

Comparative Radical-scavenging Activity of Curcumin and Tetrahydrocurcumin with Thiols as Measured by the Induction Period Method

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Abstract. *Background:* The *in vivo* radical-scavenging activity of curcumin (CUR) and THC (tetrahydrocurcumin, a metabolite of CUR) does not occur in isolation, but through an intricate antioxidant network together with co-antioxidants such as glutathione (GSH). In the present investigation, the radical-scavenging activity of CUR and THC with 2-mercapto-1-methylimidazole (MMI, a thiol) was studied using the induction period method. *Materials and Methods:* The induction period (IP) and propagation rate (Rp) for mixtures of MMI with THC or CUR were determined by differential scanning calorimetry (DSC) monitoring of the polymerization of methyl methacrylate (MMA) initiated by thermal decomposition of benzoyl peroxide (BPO, a source of peroxy radical, PhCOO·) or 2,2'-azobisisobutyronitrile (AIBN, a source of alkyl radical, R·) under nearly anaerobic conditions. *Results:* The stoichiometric number of PhCOO· radicals that could be trapped per molecule (n) was 3.4 and 3.3 for CUR and THC, and that of the R· radical was 3.1 and 2.5, respectively. At a molar ratio of antioxidant:co-antioxidant (MMI) = 1:5, a THC/MMI mixture with PhCOO· enhanced the total radical-scavenging activity, possibly due to partial regeneration of THC, whereas a CUR/MMI mixture with PhCOO· reduced it. Similarly, CUR/MMI and THC/MMI mixtures with R·, particularly the former, reduced the total radical-scavenging activity, possibly due, in part, to destructive interference between the antioxidant and the co-antioxidant. *Conclusion:* THC oxidized by peroxy radicals may be more antioxidative than the corresponding CUR in the interplay with GSH.

Curcumin (CUR) and tetrahydrocurcumin (THC), a reduced derivative of CUR, and one of its major metabolites, have received attention because of their anti-inflammatory, antioxidant and anticancer activities (1-7). The physiological and pharmacological characteristics of the protective effects of THC *in vivo* are similar to those of CUR (8). A recent comparative study of the antioxidant activities of CUR and THC has indicated that the scavenging activity of THC was significantly greater than that of CUR, using the 2,2'-diphenyl-1-picrylhydrazyl (DPPH) radical and 2,2'-azobis(2-aminopropane) dihydrochloride (DPPH)-induced red blood cell hemolysis assay (9). THC showed a more pronounced protective effect than CUR against chloroquine-induced hepatotoxicity in rats (5). We have previously proposed a quantitative model rationalizing the radical-scavenging activity of quercetin and CUR, with thiols (2-mercapto-1-methylimidazole, MMI) as co-antioxidants, in the polymerization of methyl methacrylate (MMA) initiated by the thermal decomposition of 2,2'-azobisisobutyronitrile (AIBN) and benzoyl peroxide (BPO) under nearly anaerobic conditions (4, 10). This model may be able to explain the mechanism of the antioxidant activity of polyphenols, because measurement by differential scanning calorimetry (DSC) is highly sensitive, and this system, under nearly anaerobic conditions, is relatively biomimetic, since oxygen is sparse in living cells (11). In addition, since cancer cells have anaerobic metabolism (12), our biomimetic system may be a good model for evaluating the antioxidant activity of anticancer drugs.

Antioxidants do not act in isolation, but rather as part of an intricate network, and recently the regeneration of polyphenols by synergistic reactions with ascorbates, glutathione (GSH) or other phenolic compounds such as vitamin E has been investigated in enzymatic and non-enzymatic systems (13, 14). When supplementation with polyphenols is performed, an adequate GSH level should be maintained (13). Therefore, it is of interest to investigate the antioxidant activity of polyphenols in the interplay with GSH.

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Key Words: Curcumin, tetrahydrocurcumin, radical-scavenging activity, thiol, induction period method.

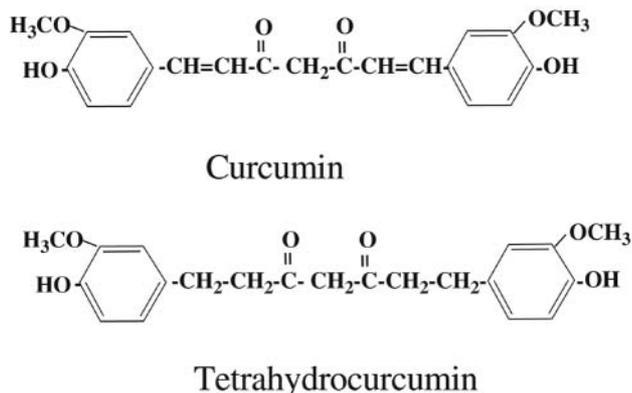


Figure 1. Chemical structure of curcumin (CUR) and tetrahydrocurcumin (THC).

The aim of the present study was to determine how CUR and THC interact with GSH in an intricate antioxidant network under cell-free conditions. The radical-scavenging activity of CUR and THC was investigated in the presence or absence of MMI, using the induction period method in the polymerization of MMA initiated by thermal decomposition of AIBN and BPO. MMI was used as a representative thiol, because GSH cannot be studied directly in this system due to its limited solubility with MMA.

Materials and Methods

Chemicals. The curcumin, MMI and MMA, were obtained from Tokyo Kasei Chem. Co., Tokyo, Japan. The THC was kindly donated by Prof. I. Yokoe, Josai University, Japan. The chemical structures of CUR and THC are shown in Figure 1. AIBN and BPO were recrystallized from chloroform and chloroform/methanol (1:1 v/v), respectively.

Induction period (IP) and initial rate of polymerization (Rp). The IP and initial rate of polymerization in the presence ($R_{p_{inh}}$) or absence ($R_{p_{con}}$) of an antioxidant were determined by the method reported previously (4, 10). The IP was calculated from the difference between the IP of specimens and that of controls. The initial rates of polymerization $R_{p_{con}}$ and $R_{p_{inh}}$ of antioxidants, co-antioxidants and antioxidant/co-antioxidant mixtures were calculated from the slopes of the plots of the first linear line representing the conversion rate of MMA polymerization (tangent drawn at the early stage of polymerization) (Figure 2).

Measurement of stoichiometric factor (n). The relative n value in Equation 1 was calculated from the IP in the presence of inhibitors:

$$n = R_i(IP)/(IH) \quad 1$$

where (IP) is the induction period in the presence of an inhibitor. The number of moles of peroxy (RhCOO) or alkyl (R) radicals trapped by the antioxidant is calculated with respect to 1 mole of

Table I. Radical-scavenging activity of tetrahydrocurcumin (THC) and curcumin (CUR) with a co-antioxidant 2-mercapto-1-methylimidazole (MMI, a thiol) in the polymerization of methyl methacrylate (MMA) initiated by thermal decomposition of benzoyl peroxide (BPO) or 2,2'-azobisisobutyronitrile (AIBN).

Phenolics, 0.01 mol%	Observed	Calculated	B-A (A/B)	$R_{p_{inh}}$ %/min
	IP (min) A	IP (min)# B		
1 mol% BPO				
THC	23.875			0.869
THC+0.01 mol% MMI	24.925	24.487**	-0.439 (1.02)	0.818
THC+0.05 mol% MMI	27.971	25.105***	-2.866 (1.11)	0.765
CUR	24.853			0.817
CUR+0.05 mol% MMI	24.723	26.083**	1.360 (0.95)	0.801
0.01 mol% MMI	0.612			0.837
0.05 mol% MMI	1.230			0.915
1 mol% AIBN				
THC	7.407			1.262
THC+0.01 mol% MMI	7.627	8.217**	0.509 (0.93)	1.318
THC+0.05 mol% MMI	10.697	11.532**	0.835 (0.93)	1.259
CUR	9.157			1.199
CUR+0.05 mol% MMI	9.722	13.282***	3.560 (0.73)	1.277
0.01 mol% MMI	0.810			1.311
0.05 mol% MMI	4.125			1.330

MMA, 9.4 mol/l; at 70 °C; conversion, 90.53-95.12%; the mean of three different experiments; standard error $\leq 5\%$; IP (induction period) = $(IP_{exp} - IP_{con})$; $R_{p_{inh}}$, R_p with an inhibitor; #simple sum of IPs (phenolics + MMI); IP_{con} for BPO and AIBN is 3.833 min and 8.916 min, respectively. Initial rate of polymerization for control ($R_{p_{con}}$) for BPO and AIBN is 0.961 %/min and 1.289 %/min, respectively. Significant differences using Student's t -test are indicated as: *** $p < 0.001$ and ** $p < 0.01$ vs. A. The procedure is described in the text.

the inhibitor moiety unit. The R_i values for AIBN and BPO at 70 °C were $5.66 \times 10^{-6} \text{ Ms}^{-1}$ and $2.28 \times 10^{-6} \text{ Ms}^{-1}$, respectively (4, 10).

Results

The observed IP (A), calculated IP (B), and B-A, A/B and $R_{p_{inh}}$ values for the BPO and AIBN systems are shown in Table I. For the BPO system at 1 mM the IP (min) declined in the order CUR (24.9) > THC (23.9) > MMI (0.6). For the AIBN system, the IP declined in the order CUR (9.2) > THC (7.4) > MMI (0.8).

For the BPO system, the observed IP, A for the THC/MMI mixture was greater than that for the calculated one, B, the simple sum of THC+MMI, whereas A for the CUR/MMI mixture was less than B. The ratio of A to B for the THC/MMI mixture at a molar ratio of 1:1 and 1:5 was 1.0 and 1.1, respectively; $A/B \geq 1$, whereas that for the CUR/MMI mixture at a molar ratio of 1:5 was 0.95; $A/B < 1$.

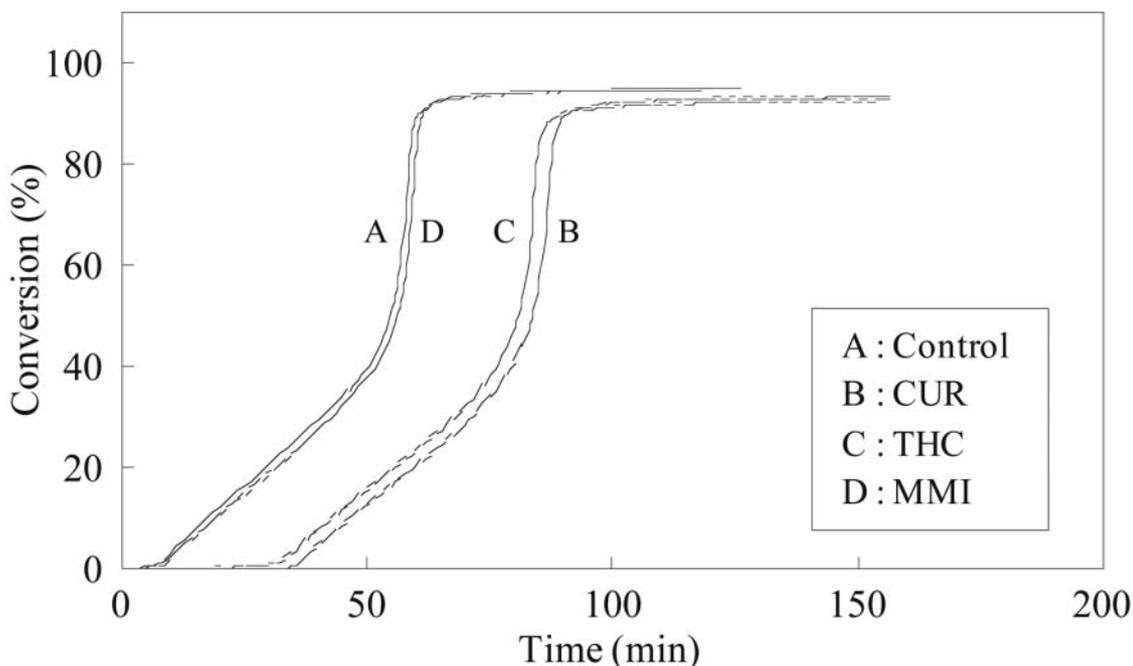


Figure 2. Time-conversion curves for the polymerization of methyl methacrylate (MMA) initiated by thermal decomposition of BPO in the absence (Control) and presence of curcumin (CUR), tetrahydrocurcumin (THC) and MMI. MMA 9.4 mol/l, BPO 0.1 mol/l, inhibitors, 1 mmol.

The radical-scavenging activity for the THC/MMI mixture was slightly but significantly more enhanced than that of the simple sum of THC+MMI. Conversely, the radical-scavenging activity of the CUR/MMI mixture was slightly but significantly less than that of the simple sum of CUR+MMI.

For the AIBN system, the observed IP for the THC/MMI mixture at 1:1 and 1:5 was less than the calculated one; $A/B=0.93$. The observed IP for the CUR/MMI mixture was markedly less than the calculated one, $A/B=0.73$; $A/B < 1$ for the CUR (or THC)/MMI mixture. The total radical-scavenging activity of the THC/MMI and CUR/MMI mixtures, particularly the latter, was greatly reduced when the $R\cdot$ radical was responsible for oxidation.

Discussion

The n values were determined using Equation 1. The n of CUR and THC for the $\text{PhCOO}\cdot$ radical was 3.4 and 3.3, respectively. n for the peroxy radical has previously been reported to be 2.7 and 3.4 for CUR and THC, respectively (5). Our result for THC showed a similar n value to that for CUR. In contrast, n of CUR and THC for the $R\cdot$ radical was 3.1 and 2.5, respectively, n for CUR being greater than that for THC. The radical-scavenging activity of CUR previously determined using electrochemical methods indicated that the number of -OH moieties for CUR appeared to be higher

than 2 during oxidation (15). This suggested that CUR undergoes chemical reactions during its oxidation, possibly involving the formation of polymeric products. The CUR radical could react with itself or with other radicals to yield stable polymeric products such as vanillin, ferulic acid and a CUR dimer (16). We have previously reported that n of CUR at higher concentrations (10 mM<) for 100 mM BPO was 1-2 (4). $n=1$ shows dimerization; radical-radical termination at position 2, $\text{R-CH=CH-CO-CH}_2\text{-CO-CH=CH-R}$ of CUR (Figure 1) (4, 17). n of CUR at lower concentrations (<1 mM) for 100 mM BPO was about 4 (data not shown).

We have previously reported that the reduction of cellular membrane mobility induced by CUR might be attributable to the generation of intracellular reactive oxygen species (ROS) induced by CUR (1-4). This is possibly due to the peroxidation of unsaturated fatty acid in the cellular membranes induced by ROS. THC, which is hydrogenated at the conjugated double bond of the central carbon seven of CUR, did not generate ROS (1). The addition of GSH prevented ROS production in CUR-treated cells, and CUR decreased the intracellular GSH level (3). Intracellular ROS generation of CUR depends on its chemical structure (1, 2). These findings probably explain the discrepancy between the reaction of CUR with THC, and that with GSH, a thiol. The present findings obtained by the induction period method indicated a great difference

between CUR/MMI and THC/MMI mixtures when oxidized by the PhCOO· radical. The total radical-scavenging activity of CUR in the interplay with GSH when oxidized by peroxy radicals derived from unsaturated fatty acids in cellular membranes may be partly reduced, possibly due to the destructive interference of CUR and GSH during oxidation. In contrast, the total radical-scavenging activity of THC in the interplay with GSH when oxidized by peroxy radicals may be unaffected or enhanced, possibly due to partial regeneration of THC. THC shows a more pronounced protective effect than CUR against chloroquine-induced hepatotoxicity in rats (6). The antidiabetic and antioxidant effects of THC in rats with streptozotocin-nicotinamide-induced diabetes are more potent than those of CUR at the same dose (7). Such protective effects of THC in rats may be due to the pronounced antioxidative activity of THC in the interplay with GSH. In the present studies, the radical-scavenging activities of CUR and THC against peroxy radicals were similar. Therefore, the greater protective effects of THC in rats, in comparison to those of CUR (6, 7), may be related to the involvement of antioxidant/co-antioxidant activity.

The $R_{p_{inh}}$ value for CUR was less than that for THC, suggesting the suppression of propagation (Table I). This indicates that CUR produces oxidation products that are capable of suppressing propagation during the induction period.

As *in vivo* experiments are too complex to be amenable to simple interpretation, we carried out physico-chemical studies using the IP method to examine the radical polymerization of MMA in the presence of antioxidants and co-antioxidants as a biomimetic model of scavenging radicals produced *in vivo*. Such studies could help to explain the mechanisms and action of chemopreventive agents.

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