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Anti- and Pro-oxidant Effects of Oxidized Quercetin, Curcumin or Curcumin-related Compounds with Thiols or Ascorbate as Measured by the Induction Period Method

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Abstract. *Phenolic antioxidants, such as quercetin (QUE), curcumin (CUR) and the CUR-related compounds eugenol (EUG) and isoeugenol (IsoEUG), do not act in isolation in vivo but form an intricate antioxidant network together with ascorbate or glutathione (GSH). To clarify the antioxidant/prooxidant activity of these compounds in their interplay with ascorbate or GSH, the induction period (IP) and propagation rate (Rp) for mixtures of 2-mercapto-1-methylimidazole (MMI, a thiol) or L-ascorbyl-2,6-dibutyrate (ASDB, an ascorbate derivative) with QUE, CUR, EUG or IsoEUG were determined from differential scanning calorimetry (DSC) monitoring of the polymerization of methyl methacrylate (MMA), initiated by thermal decomposition of 1.0 mol% benzoyl peroxide (BPO, a PhCOO· radical) under nearly anaerobic conditions. The IP (min) for 0.01 mol% test compounds declined in the order CUR (28.31) > IsoEUG (19.47) > EUG (16.83) > QUE (10.17) > MMI (2.06) > ASDB (0.16). The inhibition rate constant (k_{inh}, $M^{-1}s^{-1}$) declined in the order ASDB ($7.85x10^5$) > MMI ($5.99x10^4$) > QUE ($1.21x10^4$) > EUG ($7.93x10^3$) > IsoEUG ($7.04x10^3$) > CUR ($4.50x10^3$). The observed IP for MMI/QUE mixtures, particularly at molar ratios of 2:1 and 5:1, was significantly less than that for QUE alone as well as that calculated for MMI/QUE. The decrease in IP was similar to the observed IP in the control, suggesting the occurrence of oxygen uptake, possibly due to the formation of thiol RS radicals which, together with oxygen, produce oxo- and peroxo-sulphur radicals. The observed IPs for MMI/CUR or the MMI/IsoEUG mixtures, particularly the former, were less than the corresponding calculated IPs, suggesting co-oxidation of the MMI without oxygen uptake. In contrast, the observed IP of MMI/EUG mixtures was much greater than the corresponding calculated IP, suggesting the formation of an new antioxidative adduct between EUG-quinonemethide and MMI. The observed IP for the ASDB/QUE mixtures was greater than the corresponding calculated IP, suggesting the effectiveness of QUE as a co-antioxidant for ascorbate. In contrast, the observed IP for the ASDB/CUR mixtures was significantly less than the corresponding calculated IP, suggesting the catalytic effectiveness of CUR for ascorbate co-oxidation. Cancer cells are anaerobic in their metabolism and they selectively absorb more ascorbate than normal cells do. Thus, the present findings for the ASDB/CUR mixtures could help explain the effectiveness of CUR in chemoprevention by inducing cancer cell apoptosis. In addition, the findings for the MMI/QUE mixtures suggest the production of toxic oxo- and peroxo-sulphur radicals from thiols.*

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We have previously proposed a quantitative model rationalizing the radical-scavenging activity of monofunctional phenols (1, 2) and polyphenols (3-5) in the polymerization of methyl methacrylate (MMA) initiated by the thermal decomposition of benzoyl peroxide (BPO) under nearly anaerobic conditions. The model was able to explain the mechanism of radical-scavenging activity and to predict the chain-breaking activity of phenolic compounds (1-5), 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 (6, 7). Also, since cancer cells are anaerobic in their metabolism (the cells do not utilize oxygen (8, 9)), our biomimetic system may be a good model for evaluating the antioxidant activity of anticancer drugs.

Antioxidants do not act in isolation, but rather form an intricate network and, recently, the regeneration of polyphenols by synergistic reactions with ascorbate, glutathione (GSH), or other phenolic compounds such as vitamin E has been investigated in enzymatic and non-enzymatic systems (10-13). It is well established that ascorbate can regenerate oxidized vitamin E and that GSH can regenerate oxidized ascorbate (14, 15). Also, the prooxidant activity of polyphenolics, such as curcumin (CUR) and quercetin (QUE), after metabolic activation by peroxidase co-oxidizes ascorbate and GSH (11). Oxidized QUE reacts with thiols rather than with ascorbate (10). The aim of the present study was to determine how oxidized QUE, CUR and CUR-related compounds interact with GSH or ascorbate in an intricate antioxidant network. The antioxidant/prooxidant effects of 2-mercapto-1-methylimidazole (MMI), a thiol and L-ascorbyl-2,6-dibutyrate (ASDB), an ascorbate derivative, on the polymerization of MMA in the presence of QUE, CUR and the CUR-related compounds eugenol (EUG) and isoeugenol (IsoEUG) were studied. MMI and ASDB were used as representatives of thiols and ascorbates, respectively, because GSH and ascorbate cannot be studied directly in this system due to their limited solubility in MMA.

Materials and Methods

Materials. EUG (2-methoxy-4-allylphenol), IsoEUG (4-propenyl-2-methoxyphenol), CUR (1,7-bis-(4-hydroxy-3-methoxyphenol)-1,6-heptadiene-3,5-dione), QUE (3,3',4',5,7-pentahydroxyflavone), ASDB, MMI and MMA were obtained from Tokyo Kasei Chemical Co., Tokyo, Japan. BPO was recrystallized from chloroform/methanol (1:1 v/v).

Induction period and initial rate of polymerization. The induction period and initial rate of polymerization were determined by the method previously reported (1-5). The induction period (IP) was calculated from the difference between the IP of specimens and that of controls. The initial rates of polymerization in the absence (Rp_{con}) and presence (Rp_{inh}) of inhibitors were calculated from the slope of the plots of the first linear line of the conversion rate of MMA polymerization (tangent drawn at the early polymerization stage).

Measurement of stoichiometric factor (n). The relative *n* value in Eq. **(1)** can be calculated from the induction periods in the presence of inhibitors (1-5):

$$n = R_i[IP]/[IH] \quad \textbf{(1)}$$

where [IP] is the induction period in the presence of an inhibitor [IH]. The number of moles of peroxy radicals trapped by the relevant phenol is calculated with respect to 1 mole of inhibitor moiety unit. The R_i value of BPO at 70˚C was $2.28x10^{-6}$ Ms^{-1} (2). When polymerization of MMA is suppressed and retarded by an antioxidant, the rate can be expressed by Eq. **(2)**.

$$Rp_{inh}/Rp_{con} = (2k_tR_i)^{1/2}/\{n\ k_{inh}\ [IH]\} \quad \textbf{(2)}$$

Table I. *A free radical interaction between 2-mercapto-1-methylimidazole (MMI, a thiol) and the antioxidants quercetin (QUE), curcumin (CUR) and CUR-related compounds (eugenol (EUG), isoeugenol (IsoEUG)).*

Additives phenolics, 0.01 mol%	Observed IP (min) A	Calculated IP (min)* B	B-A	Rp_{inh} %/min
CUR	28.307			0.869
CUR + 0.01 mol% MMI	28.902	30.363	1.461	0.866
CUR+ 0.02 mol% MMI	30.455	29.078	-1.377	0.854
CUR + 0.05 mol% MMI	32.455	35.193	2.738	0.831
EUG	16.828			0.903
EUG+ 0.01 mol% MMI	19.882	18.884	-0.998	0.858
EUG+ 0.02 mol% MMI	20.385	19.599	-0.786	0.856
EUG + 0.05 mol% MMI	27.442	23.714	-3.728	0.816
Iso-EUG	19.474			0.876
IsoEUG+ 0.01 mol% MMI	20.378	21.530	1.152	0.858
IsoEUG+ 0.02 mol% MMI	21.981	22.245	0.264	0.841
IsoEUG+ 0.05 mol% MMI	24.926	26.360	1.434	0.807
QUE	10.165			0.816
QUE+ 0.01 mol% MMI	11.563	13.619	2.056	0.839
QUE+ 0.02 mol% MMI	8.557	12.936	7.222	0.859
QUE+ 0.05 mol% MMI	5.714	17.051	11.337	0.816
0.01 mol% MMI	2.056			0.884
0.02 mol% MMI	2.771			0.857
0.05 mol% MMI	6.886			0.808

BPO, 1.0 mol%; MMA, 9.4 mol/l; at 70˚C; conversion , 92.62-95.33%; the mean of three different experiments; standard error ≤5%; IP (induction period)=(IP_{exp}–IP_{con} (7.069 min)); initial rate of polymerization for control (Rp_{con}), 0.869%/ min; Rp_{inh}, Rp with an inhibitor; *sum of IP (phenolics + MMI). The IP and Rp were determined by the induction period method in polymerization of methyl methacrylate (MMA) initiated by the thermal decomposition of benzoyl peroxide (BPO) in the presence of the MMI/CUR, MMI/EUG, MMI/IsoEUG or MMI/QUE mixtures at the molar ratios of 0:1, 1:1, 2:1 or 5:1 respectively. The procedure is described in the text.

where the initial rates of polymerization in the absence and presence of antioxidants are Rp_{con} and Rp_{inh}, respectively, and k_t and k_{inh} are the rate constants of termination ($3.7x10^7$ $M^{-1}s^{-1}$ (5)) and inhibition, respectively. The rate constant k_{inh} is given by Eq. **(3)**.

$$k_{inh} = \{Rp_{con}\ (2k_tR_i)^{1/2}\}/\{n\ [IH]\ Rp_{inh}\} \quad \textbf{(3)}$$

Results

IP and k_{inh}. The IP and Rp_{inh} values are shown in Table I. The *n* value was calculated from the IP value for each compound by using Eq. **(1)**. The *n* values for inhibition declined in the order CUR (3.78) > IsoEUG (2.66) > EUG (2.30) > QUE (1.39) > MMI (0.28) > ASDB (0.02).

The k_{inh} values for these compounds were calculated by using Eq. **(3)**. The values declined in the order ASDB ($7.85x10^5$ $M^{-1}s^{-1}$) > MMI ($5.99x10^4$ $M^{-1}s^{-1}$) > QUE

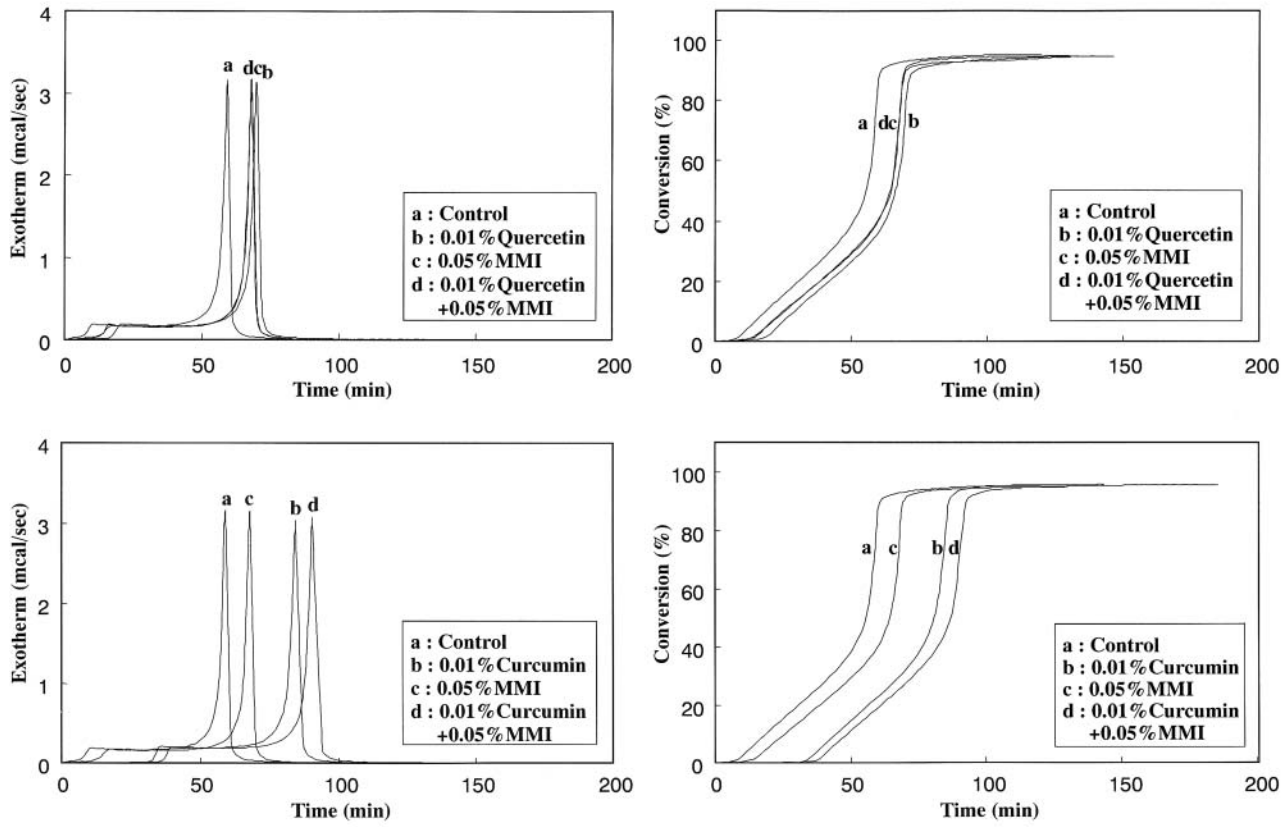

Figure 1. *Time-exothermic (left panel) and time-conversion (right panel) curves of 0.01 mol% quercetin (top) or curcumin (bottom) in polymerization of 9.4 mol MMA initiated by BPO (1 mol%), with or without 0.05 mol% MMI, a thiol, at 70˚C.*

($1.21x10^4$ $M^{-1}s^{-1}$) > EUG ($7.93x10^3$ $M^{-1}s^{-1}$) > IsoEUG ($7.04x10^3$ $M^{-1}s^{-1}$) > CUR ($4.50x10^3$ $M^{-1}s^{-1}$). The k_{inh} value of EUG was in agreement with that ($8.3x10^3$ $M^{-1}s^{-1}$) previously reported for the reaction with superoxide (16). The rate of superoxide radical reaction with QUE is $4.7x10^4$ $M^{-1}s^{-1}$, as determined by pulse radiolysis of aqueous solutions (17). The rate constant for the interaction of ascorbic acid with superoxide radical is $3.3x10^5$ $M^{-1}s^{-1}$, as determined by measuring chemiluminescence intensity at 25˚C (18). Despite the differences in experimental conditions, these rate constants are of a similar order of magnitude.

Effects of MMI, a thiol, on IP. The antioxidant behaviors of the mixtures of MMI with QUE, CUR and the CUR-related compounds EUG and IsoEUG were investigated. The results are shown in Table I, and typical exothermic and time-conversion curves for mixtures of MMI with the polyphenolics QUE and CUR at a molar ratio of 5:1 are shown in Figure 1.

The IP observed for the MMI/QUE mixture at molar ratios of 2:1 and 5:1 was significantly less than that for QUE alone and was also markedly less than the calculated value (Table I). The decrease in IP value for MMI/QUE and the IP value of the control were similar and, therefore, the decrease may be attributed to the antioxidant activity of the oxygen in the DSC pan, because oxygen scavenges radicals. It appears that QUE was oxidized by $PhCOO^{\bullet}$, the radical product of BPO, to form prooxidant QUE radicals which were able to co-oxidize MMI, accompanied by extensive oxygen uptake.

The IP observed for the MMI/CUR mixture at a molar ratio of 5:1 was greater than that for CUR alone (Figure 1), suggesting the co-antioxidant activity of MMI. However, the observed IP was less than the calculated IP (Table I), except at a molar ratio of 2:1. This indicates that MMI can show either prooxidant or antioxidant activity, depending on the conditions. MMI was an effective co-antioxidant in the MMI/IsoEUG mixture, but was less effective in the MMI/CUR mixture (Table I). MMI was co-oxidized by CUR radicals oxidized by $PhCOO^{\bullet}$, a process that occurred without oxygen uptake.

The observed IP of the MMI/EUG mixture was significantly longer than the calculated value (Table I),

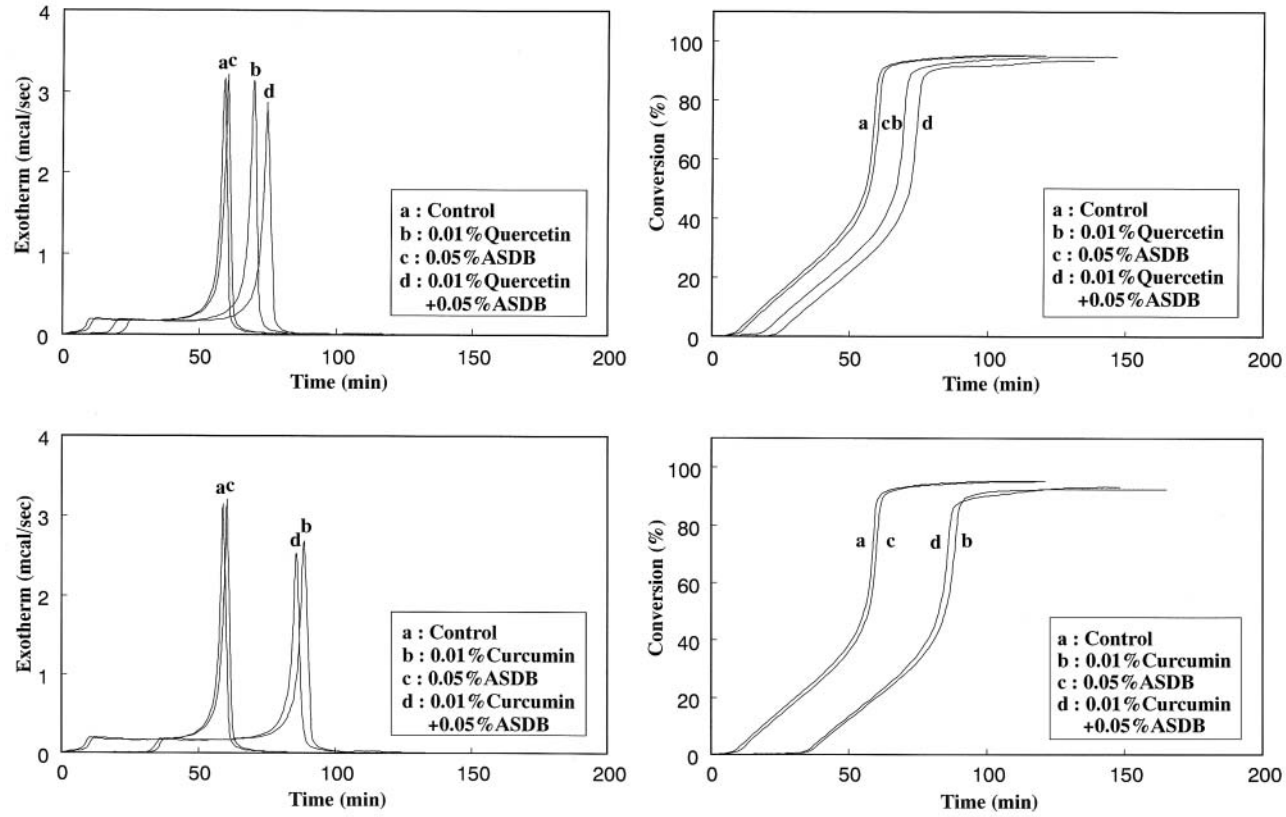

Figure 2. *Time-exothermic (left panel) and time-conversion (right panel) curves of 0.01 mol% quercetin (top) or curcumin (bottom) in polymerization of 9.4 mol MMA initiated by BPO (1 mol%), with or without 0.05 mol% ASDB, an ascorbate derivative, at 70˚C.*

suggesting synergistic antioxidant activity of the combination of EUG and MMI. This is likely to arise from the Michael addition of MMI to allyl groups in the quinonemethide of EUG (19). This newly-produced adduct, containing a phenolic OH group, could scavenge large quantities of $PhCOO^{\bullet}$. The difference in antioxidant behavior between EUG and IsoEUG may be a result of the type of radical species formed: a phenoxyl radical from EUG and a benzyl radical from IsoEUG.

Effect of ASDB, an ascorbate derivative, on IP. Typical exothermic and time-conversion curves for mixtures of ASDB with QUE or CUR at a molar ratio of 5:1 are shown in Figure 2. These results, together with those for molar ratios of 1:1 and 2:1, are summarized in Table II. The observed IP of ASDB/QUE mixtures was greater than the corresponding calculated IP, whereas the observed IP of ASDB/CUR mixtures was less than the corresponding calculated IP. The catalytic effect of QUE was to make ASDB a co-antioxidant, whereas CUR made ASDB a co-oxidant without oxygen uptake.

Discussion

Polyphenolics not only exert antioxidant, but also prooxidant, activities under certain circumstances, such as anaerobic conditions. Oxidized phenolics can be recycled by interactions with antioxidants such as ascorbate and GSH, a process called antioxidant networking (20). For example, in the interaction of reduced GSH with xenobiotic radicals, thiol RS (thiyl) radicals are produced, but the subsequent formation of GSSG (glutathione disulphide (oxidized form)) requires a secondary GSH-dependent process with enhanced oxygen uptake (21). QUE exerts antioxidant activity, but is converted into the potentially harmful oxidation products ortho-quinone and quinonemethide (QQ) (10). Ascorbate recycles QQ to the parent compound QUE, and GSH forms adducts with QQ, resulting in the finding that QQ is toxic in the absence of GSH (10). In the present study, catalytic amounts of QUE acted as a co-oxidant for MMI, leading to oxygen uptake. This suggests that, during the induction period, MMI forms thiol RS radicals which, together with oxygen, produce toxic oxo- or peroxo-sulphur radicals, $RSO^{\cdot\cdot}$ or $RSOO^{\cdot\cdot}$ (22). Thus,

Table II. *A free radical interaction between L-ascorbyl-2,6-dibutyrate (ASDB, an ascorbate) and the antioxidants, quercetin (QUE) or curcumin (CUR).*

Additives phenolics, 0.01 mol%	Observed IP (min) A	Calculated IP (min)* B	B-A	Rp_{inh} %/min
QUE	11.016			0.839
QUE+ 0.01 mol% ASDB	13.310	11.171	-2.139	0.863
QUE+ 0.02 mol% ASDB	16.030	12.513	-3.517	0.811
QUE+ 0.05 mol% ASDB	15.632	12.819	-2.813	0.819
CUR	27.480			0.800
CUR + 0.01 mol% ASDB	25.172	27.635	2.608	0.812
CUR + 0.02 mol% ASDB	26.283	27.780	1.497	0.812
CUR + 0.05 mol% ASDB	25.132	29.283	4.151	0.799
0.01 mol% ASDB	0.155			0.869
0.02 mol% ASDB	1.497			0.872
0.05 mol% ASDB	1.803			0.882

BPO, 1.0 mol%; MMA, 9.4 mol/l; at 70˚C; conversion , 92.62-95.33%; the mean of three different experiments; standard error ≤5%; IP (induction period) $=(IP_{exp}–IP_{con}$ (7.069 min)); Rp_{con} (initial rate of polymerization) for control, 0.869%/ min; Rp_{inh}, Rp with an inhibitor; *sum of IP (polyphenolic + ASDB). The IP and Rp were determined by the induction period method in polymerization of methyl methacrylate (MMA) initiated by the thermal decomposition of benzoyl peroxide (BPO) in the presence of the ASDB/QUE or ASDB/CUR mixtures at the molar ratios of 0:1, 1:1, 2:1 or 5:1, respectively.

an adequate GSH level should be maintained when supplementation with QUE is performed (10). A study of oxygen activation by polyphenolic phenoxyl radicals with GSH has previously been reported, showing that the polyphenolics were metabolized by peroxidase in the presence of GSH, but that catalytic effectiveness for oxygen activation preferentially appeared in CUR, but not in QUE (11). However, the existence of a phenoxyl-type CUR radical induced by oxidation suggests that CUR does not react with oxygen and is unlikely to cause oxygen activation (29), and our findings indicate that CUR does not cause oxygen activation. This discrepancy may result from the experimental differences between aerobic and anaerobic conditions. A similar oxygen-dependent difference in antioxidant activity has been reported for the potent antioxidants vitamin C and vitamin E, indicating that these compounds are not efficient antioxidants under anaerobic conditions (23). Under anaerobic conditions, QUE, with a catechol ring, may be more prooxidant than CUR, with a phenol ring.

QUE has been investigated as a potential chemopreventive agent against certain cancers. For example, QUE reduced the incidence of 7,12-dimethylbenz(*a*)anthracene- and *N*-nitrosomethylurea-induced rat mammary tumors (24) and of azoxymethanol-induced mouse colonic neoplasia (25). On the other hand, QUE has been reported to be carcinogenic. Dietary administration of excess QUE induced intestinal and bladder cancer in rats (26). Oxidative DNA damage induced by QUE has previously been reported (27). When an antioxidant such as QUE neutralizes a reactive species, it is converted into potentially harmful oxidative products (10, 28). Taking these reports together, QUE may have both anticarcinogenic and carcinogenic potential. The present study suggests that oxidation of QUE in the presence of excess thiols may be toxic, possibly as a result of oxygen activation and the formation of harmful thiol RS radicals.

On the other hand, the oxidized MMI/CUR mixture enhanced the prooxidation of MMI without oxygen uptake. Hepatocyte GSH depletion by polyphenol phenoxy radicals and *o*-quinone metabolites has been reported, indicating that the major GSH product for CUR is GSSG, whereas that for QUE is GSH conjugates (11). These findings may be explained by a higher co-catalytic activity of GSH in the interaction with QUE radicals compared with that with CUR radicals, which in turn may result from the difference in the reduction potentials of the phenoxyl radicals of QUE (0.6 V) (17) and CUR (0.8 V) (29). Also, regarding the reactivity of CUR or QUE with MMI, it was shown by the present findings that the IP for CUR was greater than that for QUE. QUE depletes thiol RSH through the formation of RSH conjugates.

In the present study, ASDB, an ascorbate derivative, acted as a prooxidant in the CUR antioxidant network (29). Cancer cells are anaerobic in their metabolism (8, 9) and have very poor mechanisms for absorbing adequate amounts of antioxidants. The exception is ascorbate, which is remarkably similar in chemical structure to glucose. Since cancer cells preferentially take up and metabolize glucose, they selectively absorb more ascorbate than do normal cells (30). Our findings may indicate a possible chemopreventive mechanism of CUR against cancer cells, in which CUR enhances the prooxidant activity of ascorbate which may, in turn, trigger apoptotic cell death. It is well established that CUR acts as a chemopreventive agent against cancer (31).

In vivo experiments are too complex to be amenable to simple interpretation and, therefore, we employed physico-chemical studies using the IP method in the radical polymerization of MMA in the presence of antioxidants as a biomimetic model for scavenging radicals produced *in vivo*. Such studies could help to explain the mechanisms by which polyphenolics induce cancer cell apoptosis and act as chemopreventive agents.

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