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Curcumin-induced Growth Inhibitory Effects on HeLa Cells Altered by Antioxidant Modulators

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Abstract Curcumin (diferuloyl methane), originated rhizomes of *Curcuma longa* L. has been suggested as an anti-inflammatory and anti-carcinogenic agent. In the present study, modulation of cytotoxic effects of curcumin on HeLa cells by different types of antioxidants was investigated. Cytotoxic effects of curcumin were significantly enhanced in the presence of superoxide dismutase (SOD) by decreasing IC_{50} to 15.4 from 26.0 μM after 24 hr incubation; the activity was not altered by catalase. The effect of curcumin was significantly less pronounced in the presence of 4 mM N-acetylcysteine (NAC). Low concentration (<1 mM) of NAC, however, increased the efficacy of curcumin. Cysteine and β-mercaptoethanol that have a thiol group, showed the similar biphasic patterns as NAC for modulating curcumin cytotoxicity, which was, however, constantly enhanced by ascorbic acid, a non-thiol antioxidant. In the presence of SOD, ascorbic acid, and 0.5 mM NAC, cellular levels of curcumin were significantly increased by 31-66%, whereas 4 mM NAC decreased the level. The present results indicate that thiol reducing agents showed a biphasic effect in modulating cytotoxicity of curcumin; it is likely that their thiol group is reactive with curcumin especially at high concentrations.

Keywords: curcumin, N-acetylcysteine, cytotoxicity, HeLa cell, reactive oxygen species

Introduction

Curcumin [1,7-bis(4-hydroxy-3-methoxyphenyl)-1,6-heptadiene-3,5-dione; diferuloyl methane] is a major constituent found in the ancient spices, turmeric. Turmeric is a dried powder from rhizomes of *Curcuma longa* L., a perennial herb, which grows in tropical and subtropical regions. Turmeric has been extensively used as a coloring and flavoring agent in curries and mustards. The spice has also been traditionally used as a medicinal herb for inflammation, gastrointestinal disorders, hepatic disorders, diabetic and skin wounds, rheumatism, sinusitis, and other complaints (1, 2). Compounds in turmeric have also been suggested for an antimicrobial agent against harmful intestinal bacteria (3).

Epidemiological study and studies using animal carcinogenesis models have suggested that curcumin is a possible cancer chemopreventive agent (4, 5). A ton of studies have reported the anti-carcinogenic mechanisms of curcumin. They include antioxidative activities, inhibition of ornithine decarboxylase, protein kinase C, inducible nitric oxide synthase, and thioredoxin reductase, inhibition of AP-1 and NF-κB, inhibition of epidermal growth factor receptor signaling, modulation of arachidonic acid metabolism, and the inhibition of angiogenesis (6-12).

Several studies using tumor cells have also indicated that curcumin modulated cell cycle, inhibited cell proliferation, and induced apoptosis (13-17). Previous reports have suggested that generation of reactive oxygen species (ROS) plays an important role in curcumin-induced cell death (16-19). It is reported that blockage of curcumin-induced ROS generation by N-acetylcysteine (NAC) inhibited apoptotic events including caspase-3 activation and cytochrome c release in human renal carcinoma Caki cells (17). In another study, NAC attenuated curcumin-induced c-jun N-terminal kinase activation and reversed cell death in HCT-116 human colon carcinoma cells (19). Many other curcumin-induced events in cell system including GADD153 up-regulation, heme oxygenase-1 expression, and induction of heat shock protein 70, were also reversed in the presence of antioxidants such as NAC (20-22). A recent report, however, indicated that anti-proliferative effect of curcumin is not related to the generation of ROS (23).

The causative relationship between curcumin-induced cellular events and a role of ROS, especially demonstrated in the presence of antioxidants, is not clear whether reversal of the curcumin effects was due to ROS scavenging effects by antioxidants, due to the direct modification of curcumin by antioxidants, or due to other artifacts. In the present study, several antioxidant compounds including NAC, ascorbic acid, and other thiol reducing agents, and antioxidant enzymes including superoxide dismutase (SOD) and catalase were used to investigate how they modulate cytotoxic effects of curcumin on HeLa cells, and to determine whether curcumin-induced cell death is related to ROS generation.

Materials and Methods

Chemicals and cell lines Curcumin (>95% purity) was generously provided from Dr. Mou-Tuan Huang in Rutgers University (Piscataway, NJ, USA). HeLa human cervical carcinoma cell line was a gift from Dr. Sang-Woo Bae in Korea Institute of Radiological Medical Sciences (Seoul, Korea). HeLa cells were maintained in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% fetal bovine serum, 100 unit/mL penicillin, and 0.1 mg/mL streptomycin, and were kept at 37°C in 95% humidity and 5% CO_2. SOD (from bovine erythrocyte)

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and catalase (from bovine liver) were from Sigma-Aldrich Chemical Co. (St Louis, MO, USA), and 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) was purchased from Amresco Inc. (Solon, OH, USA). All other chemicals were from Sigma-Aldrich Chemical Co.

Analysis of cell viability The inhibitory effects of curcumin on cell growth were determined by measurement of cell viability using MTT. HeLa cells were seeded in 96-well plates and treated the next day with curcumin at the indicated conditions and concentrations as described in figures and legends. After incubation for 24 hr, compound-containing medium was removed, and medium containing 0.5 mg/mL MTT was added to each well. The cells were further incubated at 37°C for 1-2 hr. The medium was then removed and replaced by 100 μL of dimethyl sulfoxide, and the absorbance was measured at 550 nm using a microplate reader (Spectra Max 250; Molecular Device, Sunnywale, CA, USA).

DPPH radical scavenging activity Radical scavenging activity of chemical antioxidants was measured using 1,1-diphnyl-2-picrylhydrazyl (DPPH). The antioxidants (20 μL) at different concentrations (1-4 mM) were mixed with 100 μL of DPPH (0.13 mg/mL in EtOH) solution. The reaction mixtures were then kept at room temperature for 30 min. The absorbance was then analyzed at 517 nm using a microplate reader. The percent scavenging activity for DPPH radical was calculated by a previous method (24)

Analysis of cellular level of curcumin To analyze cellular uptake of curcumin, HeLa cells were seeded in a 24-well plate. When the cells reached ca. 80% confluency, the cells were treated with 20 μM of curcumin in serum free DMEM media. After 4 hr incubation, cells were washed 3 times with ice cold phosphate-buffered saline. Intracellular curcumin was then extracted using 70% MeOH for 30 min and the cell extracts were centrifuged at 10,000×g for 20 min at 4°C. The extracted curcumin in supernatant was analyzed at 417 nm using a microplate reader.

Data analysis Statistical significance was evaluated using the Student's *t*-test. One-way analysis of variance (ANOVA) and the Tukey's honestly significant difference (HSD) test were used for comparing multiple results. IC_{50} (concentra-tion that caused 50% inhibition) values were calculated by using the corresponding linear regression equations determined based on the points within a linear range.

Results and Discussion

Effects of curcumin on HeLa cell growth with different antioxidant modulators Effects of curcumin on the growth of HeLa cells were investigated in the presence of different antioxidant modulators. After incubation of HeLa cell with curcumin in the absence or presence of SOD, catalase, or NAC for 24 hr, cell viability was analyzed by MTT assay. NAC, SOD, or catalase did not affect cell growth in the concentration range tested (data not shown). Curcumin inhibited HeLa cell growth with IC_{50} of 26.0 μM after 24 hr incubation (Fig. 1). The effect of curcumin was significantly enhanced in the presence of SOD (15 unit/mL) by decreasing IC_{50} to 15.4 μM. Another antioxidant enzyme, catalase also slightly increased the activity of curcumin. Several reports have indicated that curcumin-induced cell apoptosis occurred through the generation of ROS and the action of curcumin was blocked by certain antioxidants (17, 19). Present results indicate that antioxidant enzymes such as SOD and catalase could not abolish the effect of curcumin, rather increased the curcumin activity. Generation of hydrogen peroxide and ROS from curcumin in cell free media was not detectable (data not shown), suggesting that ROS generation by curcumin is cell-dependent and occurs inside cells. Since SOD and catalase are not membrane permeable due to their high molecular weights, they might not affect ROS generated by curcumin inside cells. Co-incubation with NAC (4 mM), a cell permeable anti-oxidant, however, caused dramatically decreased cytotoxic effects of curcumin (Fig. 1). This result is consistent to the previous observations that apoptosis induced by curcumin

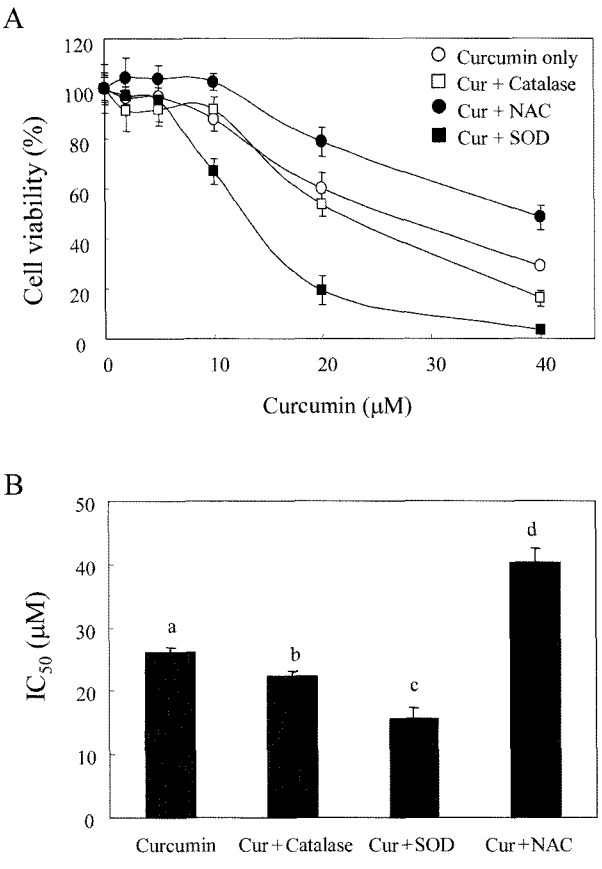

Fig. 1. Changes in effects of curcumin on HeLa cell viability by different antioxidant modulators. (A) Effects of curcumin on the viability of HeLa cells with or without SOD (15 unit/mL), catalase (30 unit/mL), or NAC (4 mM). (B) IC_{50} values of curcumin for HeLa cell growth with different antioxidant modulators. Each value represents the mean±SD (n=8). Different letters indicate a significant difference ($p<0.05$) based on 1-way ANOVA and the Tukey's HSD test.

was abolished in the presence of NAC (17, 19).

Concentration-dependent effects of these antioxidant modulators on growth inhibitory effects of curcumin were analyzed. Catalase, up to 60 unit/mL, did not affect the activity of curcumin, whereas cytotoxic effects of curcumin were markedly amplified in the presence of SOD in the range of 7.5-60 unit/mL (Fig. 2A and 2B). NAC, however, showed a biphasic pattern for modulating curcumin activities. High concentration of NAC (>2 mM) restrained the cytotoxic effect of curcumin as shown previously. Interestingly, submillimolar concentration of NAC rather significantly enhanced the effects of curcumin on cell death (Fig. 2C). Many previous reports used several millimolar concentrations of NAC for the purpose of blocking ROS generation from curcumin and showed

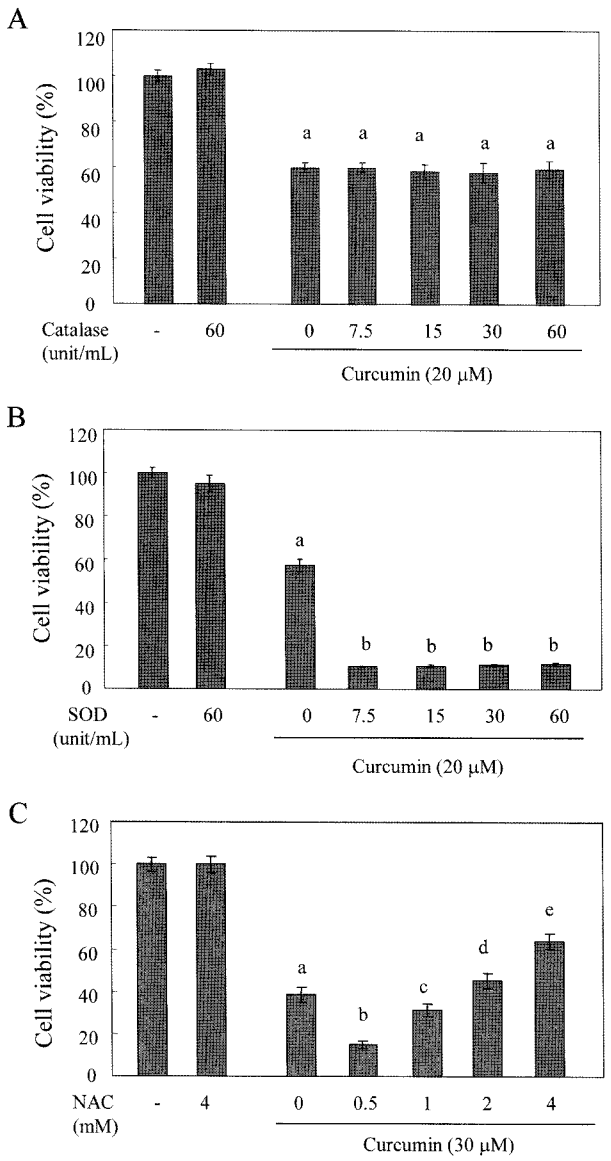

Fig. 2. Modulation of cytotoxic effects of curcumin on HeLa cells by different concentrations of catalase (A), SOD (B), or NAC (C). Each value represents the mean±SD (n=8). Different letters indicate a significant difference ($p<0.05$) based on 1-way ANOVA and the Tukey's HSD test.

the reversal of the curcumin-induced events (17, 19-22). The present result, however, was quite different from the previous observations when submillimolar concentration of NAC was applied.

Cytotoxic effects of curcumin modulated by thiol antioxidants It is suspected that the abolished curcumin activity in the presence of high concentrations of NAC might not because NAC blocked curcumin-induced ROS, but because NAC directly interacted with curcumin and caused a chemical modification of curcumin as an inactive form. To elucidate a direct interaction of curcumin with NAC, curcumin was incubated with NAC in cell-free media before being added to cell culture system. Curcumin itself was not stable in cell culture media; cytotoxic effects of preincubated curcumin for 1.5 and 4 hr were significantly less potent as compared with those of curcumin without preincubation (Fig. 3A and 3D). Preincubation of curcumin with NAC accelerated the decay of curcumin activities. Especially preincubated curcumin with 4 mM NAC during 4 hr dramatically decreased the efficacy of curcumin by increasing IC_{50} to 106.9 from 36.1 μM (Fig. 3C and 3D). Interestingly, 0.5 mM NAC within 1.5 hr preincubation with curcumin delayed the reduction of curcumin activity (IC_{50}, 31.9 mM for curcumin only vs. 22.2 μM for curcumin with 0.5 mM NAC); but longer incubation (4 hr) made curcumin much less active (IC_{50}, 60.3 μM for curcumin vs. 92.4 μM for curcumin with 0.5 mM NAC) (Fig. 3B and 3D). The results suggest that NAC especially at high concentration can directly react with curcumin and modify the activity of curcumin. At low concentration, however, NAC seems to have protective effect on curcumin activities.

It is reported that α,β-diketone structure of curcumin can interact with thiol group as an alkylating agent (12). Although reactivity of curcumin toward a thiol compound such as glutathione is low (25), high concentration of a thiol compound (e.g., higher than millimolar level) may lead to a chemical reaction with curcumin more favorably. To elucidate involvement of thiol group in modulating curcumin activity, the effects of curcumin in the presence of other reducing agents with thiol group on HeLa cell growth were analyzed. Cysteine indeed showed a similar biphasic pattern with NAC for modulating curcumin activity. Cysteine at higher than 1 mM rescued cells from curcumin toxicity, but submillimolar concentration of cysteine markedly enhanced the activity of curcumin (Fig. 4A). β-Mercaptoethanol (BME), another thiol compound, decreased HeLa cell viability concentration-dependently. In the presence of 2 mM BME, approximately 50% cells died. Despite of BME toxicity to cells, the cell viability in the presence of curcumin was gradually recovered according to increase of BME concentration (Fig. 4B). Cells with curcumin survived more than its corresponding control in the presence of 2 mM BME. Ascorbic acid that is a non-thiol antioxidant, however, constantly enhanced the cytotoxic effect of curcumin within the range of 0.25-2 mM (Fig. 4C), which was a similar pattern of SOD. The results suggest that curcumin could be reactive with thiol group of antioxidants especially at high concentrations.

Reducing abilities of the antioxidant compounds used were compared by analyzing DPPH radical scavenging

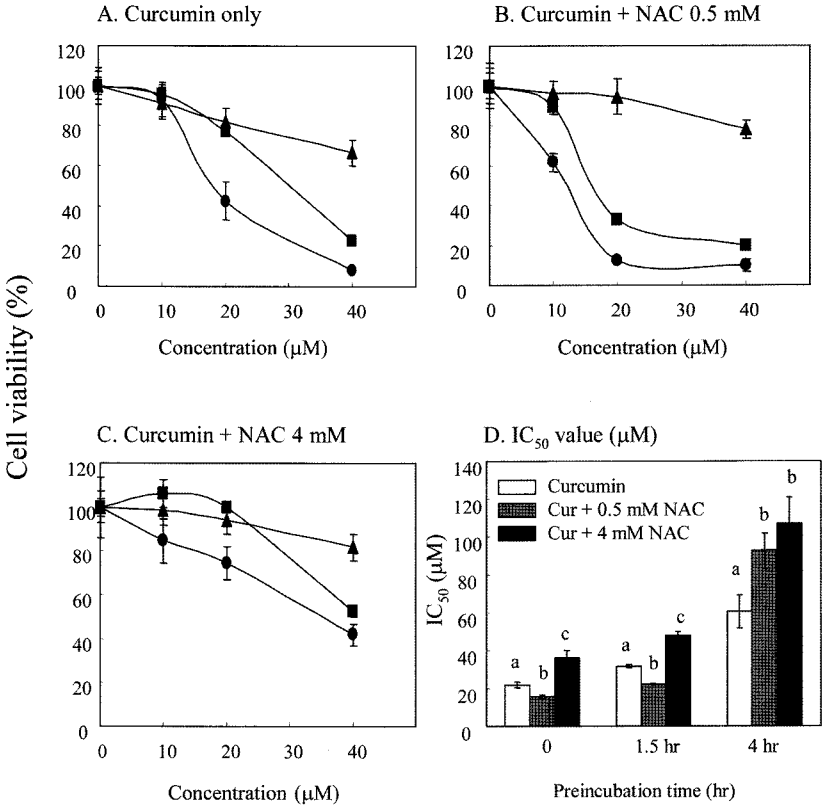

Fig. 3. Changes in effects of curcumin on HeLa cell growth by preincubation with NAC. Curcumin was incubated in the absence (A), or presence of 0.5 mM (B), or 4 mM (C) NAC for 0 (●), 1.5 (■), and 4 hr (▲) in serum free DMEM medium before being added to cells. Cells were then treated in different concentrations of the preincubated mixtures for 24 hr (D). Changes in the IC_{50} values of curcumin by different preincubation time with or without NAC. Each value represents the mean±SD (n=8). Different letters indicate a significant difference ($p<0.05$) based on 1-way ANOVA and the Tukey's HSD test.

activities. All the compounds tested showed potent DPPH radical scavenging activities. Ascorbic acid had a more potent radical scavenging activity than NAC, cysteine, or BME at 1 mM concentration, whereas NAC showed the highest activity at 4 mM. The result suggests that the reducing power of these antioxidants was not directly correlated with a modulating pattern of curcumin activity (Fig. 5).

Modulation of cellular uptake of curcumin by antioxidants Cytotoxic effects of curcumin were markedly amplified in the presence of SOD, ascorbic acid and low concentrations of thiol antioxidants. To explore cellular mechanisms involved in enhancing the activity of curcumin, effects of the antioxidant modulators on cellular uptake of curcumin were analyzed. After 4 hr incubation, cellular level of curcumin in HeLa cells was significantly increased by 66.1, 31.2, and 46.9% in the presence of 0.5 mM NAC, 1 mM ascorbic acid, or 15 unit/mL SOD, respectively. High concentration of NAC (4 mM), however, markedly decreased intracellular curcumin levels by 42% (Fig. 6). It is reported that phenolic compounds such as epigallocatechin-3-gallate were stabilized by SOD or antioxidants in cell culture condition and their activities were altered considerably in the presence of these antioxidant modulators (26). As shown previously (Fig. 3), curcumin is not stable in cell culture medium; stability of curcumin seems to be one of important factors for displaying its bioactivities including cytotoxic effects in cell culture system (27). It is proposed that molecular oxygen is reduced to superoxide anion by certain phenolic antioxidants (e.g., epigallocatechin-3-gallate) in the presence of metal ions, and oxidation of the antioxidants is propagated by the reaction of superoxide anion (26). A similar oxidation process of curcumin can be applied in the present culture condition; the addition of SOD, but not catalase, might inhibit the propagation of the chain reaction. Other nonspecific antioxidants could also impede the oxidation process of curcumin either by scavenging superoxide anion or by neutralizing oxidative intermediates from curcumin due to their electron donating abilities. Therefore SOD and ascorbic acid could increase curcumin efficacy in inhibiting cell growth because they could stabilize curcumin in cell culture condition; it was supported by significantly higher levels of curcumin in cells.

Since the reactivity of curcumin toward thiol group is not so high (25), NAC at lower concentration might primarily be involved in stabilizing curcumin, accordingly increased cellular uptake and the cytotoxic effects of curcumin. In the presence of higher concentration of NAC, the reaction of curcumin with thiol group might occur more favorably and make curcumin inactive faster. One of cytotoxic mechanisms of curcumin has been suggested that curcumin modified intracellular thiol proteins such as

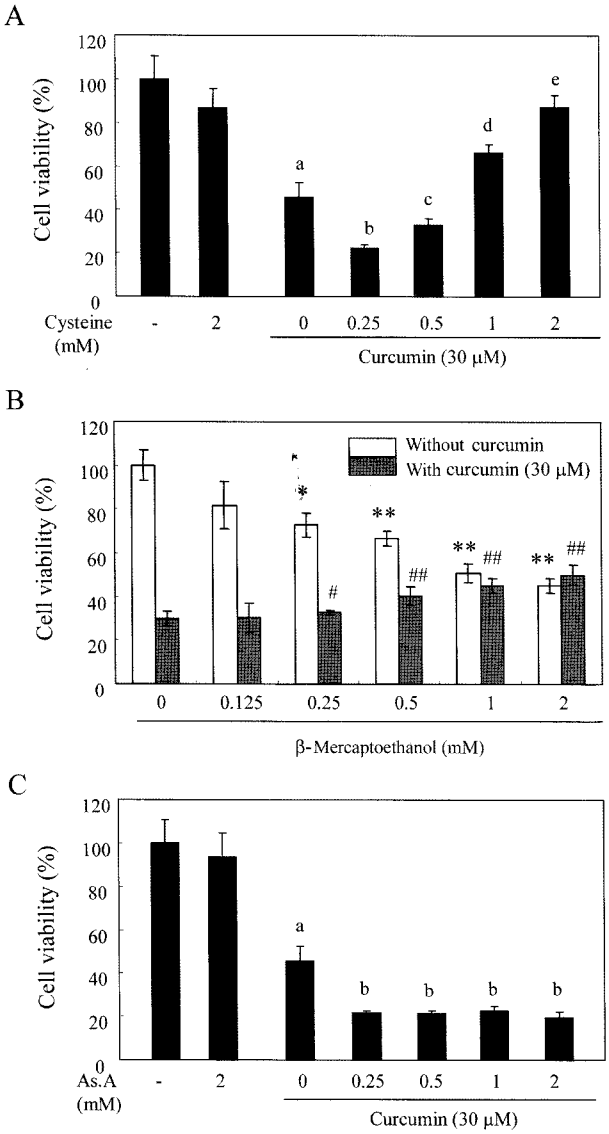

Fig. 4. Modulation of cytotoxic effects of curcumin by cysteine (A), β-mercaptoethanol (B), or ascorbic acid (C). Each value represents the mean±SD (n=8). Different letters indicate a significant difference ($p<0.05$) based on 1-way ANOVA and the Tukey's HSD test (A and C).
*,**, #,##Significantly different from its corresponding control according to Student's t test (* or $^{\#}p<0.05$; ** or $^{\#\#}p<0.01$ in B).

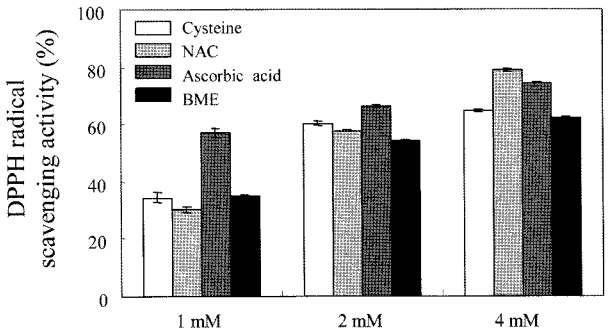

Fig. 5. DPPH radical scavenging activity of the antioxidant modulators used in the present study. Each antioxidant (20 μL) at indicated concentration was mixed with 100 μL of DPPH solution. Each value represents the mean±SD (n=4).

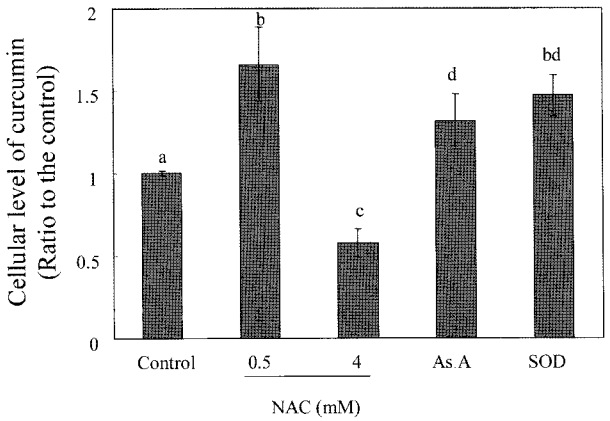

Fig. 6. Changes in intracellular levels of curcumin in the presence of NAC, ascorbic acid (1 mM), or SOD (15 unit/mL). Each value represents the mean±SD (n=3 or 4). Different letters indicate a significant difference ($p<0.05$) based on 1-way ANOVA and the Tukey's HSD test.

thioredoxin reductase (12). Decreased curcumin activity in the presence of high concentration of NAC may also be explained by that the cell permeable thiol compound competes with curcumin-targeting thiol proteins in cells. Many previous studies have used NAC and other antioxidants with curcumin for preparing ROS free system. The present results suggest that certain thiol antioxidants can directly react with curcumin rather than removing ROS. The mechanistic explanation based on results observed when curcumin with thiol antioxidants is added in cell culture system, should be carefully reconsidered. The precise characterization for chemical modification of curcumin by thiol compounds needs to be explored further.

Acknowledgments

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References

1. Ammon HP, Wahl MA. Pharmacology of *Curcuma longa*. Planta Med. 57: 1-7 (1991)
2. Ammon HP, Anazodo MI, Safayhi H, Dhawan BN, Srimal RC. Curcumin: A potent inhibitor of leukotriene B4 formation in rat peritoneal polymorphonuclear neutrophils (PMNL). Planta Med. 58: 226 (1992)
3. Lee HS. Antimicrobial properties of turmeric (*Curcuma longa* L.) rhizome-derived αγ-turmerone and curcumin. Food Sci. Biotechnol. 15: 559-563 (2006)
4. Ray A. Cancer preventive role of selected dietary factors. Indian J. Cancer 42: 15-24 (2005)
5. Surh YJ, Chun KS. Cancer chemopreventive effects of curcumin. Adv. Exp. Med. Biol. 595: 149-172 (2007)
6. Rao CV, Simi B, Reddy BS. Inhibition by dietary curcumin of azoxymethane-induced ornithine decarboxylase, tyrosine protein kinase, arachidonic acid metabolism, and aberrant crypt foci formation in the rat colon. Carcinogenesis 14: 2219-2225 (1993)
7. Arbiser JL, Klauber N, Rohan R, van Leeuwen R, Huang MT, Fisher C, Flynn E, Byers HR. Curcumin is an *in vivo* inhibitor of

angiogenesis. Mol. Med. 4: 376-383 (1998)
8. Korutla L, Cheung JY, Mendelsohn J, Kumar R. Inhibition of ligand-induced activation of epidermal growth factor receptor tyrosine phosphorylation by curcumin. Carcinogenesis 16: 1741-1745 (1995)
9. Pendurthi UR, Williams JT, Rao LV. Inhibition of tissue factor gene activation in cultured endothelial cells by curcumin. Suppression of activation of transcription factors Egr-1, AP-1, and NF-kB. Arterioscl. Throm. Vas. 17: 3406-3413 (1997)
10. Lin YG, Kunnumakkara AB, Nair A, Merritt WM, Han LY, Armaiz-Pena GN, Kamat AA, Spannuth WA, Gershenson DM, Lutgendorf SK, Aggarwal BB, Sood AK. Curcumin inhibits tumor growth and angiogenesis in ovarian carcinoma by targeting the nuclear factor-kB pathway. Clin. Cancer Res. 13: 3423-3430 (2007)
11. Hong J, Bose M, Ju J, Ryu JH, Chen X, Sang S, Lee MJ, Yang CS. Modulation of arachidonic acid metabolism by curcumin and related β-diketone derivatives: Effects on cytosolic phospholipase A_2, cyclooxygenases, and 5-lipoxygenase. Carcinogenesis 25: 1671-1679 (2004)
12. Fang J, Lu J, Holmgren A. Thioredoxin reductase is irreversibly modified by curcumin: A novel molecular mechanism for its anticancer activity. J. Biol. Chem. 280: 25284-25290 (2005)
13. Mukhopadhyay A, Banerjee S, Stafford LJ, Xia C, Liu M, Aggarwal BB. Curcumin-induced suppression of cell proliferation correlates with down-regulation of cyclin D1 expression and CDK4-mediated retinoblastoma protein phosphorylation. Oncogene 21: 8852-8861 (2002)
14. Bharti AC, Donato N, Singh S, Aggarwal BB. Curcumin (diferuloylmethane) down-regulates the constitutive activation of nuclear factor-kB and IkBa kinase in human multiple myeloma cells, leading to suppression of proliferation and induction of apoptosis. Blood 101: 1053-1062 (2003)
15. Roy M, Chakraborty S, Siddiqi M, Bhattacharya RK. Induction of apoptosis in tumor cells by natural phenolic compounds. Asian Pac. J. Cancer Prev. 3: 61-67 (2002)
16. Syng-Ai C, Kumari AL, Khar A. Effect of curcumin on normal and tumor cells: Role of glutathione and bcl-2. Mol. Cancer Ther. 3: 1101-1108 (2004)
17. Woo JH, Kim YH, Choi YJ, Kim DG, Lee KS, Bae JH, Min DS, Chang JS, Jeong YJ, Lee YH, Park JW, Kwon TK. Molecular mechanisms of curcumin-induced cytotoxicity: Induction of apoptosis through generation of reactive oxygen species, down-regulation of Bcl-X_L and IAP, the release of cytochrome c and inhibition of Akt. Carcinogenesis 24: 1199-1208 (2003)
18. Su CC, Lin JG, Li TM, Chung JG, Yang JS, Ip SW, Lin WC, Chen GW. Curcumin-induced apoptosis of human colon cancer colo 205 cells through the production of ROS, Ca^{2+}, and the activation of caspase-3. Anticancer Res. 26: 4379-4389 (2006)
19. Moussavi M, Assi K, Gomez-Munoz A, Salh B. Curcumin mediates ceramide generation via the *de novo* pathway in colon cancer cells. Carcinogenesis 27: 1636-1644 (2006)
20. Scott DW, Loo G. Curcumin-induced GADD153 upregulation: Modulation by glutathione. J. Cell Biochem. 101: 307-320 (2007)
21. Chen YC, Tsai SH, Shen SC, Lin JK, Lee WR. Alternative activation of extracellular signal-regulated protein kinases in curcumin and arsenite-induced HSP70 gene expression in human colorectal carcinoma cells. Eur. J. Cell Biol. 80: 213-221 (2001)
22. McNally SJ, Harrison EM, Ross JA, Garden OJ, Wigmore SJ. Curcumin induces heme oxygenase 1 through generation of reactive oxygen species, p38 activation, and phosphatase inhibition. Int. J. Mol. Med. 19: 165-172 (2007)
23. Sandur SK, Pandey MK, Sung B, Ahn KS, Murakami A, Sethi G, Limtrakul P, Badmaev V, Aggarwal BB. Curcumin, demethoxycurcumin, bisdemothoxycurcumin, tetrahydrocurcumin, and turmerones differentially regulate anti-inflammatory and antiproliferative responses through a ROS-independent mechanism. Carcinogenesis 28: 1765-1773 (2007)
24. Manochai B, Paisooksantivatana Y, Kim MJ, Hong JH. Antioxidant activity and total volatile oil content of cassumunar ginger (*Zingiber montanum* Roxb.) at various rhizome ages. Food Sci. Biotechnol. 16: 290-293 (2007)
25. Awasthi S, Pandya U, Singhal SS, Lin JT, Thiviyanathan V, Seifert WE Jr, Awasthi YC, Ansari GA. Curcumin-glutathione interactions and the role of human glutathione s-transferase P1-1. Chem. -Biol. Interact. 128: 19-38 (2000)
26. Hou Z, Sang S, You H, Lee MJ, Hong J, Chin KV, Yang CS. Mechanism of action of (-)-epigallocatechin-3-gallate: Auto-oxidation-dependent inactivation of epidermal growth factor receptor and direct effects on growth inhibition in human esophageal cancer KYSE 150 cells. Cancer Res. 65: 8049-8056 (2005)
27. Blasius R, Duvoix A, Morceau F, Schnekenburger M, Delhalle S, Henry E, Dicato M, Diederich M. Curcumin stability and its effect on glutathione s-transferase P1-1 mRNA expression in K562 cells. Ann. NY Acad. Sci. 1030: 442-448 (2004)