Inhibition of Cell Growth and Induction of Apoptosis via Inactivation of NF-κB by a Sulfurcompound Isolated From Garlic in Human Colon Cancer Cells

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Abstract. Compounds such as S-allylmercaptocysteine, diallyl disulfide, and S-trityl-L-cysteine isolated from garlic have been known to be effective in chemoprevention. Nuclear transcription factor-κB (NF-κB) has been known to be an implicated factor in apoptotic cell death of several cancer cells. In this study, we investigated whether a sulfurcompound (named thiacremonone) isolated from garlic could modulate NF-κB activity and thereby induce apoptotic cell death of colon cancer cells. Treatment with different concentrations (30 – 150 µg/ml) of thiacremonone for various periods (0 – 48 h) inhibited colon cancer cell (SW620 and HCT116) growth followed by induction of apoptosis in a dose-dependent manner. We also found that thiacremonone modulated tumor necrosis factor-α (TNF-α) and tetradeanoyl phorbol acetate (TPA)-induced NF-κB transcriptional and DNA binding activity. Moreover, thiacremonone suppressed NF-κB target anti-apoptotic genes (Bcl-2, cIAP1/2, and XIAP) and inflammatory genes (iNOS and COX-2), whereas it induced apoptotic genes (Bax, cleaved caspase-3, and cleaved PARP) expression. These results suggest that a novel sulfurocompound from garlic inhibited colon cancer cell growth through induction of apoptotic cell death by modulating of NF-κB.

Keywords: thiacremonone, sulfurcompound, nuclear transcription factor-κB (NF-κB), colon cancer, apoptosis

Introduction

The role of dietary compounds in cancer prevention and treatment has been widely discussed (1 – 3). In this regard, the potential chemopreventive effect of garlic has been of interest since garlic contains numerous pharmacologically active substances including sulfur and selenocompounds that have been shown to alter the activation of several carcinogens and to inhibit cancer cell growth and/or to induce cell death (4 – 6). Sulfurcompounds such as diallyl sulfide, S-allylmercapto-cysteine, and ajoene isolated from garlic have been known to increase the activity of enzymes involved in the metabolism of carcinogens (7) and have anti-oxidant activity (8) and protective activity against lipid peroxidation and hepato toxicity in vitro and in vivo (9, 10).

Several recent studies showed that these sulfurcompounds are able to inhibit the growth of several human cancer cells including breast (MCF-7), hepatoma (HepG2), and lymphocytic leukemia as well as lung cancer cells (NSCLC) in culture (11 – 14). In addition, the importance of sulfurcompounds in the preventative effect against colon cancer development have been demonstrated by several research groups (15 – 17). Jakubikova and Sedlak reported that organosulfides derived from garlic induced apoptotic cell death of human colon carcinoma cell lines (15). Garlic containing sulfurcompounds have also been demonstrated to pre-
vent chemical-induced rat aberrant crypt formation (16) and colon cancer incidence (17).

Even though sulfur compounds from natural products have been demonstrated as chemopreventive agents, their molecular mechanisms are not fully demonstrated. Recent evidences indicate that nuclear transcription factor-κB (NF-κB) activation has been connected with multiple aspects of oncogenesis, including the control of apoptosis, proliferation, differentiation, and migration of the cells (18–20). An association of colorectal cancer development and activation of NF-κB has been demonstrated. NF-κB activity was increased in the colon cancer cell lines and human tumor samples as well as nucleic of stromal macrophages in sporadic adenomatous polyps (21, 22). NF-κB can lead to further proliferation of transformed cells through enhanced production of growth factors and cytokines (23). Thus, inactivation of NF-κB in many cancer cells by a chemotherapeutic agent has been demonstrated to blunt the ability of the cancer cells to grow by the antiapoptotic function of NF-κB in mammalian cells (24–27). Although many compounds have been identified from garlic (28, 29) and their anticancer activities (4–6) have been demonstrated, new compounds and action mechanisms underlying their anticancer effects have been not fully studied. Recently, we isolated and identified a novel sulfurcompound from garlic and found that it has higher content of polyphenol and anti-oxidant activity (30). To investigate the anti-tumor activity of this novel identified sulfurcompound and its action mechanism, we examined the effect of a novel compound on NF-κB activity in colon carcinoma cell lines in parallel with the effect of this compound on colon cell growth and apoptosis.

Materials and Methods

Chemicals

A novel isolated chemical (named thiacremonone) was characterized and found to be the same as the compound isolated from Acremonium sp. strain HA33-95 by fermentation as described by Gehrt et al. (31).

![Fig. 1. Structure of thiacremonone, a novel sulfur compound isolated from garlic.](image)

The structure is shown in Fig. 1. Thiacremonone was dissolved in 0.1% DMSO and used at the treatment dose of 30 – 150 μg/ml.

Cell culture

SW620 and HCT116 human colon cancer cells were obtained from the American Type Culture Collection (Cryosite, Lane Cove NSW, Australia). SW620 and HCT116 human colon cancer cells were grown in RPMI1640 with 10% fetal bovine serum, 100 U/ml penicillin, and 100 μg/ml streptomycin at 37°C in 5% CO2 humidified air.

Cell viability assay

SW620 and HCT116 colon cancer cells were plated at a density of 10⁶ cells/well in 96-well plates per 100 μl medium. To determine the appropriate concentration that is not cytotoxic to the cells, the cytotoxic effect was evaluated in cells cultured for 12, 24, 36, and 48 h using the cell counting kit-8 assay kit according to the manufacturer’s instructions (Dojindo, Gaithersburg, MD, USA). Briefly, 10 μl of the CCK-8 solution was added to cells cultured for the designated time. The plates were incubated for 1–4 h in the incubator. The resulting color was assayed at 450 nM using a microplate absorbance reader (Sunrise, Tecan, Switzerland). Each assay was carried out in triplicate.

Gel electromobility shift assay

Gel shift assay was performed according to the manufacturer’s recommendations (Promega, Madison, WI, USA). Briefly, the sample of 1 × 10⁶ cells/ml was washed twice with 1×PBS, followed by the addition of 1 ml of PBS, and the cells were scraped into a cold Eppendorf tube. Cells were pelleted by centrifugation at 15,000 × g for 5 min, and the resulting supernatant was removed. Solution A (50 mM HEPES, pH 7.4, 10 mM KCl, 1 mM EDTA, 1 mM EGTA, 1 mM dithiothreitol, 0.1 μg/ml phenylmethylsulfonyl fluoride, 1 μg/ml pepstatin A, 1 μg/ml leupeptin, 10 μg/ml soybean trypsin inhibitor, 10 μg/ml aprotinin, and 0.5% Nonidet P-40) was added to the pellet in a 2:1 ratio (v/v) and allowed to incubate on ice for 10 min. Solution C (solution A + 10% glycerol and 400 mM KCl) was added to the pellet in a 2:1 ratio (v/v), and vortexed on ice for 20 min. The cells were centrifuged at 15,000 × g for 7 min, and the resulting nuclear extract supernatant was collected in a chilled Eppendorf tube. Consensus oligonucleotides were end-labeled using T4 polymerase, and [γ-32P]ATP for 10 min at 37°C. Gel shift reactions were assembled and allowed to incubate at room temperature for 10 min followed by the addition of 1 μl (50,000 – 200,000 cpn) of 32P-
labeled oligonucleotide and another 20 min of incubation at room temperature. Subsequently 1 µl of gel loading buffer was added to each reaction and loaded onto a 4% nondenaturing gel and electrophoresis was performed until the dye was three-fourths of the way down the gel. In EMSA competition studies, a 100-fold excess of unlabeled competitor oligonucleotide NF-κB was incubated with nuclear extract for 30 min before the addition of the labeled probe. The mixture with the labeled probe was incubated for another 30 min on ice. For the supershift assay, 0.5 µg of the indicated antibodies (p65 and p50) were further added, and the mixture was incubated for an additional 30 min on ice and then subjected to gel electrophoresis using 6% native polyacrylamide gels in 1× Tris-borate-EDTA buffer for 2 h. The gel was dried at 80°C for 50 min and exposed to film overnight at −70°C. The relative density of the DNA-protein binding bands was scanned by densitometry using MyImage (SLB, Seoul, Korea), and quantified by Labworks 4.0 software (UVP Inc., Upland, CA, USA).

Western blot analysis

Cultured cells were washed twice with 1×PBS, followed by the addition of 1 ml of PBS, and the cells were scraped into a cold Eppendorf tube. Cells were homogenized with lysis buffer [50 mM Tris pH 8.0, 150 mM NaCl, 0.02% sodium azide, 0.2% SDS, 1 mM PMFS, 10 µl/ml aprotinin, 1% igapel 630 (Sigma-Aldrich, St. Louis, MO, USA), 10 mM NaF, 0.5 mM EDTA, 0.1 mM EGTA, and 0.5% sodium deoxycholate] and centrifuged at 23,000 × g for 15 min. The protein concentration was measured by the Bradford method (Bio-Rad Protein Assay; Bio-Rad Laboratories Inc., Hercules, CA, USA), and equal amount of proteins (50 µg) were separated on a SDS/12%-polyacrylamide gel and then transferred to a Hybond ECL nitrocellulose membrane (Amersham Pharmacia Biotech Inc., Piscataway, NJ, USA). Blots were blocked for 2 h at room temperature with 5% (w/v) non-fat dried milk in Tris-buffered saline [10 mM Tris (pH 8.0) and 150 mM NaCl] solution containing 0.05% tween-20. The membrane was incubated for 5 h at room temperature with specific antibodies: mouse monoclonal inducible nitric oxide synthase (iNOS) antibody (1:500), mouse polyclonal antibodies against p65 and p50 (1:500 dilution; Santa Cruz Biotechnology Inc., Santa Cruz, CA, USA); rabbit polyclonal for cyclooxygenase (COX)-2, Bax, and Bcl-2 (1:500 dilution, Santa Cruz Biotechnology Inc.); and for caspase-3, cleaved caspase-3, cleaved caspase-9, cleaved poly (ADP-ribose) polymerase (PARP), inhibitor of apoptosis protein (cIAP) 1, and X-chromosome linked inhibitor of apoptosis protein (XIAP) (1:1000 dilution; Cell Signaling Technology, Inc., Beverly, MA, USA). The blot was then incubated with the corresponding conjugated anti-rabbit and antimouse immunoglobulin G-horseradish peroxidase (1:4:000 dilution, Santa Cruz Biotechnology Inc.). Immunoreactive proteins were detected with the ECL Western blotting detection system. The relative density of the protein bands was scanned by densitometry using MyImage (SLB) and quantified by Labworks 4.0 software (UVP Inc.).

Transfection and assay of luciferase activity

SW620 and HCT116 human colon cells (2.5 × 10⁵ cells/cm²) were plated in 24-well plates and transiently transfected with pNF-κB-Luc plasmid (5x NF-κB; Stratagene, La Jolla, CA, USA) using a mixture of plasmid and lipofectAMINE PLUS in OPTI-MEM according to manufacturer’s specifications (Invitrogen, Carlsbad, CA, USA). The transfected cells were treated with tumor necrosis factor-α (TNF-α) (10 ng/ml) or TPA (50 nM) and different concentrations (30 – 150 µg /ml) of thiacremonone for 8 h. Luciferase activity was measured by using the luciferase assay kit (Promega, Madison, WI, USA) according to the manufacturer’s instructions (WinGlow, Bad Wildbad, Germany).

Detection of apoptosis

Apoptosis assay was first performed by using 4,6-diamino-2-phenylindole (DAPI) staining. SW620 and HCT116 human colon cancer cells were cultured in the absence or presence of increasing concentrations of thiacremonone, and induction of apoptotic cell death was evaluated after 24 h. Apoptotic cells were determined by the morphological changes after DAPI staining under fluorescence microscopic observation (DAS microscope, 100× or 200×; Leica Microsystems, Inc., Deerfield, IL, USA). Apoptosis was also evaluated by TUNEL staining assay. In short, cells were cultured on 8-chamber slides. After treatment with thiacremonone (30 – 150 µg/ml) for 24 h, the cells were washed twice with PBS and fixed by incubation in 4% paraformaldehyde in PBS for 1 h at room temperature. TUNEL assays were performed by using the in situ Cell Death Detection Kit (Roche Diagonostics GmbH, Mannheim, Germany) according to manufacturer’s instructions. Total number of cells in a given area was determined by using DAPI and TUNEL staining. The apoptotic index was determined as the number of DAPI-stained TUNEL-positive cells divided by the total cell number counted ×100.

Data analyses

Statistical analyses of data were performed by using
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one-way analysis of variance followed by the Tukey test as a post hoc test. Differences were considered significant at $P<0.05$.

Results

Thiacremonone inhibited NF-κB activation

NF-κB is a factor implicated in apoptotic cell death of several types of cancer cells. To determine whether inactivation of NF-κB may be also involved in thiacremonone-induced apoptotic cell death, we determined DNA binding activity of NF-κB by the electrophoretic gel mobility shift assay. We found that high DNA binding activity of NF-κB was detected in the untreated SW620 and HCT116 colon cancer cells, and thiacremonone decreased DNA binding activity of NF-κB in a dose-dependent manner (30 – 150 µg/ml) in both SW620 (Fig. 2Aa) and HCT116 colon cancer cells (Fig. 2Ab).

![Figure 2](image-url)

**Fig. 2.** Effect of thiacremonone on NF-κB activation in SW620 and HCT116 colon cancer cells. A: Nuclear extract from SW620 (a) and HCT116 (b) colon cancer cells treated with thiacremonone (30 – 150 µg/ml) for 1 h was incubated in binding reactions of $^{32}$P-end-labeled oligonucleotide containing the κB sequence. The activation of NF-κB was investigated using EMSA as described in Materials and Methods. For competition assays, nuclear extracts from cells treated with TNF-α were incubated for 1 h before EMSA with unlabeled NF-κB oligonucleotide or labeled NF-κB oligonucleotide. For supershift assays, nuclear extracts from cells treated with TNF-α were incubated for 1 h before EMSA with specific antibodies against the p50 and p65 NF-κB isoforms. SS indicates supershift assay using p65 antibody (c). Quantification of band intensities from three independent experimental results performed by densitometry (Imaging System) and the value under each band indicated as fold difference from the untreated control group. B: Colon cancer cells were transfected with pNF-κB-Luc plasmid (5x NF-κB) and then activated with TNF-α (150 U/ml) alone or TNF-α plus different concentrations (30 – 150 µg/ml) of thiacremonone at 37°C in both SW620 (a and b) and HCT116 (c and d) colon cancer cells, and then the luciferase activity was determined. All values represent means ± S.D. of three independent experiments performed in triplicate. RLU is relative to luciferase activity in transfected unstimulated cells. *$P<0.05$ indicates statistically significant differences from the TNF-α-treated group.
This NF-κB DNA binding activity was confirmed by competition assay using unlabelled NF-κB as well as by super shift assay using antibody treatment of NF-κB subunits (p65 and p50). The supershift result indicated that p65 subunit is major target of thiacremonone (Fig. 2Ac). To determine the effect of thiacremonone on TNF-α-induced NF-κB-dependent reporter gene expression, we transiently transfected the cells with a NF-κB-regulated luciferase reporter construct, and then the transfected cells were stimulated with TNF-α alone or the combination of TNF-α and thiacremonone. Consistent with the inhibitory effect on NF-κB DNA binding activity, thiacremonone (30 – 150 µg/ml) inhibited TNF-α and TPA induced NF-κB luciferase activity dose-dependently in both SW620 (Fig. 2: Ba and Bb) and HCT116 (Fig. 2: Bc and Bd) colon cancer cells.

**Thiacremonone inhibited in SW620 and HCT116 human colon cancer cell growth**

To investigate the inhibitory effect of thiacremonone on SW620 and HCT116 human colon cancer cell growth, we analyzed cell growth by the CCK-8 assay. Morphological observation showed that the cells gradually reduced in size and changed to a round single cell shape in a dose-dependent manner by the treatment with thiacremonone in both SW620 (Fig. 3Aa) and HCT116 (Fig. 3Ba) colon cancer cells. Cancer cell growth inhibitory effects were dose-dependent (30 – 150 µg/ml) in both SW620 (Fig. 3Ab) and HCT116 (Fig. 3Bb) human colon cancer cells. IC<sub>50</sub> values (48 h) of cell growth inhibition were 105 µg/ml in SW620 colon cancer cells and 130 µg/ml in HCT116 colon cancer cells. However, thiacremone showed no cytotoxic effect in the normal colon Caco-2 cells (Fig. 3: Ca and Cb).

![Fig. 3](image-url)

**Fig. 3.** Morphological changes and cell viability of colon cancer and normal cells by thiacremonone. a: Morphological changes were observed under a microscope (magnification, ×200). SW620 (A) and HCT116 (B) colon cancer cells and normal cells (Caco-2) (C) were treated with various doses (30 – 150 µg/ml) of thiacremonone for 24 h. b: Cell viability was determined by CCK-8 assay as described in Materials and Methods. SW620 (A) and HCT116 (B) colon cancer cells as well as normal cells (Caco-2) (C) were treated with various doses (30 – 150 µg/ml) of thiacremonone for different times (12 – 48 h). Values are each the mean ± S.D. of three experiments, each performed in triplicate.
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To determine whether the inhibition of cell growth by thiacremonone was due to the induction of apoptotic cell death, we evaluated changes in the chromatin morphology of cells using DAPI staining. To further characterize the apoptotic cell death by thiacremonone, we performed TUNEL staining assays, and then the labeled cells were analyzed by fluorescence microscopy. Thiacremonone-treated cells were labeled by TUNEL assay and there was increased fluorescence intensity in both SW620 (Fig. 4Aa) and HCT116 (Fig. 4Ab) colon cancer cells. In SW620 colon cancer cells, the apoptotic cell number (DAPI-positive TUNEL-stained cells) was increased to 0%, 0% 11%, 25%, and 97% by 30, 60, 90, 120, and 150 µg/ml thiacremonone, respectively (Fig. 4Ba). In HCT116 colon cancer cells, the apoptotic cell number was increased to 0%, 0%, 29%, 95%, and 98% by 30, 60, 90, 120, and 150 µg/ml thiacremonone, respectively (Fig. 4Bb).

Thiacremonone-induced apoptotic cell death

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To further characterize the apoptotic cell death by thiacremonone, we performed TUNEL staining assays, and then the labeled cells were analyzed by fluorescence microscopy. Thiacremonone-treated cells were labeled by TUNEL assay and there was increased fluorescence intensity in both SW620 (Fig. 4Aa) and HCT116 (Fig. 4Ab) colon cancer cells. In SW620 colon cancer cells, the apoptotic cell number (DAPI-positive TUNEL-stained cells) was increased to 0%, 0% 11%, 25%, and 97% by 30, 60, 90, 120, and 150 µg/ml thiacremonone, respectively (Fig. 4Ba). In HCT116 colon cancer cells, the apoptotic cell number was increased to 0%, 0%, 29%, 95%, and 98% by 30, 60, 90, 120, and 150 µg/ml thiacremonone, respectively (Fig. 4Bb).

Thiacremonone induced the expression of apoptotic regulatory proteins

NF-κB can regulate genes controlling apoptotic cell death. NF-κB activation in colon cancer cells correlates with resistance to apoptosis and increased levels of anti-apoptotic proteins. Anticancer drugs are considered to mediate cell death by activating key elements of the apoptosis program relating NF-κB. To figure out the relationship between the induction of apoptosis by thiacremonone and expression of NF-κB target apoptotic gene expression, expressions of apoptosis-related proteins were investigated. Expressions of apoptotic proteins such as Bax, cleaved caspases 3, and cleaved PARP were increased, whereas the expressions of anti-apoptotic protein Bcl-2, cIAP1/2, XIAP, and cell cycle regulating gene cyclin D1 were decreased. We also

![Figure 4](image-url)

Fig. 4. Morphological changes and apoptotic cell death of SW620 (a) and HCT116 (b) colon cancer cells by thiacremonone. A: SW620 and HCT116 colon cancer cells were treated with various doses (30 – 150 µg/ml) of thiacremonone for 24 h; cell morphological changes were observed under a microscope (upper panels; magnification, ×200); and apoptotic cells were examined by fluorescence microscopy after TUNEL staining (fluorescent microscopy magnification, ×200) (lower panels). Total number of cells in a given area was determined by DAPI nuclear staining (fluorescent microscopy magnification, ×200) (middle panels). B: The apoptotic index was determined as the DAPI-stained TUNEL-positive cell number counted. Values are mean ± S.E.M. of three experiments, each performed in triplicate. *P<0.05 indicates statistically significant differences from the untreated group.
found that iNOS and COX-2 expression was high in these colon cancer cell lines, but thiacremonone treatment inhibited the expressions dose-dependently in both SW620 (Fig. 5a) and HCT116 (Fig. 5b) colon cancer cells.

Discussion

The reduction of malignant tumor incidence by consumption of garlic has been reported from many countries such as Italy (32), Germany (33), China (34), and USA (35). However, precise molecular mechanisms and actual components by which garlic reduced tumor incidence are not fully elucidated. It has been reported that garlic has protective effects against cardiovascular diseases (35) and blood pressure (36) and inhibits platelet aggregation (37). Some sulfur compounds isolated from garlic were clearly identified as anti-cancer compounds, such as diallylsulfide, S-allylmercapto-cysteine, and ajoene, which seems to inhibit cell proliferation and induce apoptosis in breast, lymphocytic leukemia, and hepatoma cancer cells (11 – 13). These anti-cancer effects may be due to its anti-oxidant and anti-proliferative properties of components in garlic. In our recent study, we isolated a novel sulfur compound (named Thiacremonone) from the hot water extract of garlic, and found that this compound has high anti-oxidant activity (30). Since many natural compounds that have high anti-oxidant activities have been demonstrated to prevent cancer cell growth (38) and sulfur compounds isolated from garlic showed anti-cancer effects, we were interested in investigating the anti-cancer effect of this novel isolated sulfur compound and its action mechanisms.

In the present study, we found that treatment with thiacremonone resulted in inhibition of NF-κB activation in SW620 and HCT116 human colon cancer cells. This effect was exerted in parallel with its effects of cell growth inhibition and induction of apoptosis. Several recent lines of evidence have indicated that transcription factor NF-κB is involved in the tumor development (24 – 27). Activation of NF-κB has been associated with several aspects of tumorigenesis, including cancer cell proliferation, prevention of apoptosis, and increases of angiogenesis and metastasis potential (18, 24). NF-κB transcription factor is constitutively activated in human pancreatic breast, prostate, and lung cancer cells as well as colorectal carcinomas, which provides a favorable condition for cancer cell growth (24, 25, 39 – 42). In addition, NF-κB is also activated in human colon tumor samples and adenomatous polyps (22). The present data therefore indicated that the inhibition of NF-κB by thiacremonone might be at least a critical mechanism in the inhibition of SW620 and HCT116 human colon cancer cell growth. Similar to our findings, epoxyquinone A monomer, a synthetic derivative of the natural product epoquinol A, inhibited NF-κB through binding to p65 subunit, and NF-κB inhibition was correlated with its inhibitory effect on colon cancer cell growth (26). Aggarwal et al. also reported that curcumin inhibited colon cancer cell growth through NF-κB inactivation (27). Recently, we also found that inhibition of NF-κB

![Fig. 5. Effect of thiacremonone on expression of apoptosis regulatory proteins. The cells were treated with different concentrations (30–150 µg/ml) of thiacremonone at 37°C for 24 h. Equal amounts of total proteins (50 µg/lane) were subjected to 12% SDS-PAGE. Expressions of Bax, cleaved caspase-3, cleaved PARP, Bcl-2, cIAP1/2, XIAP, and β-actin were detected by Western blotting using specific antibodies. β-Actin protein was used as an internal control. a: SW620 colon cancer cells. b: HCT116 colon cancer cells. Each band is representative of three independent experiments.](image-url)
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by 2’-hydroxynamaldehyde resulted in inhibition of colon cancer cell growth through induction of apoptotic cell death of human colon cancer cells (24). These data suggest that constitutive activation of the NF-κB signaling pathway could be an important step during tumor development in the colon, and thereby inhibition of NF-κB activation could be one mechanism mediating prevention of colon cancer cell growth by the garlic component thiacremonone.

The precise mechanism for how thiacremonone inactivates NF-κB is not clear. The anti-oxidative property of thiacremonone may be related with the inhibitory effect against NF-κB. It is noteworthy that many anti-oxidants can downregulate NF-κB activity (43, 44). Curcumin (diferuloylmethane) is a chemical derived from several Curcuma species (turmeric), possessing anti-inflammatory and antioxidant properties. Curcumin induced apoptosis in human colon cancer colo 205 cells through inhibition of NF-κB activation (43). Mangiferin, a natural polyphenol, is known to exhibit antioxidative effects, and induced apoptosis through inhibition of NF-κB activation induced by TNF-α in human breast cancer cells (MCF-7) (44). Therefore, the high antioxidant property of thiacremonone may interfere with the NF-κB signal causing apoptotic cell death. We also found that consistent with the expression pattern of anti-apoptotic proteins and cyclin D, iNOS and COX-2 expressions were high in these colon cancer cell lines, but thiacremonone treatment of these two cancer cell lines inhibited the expressions dose-dependently. These data suggest that inhibition of NF-κB resulted in iNOS and COX-2 expression that give secondary environments favorable for the progression of cancer cell growth. Thus, the inhibitory effect of thiacremonone on the inflammatory reaction could be related to its anti-cancer effects. In fact, we found that thiacremonone has an inhibitory effect on iNOS and COX-2 expressions accompanied with the inhibitory effect on NF-κB in cultured macrophage cells (Raw 264.7 cells) (unpublished data). Another possibility for the inhibitory effect of thiacremonone on NF-κB activity is a direct interaction between the sulfur moiety of thiacremonone and NF-κB, which results in adduct formation with the thiol groups of NF-κB proteins and subsequent alteration of NF-κB properties, including DNA binding activity (45). Several compounds have been found to directly react with cysteine sulhydryl groups of the NF-κB subunit and thus alters expression of the NF-κB target gene product (24, 46). In NF-κB/p65, cysteine residues participate in DNA binding by forming a hydrogen bond with the sugar/phosphate backbone of the κB-DNA motif. Therefore, it is highly possible that thiacremonone caused a decrease in DNA binding activation of NF-κB through reacting directly with cysteine sulhydryl groups of the NF-κB subunit molecules. In fact, we found that Thiacremonone did not show any growth inhibitory effect on the SW620 human colon cancer transiently transfected with p65 mutant (data not shown).

NF-κB plays a key role in cell protection against diverse apoptotic stimuli, including chemotherapeutic treatments through inactivation of the antiapoptotic gene program in cells (24, 47). Pro- and anti-apoptotic genes that are regulated by NF-κB include inhibitor of apoptosis proteins such as cIAP1/2, XIAP, and Bcl-2 and activator of apoptosis proteins such as Bax, caspase-3 and -9, and PARP (24, 47). Apoptosis is an important mechanism for eliminating unwanted cells in a wide variety of physical processes, and regulation of this process is implicated in pathogenesis of many chronic diseases, including cancer development (48). Thiacremonone treatment in SW620 and HCT116 human colon cancer cells altered the expression of apoptosis regulatory proteins. Thiacremonone repressed the expression of anti-apoptotic proteins (Bcl-2, IAP1/2, and XIAP), whereas it increased the expression of proapoptotic proteins (Bax, cleaved caspase-3, and cleaved PARP). Thus, thiacremonone may induce an alteration of apoptosis and anti-apoptosis regulatory protein expression that provide the favorable circumstance of cancer cells going to death status. However, we did not observe any cytotoxic effect in normal colon cells treated with thiacremonone (up to 300 μg/ml).

Taken together, our data showed that induction of apoptosis could be involved in thiacremonone-induced cell growth inhibition in colon cancer cells, and inhibition of NF-κB could provide a specific and causative mechanism for the inhibition of colon cancer cell growth by thiacremonone, a novel recently identified sulfur-compound from garlic.

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