeric (*Curcuma longa Linn*), is known as a potent antioxidant comparable to α-tocopherol. Its antioxidant activities have been studied in several *in vitro* models.[1] Despite its poor bioavailability,[2—4] the therapeutic benefits of curcumin in animals have been demonstrated in several oxidative stress models such as Alzheimer's disease,[5] ethanol induced oxidative injury in brain, liver, heart and kidney,[6,7] and myocardial ischemic damage.[8] It is possible that the metabolites of curcumin could mediate major antioxidant activities *in vivo*.

In mouse, curcumin is first biotransformed to dihydrocurcumin (DHC) and tetrahydrocurcumin (THC) and these compounds are subsequently converted to monoglucuronide conjugates including curcumin-glucuronide, dihydrocurcumin-glucuronide and tetrahydrocurcumin-glucuronide.[9] In human and rat hepatocytes, curcumin is metabolized into curcumin glucuronide, curcumin sulfate, THC, hexahydrocurcumin (HHC) and octahydrocurcumin (OHC).[10,11]

Antioxidant activities of THC have already been studied both *in vitro* and *in vivo*. Venkatesan *et al.*[12] reported that THC had higher activity than curcumin in protecting the nitrite induced oxidation of haemoglobin and lysis of erythrocytes. THC has also been demonstrated to be more potent than curcumin in protection against ferric nitrilotriacetate (Fe-NTA) induced oxidative renal damage in mice.[13] THC produces this protective effect to cells against oxidative stress by scavenging of free radicals,[14] inhibition of lipid peroxidation and formation of hydroperoxides.[15] To the best of our knowledge, the antioxidant activities of HHC and OHC have not been reported.

We are also interested in the antioxidant activities of natural demethoxyl derivatives of curcumin, demethoxycurcumin (Dmc) and bisdemethoxycurcumin (Bdmc), which are always found together with curcumin in tumeric extracts and in commercial preparation of curcumin. Antioxidant activities of Dmc and Bdmc have been reported in the model systems of hydroxyl radical induced DNA damage,[16] DPPH radical scavenging activity[17] and recently in lipid peroxidation.[18] However, to date, there has been no comparative study on the antioxidant activities of curcumin with its natural demethoxy derivatives and metabolite hydrogenated derivatives. In particular, the ability to provide protection against lipid and cell membrane damage of all of those derivatives has not yet been reported.

The aim of this study is to compare the antioxidant activities of curcumin, its demethoxy derivatives (Dmc and Bdmc), and hydrogenated derivatives (THC, HHC and OHC) using three *in vitro* models: radical scavenging activity by DPPH (2,2-diphenyl-1-picrylhydrazyl) assay, AAPH [2,2′-azobis(2-amidinopropane)dihydrochloride] induced linoleic acid oxidation and AAPH induced red blood cells hemolysis. The relationship between chemical structure and ability to protect against lipid and cell membrane damage was also demonstrated in this study.

MATERIALS AND METHODS

Separation of Curcumin, Demethoxycurcumin and Bis-

* To whom correspondence should be addressed. e-mail: scnpm@mahidol.ac.th

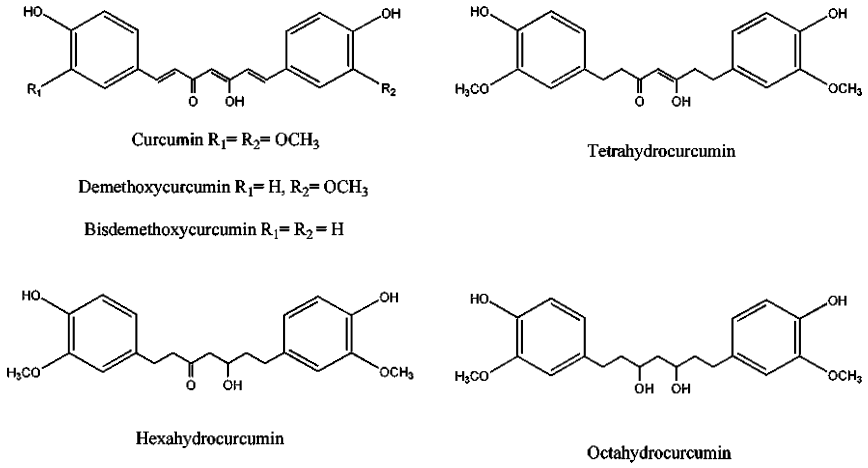

Fig. 1. Chemical Structures of Curcumin and Its Demethoxy and Hydrogenated Derivatives

demethoxycurcumin Curcumin, demethoxycurcumin (Dmc) and bisdemethoxycurcumin (Bdmc) were separated from curcuminoids (Government Pharmaceutical Organization, Bangkok, Thailand). Curcuminoids were subjected to silica gel column chromatography using 10% (v/v) MeOH/$CHCl_3$ as the eluting solvent. The eluted solution was subjected to thin layer chromatography on silica gel using chloroform–ethanol (25 : 1 (v/v)) as the mobile solvent. Three orange bands were separated, and were eluted with acetone. After evaporation and crystallization from ethanol, the top band gave curcumin as orange yellow needles. The middle band crystallized from methanol gave demethoxycurcumin as orange crystals and the lowest band crystallized from an ethyl acetate–methanol mixture gave bisdemethoxycurcumin as orange powder.[16] The compounds were identified by using MS and NMR spectra. Curcumin, MS *m/z*: 368 (M^+), ^{1}H-MNR ($CDCl_3$), δ=7.26 (2 H, d, *J*=16 Hz), 7. 13 (2 H, d, *J*=8 Hz), 7.05 (2 H, S), 6.92 (2 H, d, *J*=8 Hz), 6.70 (2 H, d, *J*=16 Hz), 5.85 (1 H, s), 3.95 (6 H, s).[19] Demethoxycurcumin, MS *m/z*: 338 (M^+), ^{1}H-MNR ($CDCl_3$), δ=7.63 (2 H, d , *J*=16 Hz), 7.48 (H, d, *J*=8 Hz), 6.46 (2 H, d, *J*=16 Hz), 7.26 (H, d, *J*=8 Hz), 7.01 (H, d, *J*=8 Hz), 7.13 (H, d, *J*=8 Hz), 6.87 (d, *J*=8 Hz), 3.91 (3 H, s).[20] Bisdemethoxycurcumin, MS *m/z*: 308 (M^+), ^{1}H-MNR ($CDCl_3$), δ=7.61 (2 H, d, *J*=16 Hz), 7.43 (4 H, d, *J*=8 Hz), 6.90 (4 H, d, *J*=8 Hz), 6.5 (2 H, d, *J*=16 Hz), 5.81 (1 H, S, 1-H).[18]

Synthesis of Tetrahydrocurcumin, Hexahydrocurcumin and Octahydrocurcumin Curcumin was converted to tetrahydrocurcumin (THC) by hydrogenation with palladium-carbon (Pd/C) as the catalyst.[19] Hexahydrocurcumin (HHC) and octahydrocurcumin (OHC) were synthesized from tetrahydrocurcumin by reduction with sodium borohydride,[20] and the products were confirmed using MS and NMR spectra. Tetrahydrocurcumin, MS *m/z*: 372 (M^+), ^{1}H-MNR ($CDCl_3$), δ=6.82 (2 H, d, *J*=8 Hz), 6.69 (2 H, s), 6.65 (2 H, d, *J*=8 Hz), 5.51 (2 H), 5.43 (1 H, s), 3.87 (6 H, s), and 2.5-3 (8 H, m).[19] Hexahydrocurcumin, MS *m/z*: 374 (M^+), ^{1}H-MNR ($CDCl_3$), δ=6.81 (2 H, d, *J*=8 Hz), 6.61 (2 H, s), 6.65 (2 H, d, *J*=8 Hz), 5.50 (2 H), 4.00 (1H), 3.81 (6H, s), 2.5—2.9 (8H, m), 1.7 (2 H, m).[19] Octahydrocurcumin, MS *m/z*: 376 (M^+), ^{1}H-MNR ($CDCl_3$), δ=1.68 (2 H, m), 2.15 (4 H, m), 2.45—2.78 (4 H, m), 3.80 (6 H, s), 3.91 (2 H, br s), 5.98 (2 H, s), 6.64 (2 H, d, *J*=8 Hz), 6.68 (2 H, br s), 6.80 (2 H, bd, *J*=8 Hz).[21]

The structures of curcumin and the demethoxy and hydrogenated derivatives are shown in Fig. 1.

DPPH Radical Scavenging Assay The hydrogen donating or radical scavenging ability of curcumin and its derivatives was evaluated by using a stable radical, DPPH.[22] Curcumin and its derivatives were dissolved in methanol into various concentrations. An aliquot of 0.2 ml of antioxidant was mixed with 0.1 ml of 0.16 mM DPPH solution (Sigma, St. Louis, MO, U.S.A.) in a total volume of 3 ml made up of methanol. Final concentrations of the test antioxidants were 12.5 to 200 μM. The decrease in absorbance at 515 nm was determined continuously for 15 min with a spectrophotometer (UV–Visible Spectrometer GBC Cintra 4.0). Trolox (Sigma, St. Louis, MO, U.S.A.) was used as a standard antioxidant. All determinations were performed in triplicate. The percentage of inhibition (% inhibition) was calculated following the equation: % inhibition=[1−(Ab_{515} sample/Ab_{515} control)]×100. The IC_{50} value (the inhibition concentration of sample at 50% fall in absorbance of DPPH) was used to compare DPPH scavenging activity.

Inhibition of AAPH Induced Linoleic Acid Oxidation Inhibitory activity toward lipid peroxidation was determined according to the method of Liegois *et al.*[23] The linoleic acid peroxidation was thermally initiated at physiological temperature by a water soluble azo initiator, AAPH. The linoleic acid emulsion was prepared by adding drop wise 0.25 ml linoleic acid (Cayman Chemical, Ann Arbor, MI, U.S.A.) to 5 ml of 0.05 M borate buffer (pH 9) containing 0.25 ml of Tween 20. The resulting dispersion was clarified by adding 1 ml of 1 N sodium hydroxide. The volume was adjusted to 50 ml with additional borate buffer. The 16 mM linoleic acid solution was stored at 4°C in the dark under a nitrogen atmosphere until needed. Thirty microliters of the 16 mM linoleic acid dispersion was added to a cuvette containing 2.81 ml of 0.05 M phosphate buffer, pH 7.4, prethermostated at 40°C. The oxidation reaction was initiated at 37°C under air by the addition of 150 μl of 40 mM AAPH solution (Cayman Chemical, Ann Arbor, MI, U.S.A.). Oxidation was carried out in the presence of 10 μl aliquots of the test antioxidants in methanol (final concentration in test solution=1—12 μM). In the assay without antioxidant, lipid oxidation was measured in the presence of the same amount of methanol.

The rate of oxidation at 37°C was monitored by recording the increase in absorption at 234 nm caused by conjugated diene hydroperoxide formation. A molar extinction coefficient of 28000 $m^{-1} cm^{-1}$ was used to calculate diene formation. In all cases, the measurements were run in duplicate against the buffer and compared with a separate AAPH-free control to check for any spontaneous oxidation. AAPH has a relatively high absorbance below 260 nm, therefore, its absorbance measured in a separate cuvette in the absence of linoleic acid was subtracted from each experimental point.

Inhibitory Effects on Red Blood Cell Hemolysis The inhibitory activity of free radical induced red blood cell damage was evaluated as described by Dang *et al.*[24] Blood samples were obtained by venipuncture from healthy volunteers and collected in heparinized tubes. After centrifugation at 2500 rpm for 10 min, the plasma and buffy coat were removed. Erythrocytes were washed three times with 10 mM phosphate buffer saline (PBS), pH 7.4. During the last washing the cells were centrifuged at exactly 2500 rpm for 10 min to obtain a constantly packed cell volume. The 5% (v/v) suspension of washed erythrocytes was incubated at 37°C for 5 min and then a PBS solution of AAPH was added to initiate hemolysis. The reaction mixture was shaken gently while being incubated at 37°C. At 30 min intervals, a volume of 400 μl of reaction mixture was removed and diluted with 3.6 ml of 0.9% (w/v) NaCl, and centrifuged at 2000 rpm for 10 min to separate the red blood cells. The absorbance (A) of the resulting supernatant was measured at 540 nm using a spectrophotometer (UV–Visible Spectrometer GBC Cintra 4.0). Similarly, the reaction mixture was treated with 3.6 ml of distilled water to obtain complete hemolysis, and the absorbance (B) of the supernatant was analyzed under the same conditions at 540 nm. To study the antioxidant effect, the test antioxidants were dissolved in dimethyl sulfoxide (DMSO) to a final concentration of 10—30 μM. The final concentration of DMSO was 0.1% (v/v). An aliquot of 5 μl of the antioxidant was added and incubated at 37°C for 5 min before addition of AAPH. The percentage hemolysis was calculated from ratio of (A/B)×100. Antioxidant activity is expressed as the time to 50% of maximal hemolysis (HT_{50}) by plotting between the percentage hemolysis and incubation time. Since DMSO can scavenge free radical,[25] the effect of DMSO at the same concentration of the antioxidant solution was also determined.

Statistical Analysis Statistical analyses were carried out using SPSS version 11.5. Differences among the tested antioxidants were analyzed by using one-way ANOVA using Tukey's test as a post test. Values are expressed as the mean±S.D. and differences between groups were considered to be significant at $p<0.05$

RESULTS

DPPH Radical Scavenging Activities The results of the DPPH scavenging activity of curcumin, its demethoxy derivatives (demethoxycurcumin, Dmc and bisdemethoxycurcumin, Bdmc) and hydrogenated derivatives (tetrahydrocurcumin, THC; hexahydrocurcumin, HHC; octahydrocurcumin; OHC) are shown in Table 1. The IC_{50} value indicated that the scavenging activity of curcumin was comparable to trolox, the water soluble derivative of vitamin E. Dmc and Bdmc were about 2 and 7 fold less potent than curcumin. Interestingly THC, HHC and OHC were remarkably more potent than curcumin. The scavenging activity of curcumin and its derivatives significantly decreased in the order: THC> HHC=OHC>trolox>curcumin>Dmc≫Bdmc.

Table 1. IC_{50} Values of Curcumin, Its Derivatives and Trolox on DPPH-Radical Scavenging Activity

Antioxidant	IC_{50} (μM)[a]
Curcumin	35.1*
Dmc	53.4*,**
Bdmc	>200*,**
THC	18.7*,**
HHC	21.6*,**,†
OHC	23.6*,**,†
Trolox	31.1

a) 50% inhibition concentration; concentration of antioxidant required to quench 50% of DPPH radical. A lower IC_{50} indicates greater antioxidant activity. * $p<0.05$ compared with trolox. ** $p<0.05$ compared with curcumin. † $p<0.05$ compared with THC.

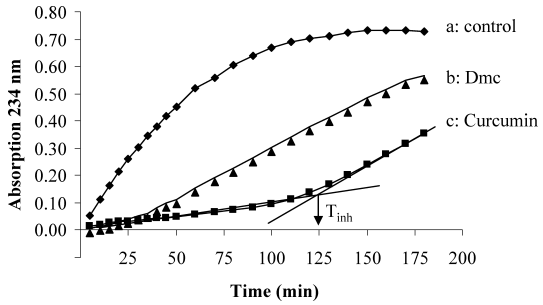

Fig. 2. Formation of Conjugated Dienes during the Oxidation of Linoleic Acid (0.16 mM) at pH 7.4 and 37°C, Initiated with 2 mM AAPH

Line (a) control, without antioxidant; (b) in the presence of Dmc (3 μM); (c) in the presence of curcumin (3 μM).

Table 2. Antioxidant Activities of Curcumin, Its Derivatives and Trolox by AAPH Induced Linoleic Acid Oxidation

Antioxidant	R_{inh}/R_o[a]	Antioxidant activity (min/μM)[b]	n[c]
Curcumin	0.09±0.01*	46.2±8.1*	2.7
Dmc	0.26±0.02*,**	20.3±2.8**	2.0
Bdmc	0.30±0.01*,**	10.0±1.2**	1.4
THC	0.13±0.01*,**	69.3±6.6*,**	3.4
HHC	0.20±0.03*,**,†	30.8±3.3*,**,†	3.8
OHC	0.19±0.02*,**,†	40.3±8.3*,†	3.1
Trolox	0.51±0.03	16.0±1.2	2.0

Values are expressed as the mean±S.D. of 3 independent experiments. * $p<0.05$ compared with trolox. ** $p<0.05$ compared with curcumin. † $p<0.05$ compared with THC. *a*) The initial rates of oxidation in the presence of 3μM antioxidant (except Bdmc 12 μM) compared to the control. *b*) Slope of the plot between (inhibition time) T_{inh} *versus* antioxidant concentrations. *c*) n represents the stoichiometric number of peroxyl radicals trapped per molecule of antioxidant.

Inhibition of AAPH-Induced Linoleic Acid Oxidation A kinetic curve of the conjugated diene formation during linoleic acid oxidation is shown in Fig. 2. Addition of curcumin to the reaction decreased the rate of conjugated diene formation. From this curve the initial oxidation rate in the presence (R_{inh}) and absence of antioxidant (R_o), as well as the inhibition time (T_{inh}) of each concentration of antioxidants, were obtained. The first antioxidant index was the ratio R_{inh}/R_o as shown in Table 2. A lower R_{inh}/R_o value indicates a higher antioxidant activity. Comparing across the same con-

Table 3. Comparison of Time to 50% of Maximal Hemolysis (HT_{50}) of Curcumin, Its Derivatives and Trolox at 30 μM

Antioxidant	HT_{50} (min)
Control	125.5±0.6
DMSO	133.7±1.7
Curcumin	184.8±7.5*
Dmc	181.1±1.3*
THC	215.1±0.3*,**
HHC	212.7±2.4*,**
OHC	224.7±3.1*,**,†
Trolox	195.7±2.0

Values are expressed as the mean±S.D. of 3 independent experiments. *$p<0.05$ compared with trolox. **$p<0.05$ compared with curcumin. †$p<0.05$ compared with THC.

centration (3 μM), THC, HHC, OHC, curcumin and Dmc were about 2 to 6 fold more potent than trolox in inhibiting conjugate diene formation. However, high concentrations of Bdmc were needed for its antioxidant activity.

The much more informative parameters were antioxidant activity and the stoichiometric coefficient *n*, which represents the number of peroxyl radicals trapped by each molecule of antioxidant (Table 2). The antioxidant activity was obtained from the slope of the curve between Tinh *versus* the antioxidant concentration. This value indicates the quantitative ability of 1 μM antioxidant to inhibit lipid peroxidation in a period of time. Curcumin and its derivatives, except DMc and Bdmc, showed significantly higher antioxidant activities than trolox. THC has the highest activity among the test compounds. The antioxidant activity significantly decreased in the order: THC>curcumin>=OHC>=HHC>Dmc>=trolox>Bdmc.

Using trolox as a reference inhibitor removing 2 peroxyl radicals per added molecule, *n* of each antioxidant (x) was calculated by the following equation: $R_{i(x)} \cdot T_{inh(x)} \cdot 2[\text{trolox}]/R_{i(\text{trolox})} \cdot T_{inh(\text{trolox})} \cdot [x]$. The results showed that 1 molecules of THC, HHC or OHC can trap more than 3 molecules of peroxyl radicals. The ability to trap peroxyl radicals by curcumin and Dmc was equal to trolox (*n* about 2), whereas that of Bdmc was lower.

Inhibition of AAPH Induced Red Blood Cell Hemolysis The protective effect of curcumin and its derivatives against AAPH induced red blood cell hemolysis is expressed as the time to 50% of maximal hemolysis (HT_{50}) and is shown in Table 3. All of the test antioxidants significantly prolonged the HT_{50} compared to the control. As with the other models, the hydrogenated derivatives, THC, HHC and OHC showed a significantly higher protective activity than trolox, curcumin and Dmc. On the other hand, curcumin and Dmc had a significantly lower activity than trolox. Bdmc had no activity at the test concentrations up to 30 μM. It should be noted that DMSO, as a solvent, had only a slight effect in this model. The protective activity on AAPH induced red blood cell hemolysis significantly decreased in the order: OHC>THC=HHC>trolox>curcumin=Dmc.

DISCUSSION

The antioxidant activities of curcumin, the natural demethoxy derivatives (Dmc and Bdmc) and metabolite hydrogenated derivatives (THC, HHC and OHC) have been compared using three *in vitro* models. The DPPH scavenging assay is a widely used method to primarily evaluate free radical scavenging activity. The effects of antioxidants on DPPH stable radical are thought to be due to their hydrogen donating ability.[26] Biomolecules such as lipid, protein and DNA are the target sites of free radical damage in living organisms, and, therefore, a potential antioxidant *in vivo* should have inhibitory effects against lipid peroxidation and membrane damage. In this study, AAPH induced linoleic acid peroxidation and RBC hemolysis were used as lipid peroxidation and biomembrane damage models, respectively. The peroxidation was initiated by AAPH that could decompose at physiological temperature and generate alkyl radicals to initiate lipid peroxidation. Since AAPH is water-soluble and the generation rate of free radicals from the decomposition of AAPH can be easily controlled and measured,[23] the highly informative parameter, namely the number of peroxyl radical trapped per molecule of the antioxidant (*n*), can be estimated.

In all three of our study models, curcumin demonstrated antioxidant activities comparable to trolox. Hydrogenated derivatives of curcumin showed a remarkably higher activity than curcumin, suggesting that the hydrogenation at conjugated double bonds of the central seven carbon chain and β-diketone of curcumin improved antioxidant activities. The number (*n*) of THC, HHC and OHC was 3.4, 3.8 and 3.1, respectively while it was 2.7 for curcumin. Even though the hydrogenated derivatives of curcumin have less polarity than curcumin, they showed higher protective activity toward AAPH induced red blood cell hemolysis. Sugiyama *et al*.[27] have reported that THC showed a greater inhibitory effect than curcumin on lipid peroxidation of erythrocyte membrane ghosts induced by *tert*-buthylhydroperoxide. Moreover, this study has suggested that the β-diketone moiety of THC exhibits antioxidant activity by cleavage of the C–C bond at the active methylene carbon between two carbonyls in the β-diketone moiety.

On the other hand, lower antioxidant activity was found in the demethoxy derivatives. Bdmc has negligible antioxidant activity, especially in the red blood cell hemolysis model. The number (*n*) of Dmc and Bdmc was 2.0 and 1.4, respectively. In agreement with other studies[17,18,24,28,29] curcumin was more effective than Dmc and Bdmc at scavenging DPPH radicals, and inhibiting free radical induced lipid peroxidation, red cell hemolysis, and protein oxidation.

It is controversial with respect to whether the phenolic hydrogen or the central methylenic hydrogen in the hepadienone moiety is responsible for the antioxidant mechanisms of curcumin. Jovanovic *et al*.[30] studied the antioxidant mechanism of curcumin by laser flash photolysis and pulse radiolysis, and demonstrated that in acidic and neutral aqueous solutions (pH 3 to 7) curcumin is a superb H-atom donor, donating the H-atom from the central methylenic group rather than from phenolic group. In contrast, Priyadasrini *et al*.[31] reported that curcumin showed much greater ablility to inhibit lipid peroxidation than its dimethoxy counterpart, suggesting that the phenolic group is essential for the antioxidant activity. Recently, Chan *et al*.[29] demonstrated that the antioxidant activity of curcumin is due not only to the number of phenolic groups but also the *ortho*-methoxyphenolic functionality. The *ortho*-methoxy group can form an intramolecular hydrogen bond with the phenolic hydrogen,

making the H-atom abstraction from the *ortho*-methoxyphenols surprisingly easy. In our study, therefore, the decreasing antioxidant activities of Dmc and Bdmc could be caused by the lack of an *ortho*-methoxy group.

Our comparative study demonstrated that the antioxidant activities of curcumin and its derivatives can arise both from the *ortho*-methoxyphenol and from a central methylenic hydrogen in the central seven carbon chain and β-diketone moiety. However, we found that the decrease of one methoxy group of curcumin caused a great decrease in antioxidant activities while the addition of a H atom to THC caused only a slightly change in antioxidant activities. Even though there are 2 and 4 additional H atoms in HHC and OHC, respectively, the increased antioxidant activities of THC, HHC and OHC were not in the order of the addition of H atoms.

In conclusion, the results demonstrate that the *ortho*-methoxyphenolic group is important for the antioxidant activity of curcumin. However, hydrogenation of the heptadiene moiety of curcumin remarkably enhanced antioxidant activity. All of the metabolite hydrogenated derivatives of curcumin (THC, HHC and OHC) showed greater DPPH scavenging activity, inhibition of linoleic acid peroxidation and free radical induced red blood cell hemolysis than their curcumin parent compound. The good antioxidant and pharmacological properties of curcumin *in vivo* could be mediated principally *via* its active metabolites such as THC, HHC and OHC.

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REFERENCES

1) Maheshari R. K., Singh A. K., Gaddipati J., Srimal R. C., *Life Sci.*, **78**, 2081—2087 (2006).
2) Ravindranath V., Chandrasekhra N., *Toxicology*, **16**, 259—265 (1980).
3) Cheng A. L., Lin J. K., Hsu M. M., Shen T. S., Ko J. Y., Lin J. T., Wu M. S., Yu H. S., Jee S. H., Chen G. S., Chen T. M., Chen C. A., Lai M. K., Pu Y. S., Pan M. H., Wang U. J., Tsai C. C., Hsieh C. Y., *Proc. Am. Soc. Clin. Oncol.*, **17**, 558a (1998).
4) Shientarma R. A., Hill K. A., McLelland H. R., Ireson C. R., Euden S. A., Manson W. P., *Clin. Cancer Res.*, **7**, 1834—1900 (2001).
5) Ringman J. M., Frautschy S. A., Cole G. M., Masterman D. L., Cummings J., *Current Alzheimer Res.*, **2**, 131—136 (2005).
6) Rajakrihna V., Viswanathan P., Rajasekharan K. N., Menon V. P., *Phytother. Res.*, **1**, 571—574 (1999).
7) Rukkumani R., Aruna K., Varma P. S., Rajasekaran K. N., Menon V. P. M., *J. Pharm. Pharmaceu. Sci.*, **7**, 274—283 (2004).
8) Manikandan P., Sumitra M., Aishwarya S., Manohar B. M., Lokanadam B., Puvanakrihnan R., *Int. J. Biochem. Cell Biol.*, **36**, 1967—1980 (2004).
9) Lin J. K., Pan M. H., Lin-Shian S. Y., *Biofactor*, **13**, 153—158 (2000).
10) Radindranath V., Chandrasekhara N., *Toxicology*, **22**, 337—344 (1982)
11) Ireson C. R., Jones D. J. L., Orr S., Coughtrie M. W. H., Boocock D. J., Williams P., Farmer P. B., Steward W. P., Gescher A. J., *Cencer Epidemiol. Biomarkers Prev.*, **11**, 105—111 (2002).
12) Venkatesen P., Unnikrishnan M. K., Sudheer Kumar M., Rao M. N. A., *Curr. Sci.*, **84**, 74—78 (2003).
13) Okada K., Wangpoengtrskul C., Tanaka T., Toyokuni S., Uchida K., Osawa T., *J. Nutr.*, **131**, 2090—2095 (2001).
14) Khope S. M., Priyadarisni K. I., Guha S. N., Satav J. G., Venkatesan P., Rao M. N., *Biosci. Biotechnol. Biochem.*, **64**, 503—509 (2000).
15) Pari L., Murugan P., *Pharm. Res.*, **49,** 481—486 (2004).
16) Ahsan H., Parveen N., Khan N. U., Hadi S. M., *Int. Chem. Biol.*, **121**, 161—175 (1999).
17) Wei Q. Y., Chen W. F., Zhou B., Yang L., Liu Z. L., *Biochem. Biophys. Acta*, **1760**, 70—77 (2006).
18) Jayaprakasha G. K., Rao J. L., Sakariah K. K., *Food Chem.*, **98**, 720—724 (2006).
19) Roughley P. J., Whiting D. A., *J. Chem. Soc., Perkin Trans. 1*, **20**, 2379–2388 (1973).
20) Vevkateswarlu S., Ramachandra M. S., Rambabu M., Subbaraju G. V., *Indian J. Chem.*, **40B**, 495—497 (2001).
21) Ohtsu H., Xiao Z., Ishida J., Nagai M., Wang H. K., Itokawa H., Su C. Y., Shin C., Shih C., Chiang T., Chang E., Lee Y., Tsai M. Y., Chang C., Lee K. H., *J. Med. Chem.*, **45**, 5037—5042 (2002).
22) Chan C. W., HO C. T., *J. Food Lipid*, **2**, 35—46 (1995).
23) Liegois C., Lermusieau G., Collin S., *J. Agric. Food Chem.*, **48**, 1129—1134 (2000).
24) Dang S. L., Chen W. F., Zhou B., Yang L., Liu Z. L., *Food Chem.*, **98**, 112—119 (2005).
25) Schaich K. M., Borg D. C., *Free Radic. Res. Commun.*, **9**, 267—278 (1990).
26) Gulcin L., Alici H. A., Cesur M., *Chem. Pharm. Bull.*, **53**, 281—285 (2005).
27) Sugiyama Y., Kawakishi S., Osawa T., *Biochem. Pharm.*, **52**, 519—525 (1996).
28) Song E. K., Cho H., Kim J. S., Kim N. Y., An N. H., Kim J. A., Lee S. H., Kim Y. C., *Planta. Med.*, **67**, 876—877 (2001).
29) Chen W. F., Deng S. L., Zhou B., Yang L., Liu Z. L., *Free Radic. Biol. Med.*, **40**, 526—535 (2006).
30) Jovanovic S. V., Steenken S., Boone C. W., Simic M. G., *J. Am. Chem. Soc.*, **121**, 9677—9678 (1999).
31) Priyadarsini I. K., Maity D. K., Naik G. H., Sudheer K., Unnikrishnan M. K., Satav J. G., Mohan H., *Free Radic. Biol. Med.*, **35**, 475—484 (2003).