Antiproliferative Effects of S-Allylmercaptocysteine on Colon Cancer Cells When Tested Alone or in Combination with Sulindac Sulfide

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ABSTRACT

Epidemiological studies link increased garlic (Allium sativum) consumption with a reduced incidence of colon cancer in various human populations. Experimental carcinogenesis studies in animal models and in cell culture systems indicate that several allium-derived compounds exhibit inhibitory effects and that the underlying mechanisms may involve both the initiation and promotion phases of carcinogenesis. To provide a better understanding of the effects of allium derivatives on the prevention of colon cancer, we examined two water-soluble derivatives of garlic, S-allylcysteine (SAC) and S-allylmercaptocysteine (SAMC), for their effects on proliferation and cell cycle progression in two human colon cancer cell lines, SW-480 and HT-29. For comparison, we included the compound sulindac sulfide (SS), because sulindac compounds are well-established colon cancer chemopreventive agents. We found that SAMC, but not SAC, inhibited the growth of both cell lines at doses similar to that of SS. SAC also induced apoptosis, and this was associated with an increase in caspase-3-like activity. These effects of SAC were accompanied by induction of jun kinase activity and a marked increase in endogenous levels of reduced glutathione. Although SS caused inhibition of cell cycle progression from G1 to S, SAC inhibited progression at G2-M, and a fraction of the SW-480 and HT-29 cells were specifically arrested in mitosis. Codmanistration of SS with SAC enhanced the growth inhibitory and apoptotic effects of SS. These findings suggest that SAC may be useful in colon cancer prevention when used alone or in combination with SS or other chemopreventive agents.

INTRODUCTION

Colon cancer is one of the leading causes of cancer morbidity and mortality worldwide (1). Over the past decade, the use of garlic either as a food or as dietary supplement for the chemoprevention or treatment of gastric or colon cancer (2, 3) has received increasing attention. The rationale for its chemopreventive efficacy is based on the ability of both lipid- and water-soluble allium-derived compounds to: (a) enhance the activity of specific mixed-function oxidases that depress the activation of carcinogens (4–7); (b) induce phase II conjugating enzymes, such as glutathione S-transferases and glucuronyl transferases, which enhance detoxification and excretion of potential carcinogens and reduce the formation of DNA adducts; and (c) increase the synthesis of GSH, an endogenous tripeptide thiol that directly protects cells from damage by free radicals. In addition to inhibiting cancer initiation, specific allylsulfide derivatives also inhibit the growth of transplantable tumors and exert antiproliferative activity against a number of mammalian tumor cell lines (8–12). It appears that thioalyl derivatives can inhibit the growth of transformed cells by various mechanisms, including alterations in mitogenic programming, inhibition of protein kinases involved in signal transduction, blocking the function of calcium ion channels (9), and modifying steroid hormone responsiveness in breast and prostate carcinoma cells (13).

To further elucidate the antiproliferative activity of naturally occurring water-soluble constituents of garlic, we examined the effects of both SAC and SAMC on cell proliferation, cell cycle kinetics, and apoptosis in two well-characterized human colon cancer cell lines, SW-480 and HT-29. In addition, because several signaling molecules (the c-Fos/Jun complex, bcl-2, and jun kinase) contain cysteine domains (14, 15) that are sensitive to changes in sulfhydryl/disulfide oxidation, we also assessed the effects of these compounds on cellular levels of GSH. We hypothesized that changes in the intracellular redox environment induced by allylsulfides may alter the activity of specific signal transduction factors. Because of the current interest in the chemoprevention of colon cancer using sulindac or its metabolites (16, 17), we also compared the effects of these two allylsulfides with those of SS in SW-480 and HT-29 cells.

MATERIALS AND METHODS

Allium Derivatives and SS. SAC and SAMC are water-soluble allium derivatives and were generously supplied by Wakunaga of America Co., Ltd. (Mission Viejo, CA). Whereas SAC is a major constituent of "Aged Garlic Extract," a proprietary mixture of a commercially available garlic supplement, SAMC can be formed in vivo from the interaction of the garlic constituent, allicin, and the amino acid, cysteine. Stock solutions of both derivatives (5 mm) were prepared fresh in PBS followed by mild sonication. Before addition to cell cultures, both solutions were cold-filtered through sterile 0.2-mm Millipore syringe filters. SS was obtained from Cell Pathways, Inc., Philadelphia, PA.

Cell Lines and Culture Conditions. SW-480 and HT-29 human colon cancer cell lines were obtained from the American Type Culture Collection and maintained in DMEM with 10% fetal bovine serum (Life Technologies, Inc.) in 180-ml tissue culture flasks (Nunclon, Rochester, NY) at 37°C in an atmosphere 5% CO2 and air. Cells were fed fresh medium with serum every 2–3 days and split when 50% confluent. All studies were performed with exponentially growing cells at 50% confluence. SW-480 and HT-29 cells were seeded in triplicate in 6-well (3.5-cm diameter) cell culture plates (Becton Dickinson) at a concentration of 3 × 104 cells/well. After 24 h, cells were treated with increasing concentrations of SAC or SAMC (50–250 μM) or SS (100 and 200 μM). Cells were harvested by trypsinization at 0, 24, and 48 h postadministration of each compound and cell numbers were determined using a Coulter Counter (Coulter Electronic, Inc.).

Assessment of Cell Cycle Distribution and Apoptosis. SW-480 and HT-29 cells that were cocultured with either SAC or SS and/or SS were analyzed, in parallel with cells grown in the absence of these compounds, using a standard flow cytometric procedure (18, 19) to determine effects on cell cycle distribution and apoptosis. Both adherent and floating cells were collected and fixed with 70% ethanol. The conditions for PI staining and data acquisition have been described previously (20). Apoptotic cells were considered to

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3 The abbreviations used are: GSH, reduced glutathione; SAC, S-allylcysteine; SAMC, S-allylmercaptocysteine; SS, sulindac sulfide; PI, propidium iodide; JNK1, c-Jun-NH2-terminal kinase; FISH, fluorescence in situ hybridization.
constitute the sub-G1 cell population. All experiments were performed in triplicate and gave similar results. Cells were also fixed and stained with a 1:250 dilution (20 μg/ml) of a MPM-2 mouse antibody (Upstate Biotechnology, Lake Placid, NY) and FITC-labeled goat antimouse IgG, diluted 1:100 (2 μg/ml; Rockland, Gilbertsville, PA). The PI-staining after MPM-2-staining was performed with a concentration of PI (5 μg/ml) that was lower than that used for routine flow cytometric studies.

**FISH.** SW-480 and HT-29 cells, before and after a 24-h treatment with 200 μM SAMC, were harvested when 50% confluent, and deposited on slides. FISH analysis was performed using standard methods (21). Spectrum orange- and spectrum green-labeled centromeric probes for chromosomes 11, 12, 16, and 17 were obtained from Vysis (Downer Grove, IL). Double-color FISH was performed to identify and enumerate chromosome numbers, and the signals were captured and analyzed using an Applied Imaging CytoVision Capture workstation (Santa Clara, CA). At least 1000 cells were analyzed per experiment.

**Protein Extraction and Western Blotting.** Cells in their log phase of growth were collected and proteins extracted for immunoblotting as described previously (20). Samples of 50–100 μg were subjected to SDS-PAGE, transferred to nylon membranes, incubated overnight at 4°C in blocking buffer (50 mM Tris, 200 mM NaCl, 0.2% Tween 20, and 3% BSA) and reincubated for 1 h with the indicated antibodies. Polyclonal antisera to the Bcl-2 gene family of proteins (Bcl-2, Bax, Bcl-x, and Bak) were kindly provided by Dr. John C. Reed (Burnham Institute, La Jolla, CA) and used at a concentration of 1:1500, as described previously (22). Immunoblotting of actin (1:5000 dilution; Sigma Chemical Company, St. Louis, MO) was performed to verify equivalent loading of protein on the gels.

**GSH Determinations.** Cellular concentrations of GSH were measured using a Perkin-Elmer high-performance liquid chromatograph equipped with a four-channel coulometric array detector (ESA, Inc., Chelmsford, MA; Ref. 23). Cells were plated in 150-mm culture dishes at concentrations to yield 50% confluence within 48 h. Media were aspirated and replaced with fresh media containing either PBS, 200 μM SAC, 200 μM SAMC, or 200 μM SS or combinations of SAC/SS (200 μM/100 μM) and SAMC/SS (200 μM/100 μM). At 3 and 24 h postincubation, cells were harvested by gentle scraping, washed twice with ice-cold PBS, and centrifuged to obtain a final cell pellet. After removal of the supernatant fraction, the cells (5–10 × 10^6) were lysed in 0.25 ml of 200 mM methane sulfonic acid containing 5 mM diethylenetriaminepentaaetic acid and centrifuged at 10,000 × g for 10 min. Precipitates were dissolved in 0.1 N NaOH and saved for protein determinations, which were measured by a spectrophotometric quantitation method using the BCA protein assay reagent (Pierce Chemical Co., Rockford, IL). After a 1.3 dilution of the supernatant fractions with the mobile phase, aliquots were injected onto an Ultrasphere 5 microns, 4.6 × 250 mm, C18 column, and then eluted with a mobile phase of 50 mM NaH_2PO_4, 0.05 mM octane sulfonic acid, and 2% acetonitrile (pH 2.7) at a flow rate of 1 ml/min. The four-channel CoulArray detectors were set at 525, 625, 725, and 825 mV, respectively. Peak areas were analyzed using ESA, Inc., software. The final concentrations of GSH were reported as nmol/mg protein.

**Assays for JNK1 Activity.** Assays for JNK1 kinase activity were performed at 30, 60, and 120 min posttreatment of the cells with 200 μM of SAC, SAMC, or SS. Cells pellets were suspended in 500 μl M2 lysis buffer (20 mM Tris (pH 7.6) containing 0.5% NP-40, 250 mM NaCl, 3 mM EDTA, 3 mM EGTA, 1 mM DTT, 0.5 mM phenylmethylsulfonyl fluoride, 10 μM leupeptin, 20 μM of a 0.2 mM solution of α-glycerophosphate, and 5 μM of 0.1 mM sodium vanadate). Cellular JNK1 was precipitated by incubation of 200 μg of the cell protein extract with an anti-JNK antisera (Santa Cruz, Santa Cruz, CA) and protein A-Sepharose (Sigma, St. Louis, MO) beads in M2 lysis buffer for 2 h with rotation at 4°C. The precipitates were washed twice with M2 buffer and twice with kinase buffer (20 mM Tris (pH 7.5) containing 20 mM α-glycerophosphate, 10 mM p-nitrophenylphosphate, 10 mM MgCl_2, 1 mM DTT, 50 μM sodium vanadate, and 20 μM ATP) leaving a 25 μl final volume of beads and buffer. The kinase assay mix, containing 1 μg of GST-c-Jun (New England Biolabs, Beverly, MA) as the substrate and 1 μl of γ-32P-ATP (Amersham, Piscataway, NJ) in 25 μl of kinase buffer per reaction, was added to each tube on ice (50 μl total volume). The kinase reaction was then performed at 30°C for 20 min and terminated by adding SDS sample buffer. The reaction mixtures were boiled for 5 min and analyzed by SDS-PAGE (10%). The extent of protein phosphorylation was determined using autoradiography.

**Assays for Caspase Activity.** After treatment with SAC, SAMC, or SS, the cells were washed twice with ice-cold PBS and lysed in caspase buffer [50 mM HEPES-KOH (pH 7.4) containing 0.1% 3-[3-cholamidopropyl]dimethylammonio]-1-propanesulfonic acid, 100 mM NaCl, 1 mM EDTA, 10 mM DTT, and 10% glycerol]. The lysates were sonicated for 5 s and then placed on ice for 10 min. The homogenates were centrifuged at 14,000 × g for 2 min and the supernatant fraction saved for analysis. Caspase activity was determined using a fluorometric assay (24). Briefly, 50 μg of total protein, determined by the Bio-Rad Protein Assay (Bio-Rad, Richmond, CA), were incubated with 10 μg of a fluorogenic peptide substrate, Ac-DEVD-AFC (PharMingen, San Diego, CA), with caspase buffer, in a total volume of 1 ml. After a 1-h incubation at 37°C, the release of 7-amino-4-methylcoumarin was determined spectrophotometrically using an Applied Imaging CytoVision Capture workstation (Santa Clara, CA). At least 1000 cells were analyzed per experiment.

**RESULTS**

**Effects of SAC and SAMC on Growth and Cell Cycle Kinetics.** The addition of SAMC to either SW-480 or HT-29 cell lines was associated with a marked dose-dependent inhibition of growth, with an IC50 value of ~160 μM for SW-480 cells and ~175 μM for HT-29 cells (Fig. 1). To examine the mechanism responsible for this growth inhibition, cell cycle distribution was evaluated using flow cytometry. The addition of 200 μM SAMC caused an accumulation of cells in G2-M with both the SW-480 and HT-29 cells, although this effect was less pronounced in HT-29 cells. Thus, at 24 h, 71% of the SAMC-treated SW-480 cells were in G2-M versus 16% of the control cells; and 30% of the SAMC-treated HT-29 cells were in G2-M versus 12% of the control cells. There was a reciprocal decrease in the number of cells in G3 in both cell types (Fig. 2A). These changes were much less striking at 48 h (Fig. 2A), perhaps because of the onset of apoptosis, as described below.

It is of interest that similar concentrates of SAC produced no significant growth inhibition of either SW-480 or HT-29 cells (Fig. 1) and no significant change in cell cycle distribution (data not shown). We included SS for comparison and found that it also inhibited growth, with an IC50 value similar to that of SAMC (Fig. 1), and it also inhibited cell cycle progression; but with this agent, the arrest was in G1 (data not shown), as reported previously (25).

**Evidence that SAMC Specifically Arrests Cells in the G2 or M Phases.** The above studies indicated that, in contrast to SS, SAMC arrests cells in G2-M (Fig. 2A). Conventional DNA flow cytometry does not distinguish cells that are in the G2 phase from cells that are in the M phase, because cells in both phases have the same DNA content. Therefore, we used an MPM2 antibody that reacts specifically with phosphoproteins present in M-phase cells (26) in combination with DNA flow cytometry to further analyze the effects of SAMC. Using this technique, we found that after treating the SW-480 cells with 200 μM SAMC, there was a significant increase of cells in the G2 phase but not in the M phase when compared with control untreated cells (Fig. 2B). However, with 300 μM SAMC there was an increase of cells in both G2 and M (Fig. 2B). With the HT-29 cells, 200 μM SAMC caused a reduction of cells in the G2 phase and an increase of cells in the M phase. These changes were further exag-
gerated when HT-29 cells were treated with 300 μM SAMC (Fig. 2B). Thus, SAMC causes SW-480 cells to accumulate in both G2 and M, but causes HT-29 cells to accumulate in M. As expected, SS (200 μM) did not cause an increase of cells in G2 or in M (Fig. 2B), but instead most of the treated cells accumulated in G1 (data not shown).

It is well known that cells in anaphase have twice the number of centromeres as those in metaphase or in stages before metaphase. Therefore, using the criterion of number of centromeres, it was of interest to determine whether some of the cells that accumulated in the M phase after treatment with SAMC were actually in anaphase.

Exponentially growing cultures of either SW-480 or HT-29 cells were treated with SAMC (200 μM) for 24 h or with only the solvent, and then double-color FISH analysis was performed with two sets of centromeric probes, CEP12/16 and CEP 11/17. The number of targeted centromeres in untreated SW-480 and HT-29 cells varied between 2 and 4, which is consistent with the aneuploid nature of these cells. In the SAMC-treated SW-480 cells there was an increase in the number of centromeres. Thus, the frequency of cells in anaphase was 7.6% in the SAMC-treated SW-480 cells compared with 1.9% in the untreated cells (Fig. 3). However, HT-29 cells did not display an increase in the number of cells in anaphase after treatment with SAMC (data not shown). These data suggest that the SAMC-induced G2-M blockade in SW-480 cells, as demonstrated by flow cytometry (Fig. 2A), is associated with a cell cycle delay in anaphase.

SAMC-induced Apoptosis. Spontaneous apoptosis occurred in 2–5% of the exponentially growing SW40 and HT-29 colon cancer cells, as assessed by analysis of the subdiploid DNA peak on DNA flow cytometry. The addition of SAMC (200 μM) to exponentially growing cells induced a 2- to 5-fold increase in the percentage of cells with a sub-G1 population in both SW-480 and HT-29 cells (Fig. 4). This effect was greater in the SW-480 cells than in the HT-29 cells, which is consistent with the fact that HT-29 cells were also somewhat more resistant with respect to growth inhibition (Fig. 1). With both cell types, the increase in apoptosis was apparent at 24 h, and the extent of apoptosis increased further at
Fig. 4. Induction of apoptosis. Exponentially dividing cultures of SW-480 and HT-29 cells were incubated with 200 μM SAC, 200 μM SAMC, 200 μM SS, or a combination SS plus SAC or SS plus SAMC (200 μM of each) for either 24 or 48 h. The extent of apoptosis was assessed by the size of the sub-G₁ population seen during DNA flow cytometry and is expressed as the fold increase relative to the untreated control. The values are means +/− standard deviations of triplicate assays (*, P < 0.001).

48 h (Fig. 4). On the other hand, SAC did not induce apoptosis (Fig. 4), which is consistent with its failure to cause growth inhibition in these cell lines (Fig. 1). SS (200 μM) produced about twice the level of apoptosis obtained with the same concentration as SAMC (Fig. 4). It was, therefore, of interest to examine the combined effects of SAMC and SS on apoptosis when each was tested at 200 μM. With the SW-480 cells, the combined effects of SAMC plus SS on apoptosis were close to additive, and with HT-29 cells the combined effects were supra-additive (Fig. 4). As expected, the effects of SAC plus SS were similar to those of SS alone, because SAC alone did not induce apoptosis (Fig. 4).

Caspase-3-like Activity. Because caspases play a central role in mediating apoptosis (reviewed in Refs. 24 and 27), we asked whether caspases were involved in the cell death response induced by the garlic derivatives, SAC and SAMC. Caspase-3-like activity was measured after treatment of SW-480 and HT-29 cells with 200 μM SAC, SAMC, or SS for 3, 6, and 24 h (Fig. 5). As expected, SAC did not induce caspase-3 activity in both cell lines at all three time points. In SW-480 cells, SAMC and SS induced a 2.5- and 3.1-fold increase,
respectively, in caspase-3 activity at 24 h with no induction at earlier time points. In the HT-29 cell line, the increase in caspase-3 activity at 24 h after SAMC treatment was significantly less than in SW-480 cells, inasmuch as there was only a 1.3-fold increase at 24 h. SS caused a 3.3-fold increase in caspase-3 activity in HT-29 cells at 24 h. Thus, there was a direct association between induction of caspase-3-like activity and apoptosis. Additional studies using caspase inhibitors may help to clarify whether the activation of caspase-3-like proteases are required for the induction of cell death by SAMC.

Expression of Bcl2 Family Proteins. Western blot analysis of protein extracts obtained from untreated exponentially growing cultures indicated that the SW-480 and HT-29 cells expressed detectable levels of the following apoptosis-related proteins: Bcl-2, Bax, Bak, and BclXL. However, treatment of either of these two cell lines with 200 μM SAC or SAMC for 24 or 48 h did not show any significant changes in the levels of these proteins (data not shown).

Effect of Garlic Derivatives on Cellular Levels of GSH. Because several signal proteins contain redox-sensitive sites that can be modified by oxidative stress, we measured the concentration of GSH in cells treated for either 3 or 24 h with 200 μM SAC, SAMC, or SS. Fig. 6 indicates that with both SAC and SAMC there was an increase in GSH within 3 h and an additional increase with SAMC at 24 h, in both SW-480 and HT-29 cells. The effect of SAC was about twice that of SAC. SS had no effect at 3 h, but it caused a 2- to 3-fold increase at 24 h. In HT-29 cells, the combined effects of SS plus SAC or SS plus SAMC on GSH levels were approximately additive.

Effects of SAC, SAMC, and SS on JNK 1 Activation. There is evidence that activation of JNK1 activity plays a role in apoptosis induced by various agents (reviewed in Ref. 27). In recent studies, we found that SS, sulindac sulfone (exisulind), and related derivatives cause a rapid and sustained activation of JNK1, and that this activation plays a critical role in the induction of apoptosis by these drugs (27). Therefore, it was of interest to examine the effects of SAC and SAMC on JNK1 kinase activity. Assays for jun kinase activation were done at 30, 60, and 120 min after the addition of the drugs to exponentially dividing cultures, and the results are summarized in Fig. 7. Treatment of the cells with 200 μM SAC led to an ~2.6-fold increase in JNK 1 activity in SW-480 cells at the 120-min time point (Fig. 7). With the HT-29 cells, the induction of JNK 1 activity was only about 1.8-fold (data not shown). Treatment with 200 μM SAC had no effect in either cell type, and treatment with 200 μM SS led to an ~1.9-fold activation within 30 min in both cell types. The fact that SAMC gave a stronger induction of JNK1 activity in SW-480 cells than in HT-29 cells is again consistent with its more potent antiproliferative effects in SW-480 cells (Figs. 1 and 2).

DISCUSSION

Our results indicate that SAC inhibits growth, arrests cells in G2-M, and induces apoptosis in both SW-480 and HT-29 human colon cancer cells lines, and that this is associated with an increase in intracellular levels of GSH and activation of JNK1. We also found that combined treatment with SAC and SS had an approximately additive effect on the induction of apoptosis. By contrast, treatment of these cells with equimolar concentrations of the garlic derivative SAC did not inhibit growth or cell cycle progression, or induce apoptosis, in either of these cell lines when tested alone or in combination with SS.

Our results with SAC are consistent with previous reports (9) that also failed to show growth-inhibitory effects of SAC in HCT15 colon cancer cells or in lung and skin carcinoma cell lines. However, in previous studies, comparable doses of SAC did have antiproliferative effects on neuroblastoma (11), melanoma (10), and prostate carcinoma cells (28). Therefore, SAC may be effective as an antiproliferative agent in specific cell lines and/or under specific conditions.

The inhibitory effects on cell proliferation obtained with SAC in the present study are consistent with results obtained previously with allylsulfide derivatives. Thus, previous studies indicated that erythroleukemia (HEL; Ref. 29), promyelocytic leukemia (HL60; Ref. 30), and colon carcinoma (HCT15, SW-480, and HT-29) cells (9, 31) exposed to the diallylsulfide ajoene (E,Z)-4,5,9-trihidiododeca-1,6,11-triene-9-oxide), or to SAMC, exhibited marked decreases in the number of cells in the G1 phase and a corresponding accumulation of the cells in G2-M. By contrast, human umbilical vascular endothelial cells and smooth-muscle cells appeared to arrest in G1 when treated with these compounds (12).

The inhibitory effects of SAC on HT-29 cells were less pronounced than those on SW-480. HT-29 cells have a normal k-ras gene and express Cox-1 and Cox-2, whereas SW-480 cells have an oncogenic k-ras mutation and do not express Cox-2 (31). Studies by Singh et al. (33) demonstrated that diallylsulfide appears to suppress the growth of H-ras oncogene-transformed tumors in nude mice by decreasing the association of the p21H-ras protein with the plasma membrane. Because, however, HT-29 cells are responsive to the inhibitory effects of SAC (Fig. 1), this inhibition is not entirely dependent on ras transformation. Likewise, Cox-2 may not play a critical role, because HCT116 colon cancer cells do not express Cox-2, (34), and yet we found that they are sensitive to induction of
apoptosis and changes in cell cycle progression when treated with SAMC. Therefore, the reason for the greater resistance of HT-29 cells to SAMC is not apparent at the present time.

It is of interest that when treated with SAMC, both the SW-480 and HT-29 cells accumulated in the G_{2-M} phases of the cell cycle when examined by DNA flow cytometry (Fig. 2A). This is in contrast to the effects of sulindac, SS, and sulindac-sulfone, which usually causes cells to arrest in G_{1} (25). Using FISH analysis to examine the number of centromeres (Fig. 3), and flow cytometry after staining the cells with an antibody that reacts only with phosphoproteins present in the M phase (Fig. 2B), we obtained evidence that after treatment with 300 μM SAMC, an appreciable fraction of the SW-480 and HT 29 cells were actually arrested in the M phase. Studies are in progress to determine whether this reflects an effect of SAMC on microtubules per se, or on other proteins that control the M phase.

Recent studies have indicated that c-jun H-terminal kinases, also referred to as stress-activated protein kinases, are frequently involved in cellular responses to various environmental stresses, including agents that induce apoptosis (35, 36). Yu et al. (37, 38) found that phenethylisothiocyanate, which is present in cruciferous vegetables, induces apoptosis with concomitant activation of JNK1 and suggested that this plays a role in the chemopreventive effect of this compound. In the present study, we found that treatment of either SW-480 or HT-29 cells with SAMC, but not with SAC, caused, within 30 to 60 min, the activation of JNK1. SS had a similar and even more rapid effect (Fig. 7; Ref. 27). Studies are in progress to examine the significance of this finding with respect to growth inhibition, cell cycle arrest, and apoptosis.

It has been suggested that the anticarcinogenic effect of allylsulfide derivatives is mediated by the induction of GSH (28, 39, 40) because this endogenous tripeptide thiol compound can detoxify various carcinogens, serves as an intracellular antioxidant, and also regulates DNA and protein synthesis (41). Indeed, we found that the treatment of SW-480 or HT-29 cells with SAMC led to increased cellular levels of GSH. However, this was also seen with SAC (Fig. 6); and yet the latter compound did not have an antiproliferative effect. Therefore, it seems unlikely that the cell cycle arrest and apoptotic effects of SAMC are solely attributable to the increase in GSH. At the same time, SAMC, SAC, and other compounds in garlic may, by enhancing cellular levels of GSH, play a protective role in the early phases of carcinogenesis by scavenging free radicals. Thus, specific allylsulfide compounds in garlic, like SAC, might inhibit initiation of the carcinogenic process whereas others, like SAMC, might, through their antiproliferative effects, inhibit tumor promotion and/or progression. Our finding that SS also induces GSH in colon cancer cells within 24 h after treatment is consistent with previous data indicating that nonsteroidal anti-inflammatory drugs can induce GSH and glutathione S-transferase in the digestive tract (42).

Finally, we examined whether SAC or SAMC can augment the apoptotic effect of SS. Sulindac and sulindac derivatives are known to cause growth inhibition and to induce apoptosis in colon cancer cells (43, 44), and sulindac has been used for the suppression of adenoma formation in patients with familial adenomatous polyposis (45). We found that, when added together, SAMC and SS had an approximately additive effect on the extent of apoptosis in both SW-480 and HT-29 cells (Fig. 4). Recent studies indicate that the combination of lovastatin with SS is also additive with respect to induction of apoptosis in colon cancer cells (46).

Taken together, these cell culture studies suggest that SAMC may be useful in colon cancer chemoprevention when used alone or in combination with other compounds, e.g., sulindac or its derivatives. Obviously, additional studies are required to evaluate the efficacy of SAMC in suitable experimental animal systems.

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