Published in final edited form as:

Nutr Cancer. 2009 ; 61(3): 332–339. doi:10.1080/01635580802549982.

Phytochemical induction of cell cycle arrest by glutathione oxidation and reversal by N-acetylcysteine in human colon carcinomacarcinoma cells

R. Y. Odom¹, M. Y. Dansby^{1,2}, A. M. Rollins-Hairston¹, K. M. Jackson³, and W. G. Kirlin¹

¹Dept. of Pharmacology and Toxicology, Morehouse School of Medicine, Atlanta, GA

²Dept. of Physiology, Emory University School of Medicine, Atlanta, GA

³Dept. of Chemistry, Spelman College, Atlanta, GA

Abstract

Cancer prevention by dietary phytochemicals has been shown to involve decreased cell proliferation and cell cycle arrest. However, there is limited understanding of the mechanisms involved. Previously, we have shown that a common effect of phytochemicals investigated is to oxidize the intracellular glutathione (GSH) pool. Therefore, the objective of this study was to evaluate whether changes in the glutathione redox potential in response to dietary phytochemicals was related to their induction of cell cycle arrest. Human colon carcinoma (HT29) cells were treated with benzyl isothiocyanate (BIT), diallyl disulfide (DADS), dimethyl fumarate (DMF), lycopene (LYC), sodium butyrate (NaB) or buthione sulfoxamine (BSO, a GSH synthesis inhibitor) at concentrations shown to cause oxidation of the GSH: glutathione disulfide pool. A decrease in cell proliferation, as measured by [³H]-thymidine incorporation, was observed that could be reversed by pretreatment with the GSH precursor and antioxidant N-acetylcysteine (NAC). Cell cycle analysis on cells isolated 16 h after treatment indicated an increase in the percentage (ranging from 75% to 30% for benzyl isothiocyanate and lycopene, respectively) of cells at G2/M arrest compared to control treatments (dimethylsulfoxide) in response to phytochemical concentrations that oxidized the GSH pool. Pretreatment for 6 h with N-acetylcysteine (NAC) resulted in a partial reversal of the G2/M arrest. As expected the GSH oxidation from these phytochemical treatments was reversible by NAC. That both cell proliferation and G2/M arrest, were also reversed by NAC leads to the conclusion that these phytochemical effects are also mediated, in part, by intracellular oxidation. Thus, one potential mechanism for cancer prevention by dietary phytochemicals is inhibition of the growth of cancer cells through modulation of their intracellular redox environment.

Keywords

Phytochemicals; cell cycle arrest; colon cancer; oxidative stress; redox potential; glutathione

Introduction

Colon cancer is the third leading cause of cancer in men and women. According to American Cancer Society estimates there will be 112,340 new cases of colon cancer will be diagnosed,

Corresponding author Ward G. Kirlin, Ph.D. Morehouse School of Medicine Department of Pharmacology and Toxicology 720 Westview Dr. S.W. Atlanta, GA 30310 kirlin@msm.edu Phone: (404) 752-1709 Fax: (404) 752-1164. Mailing address for reprints: Ward G. Kirlin Morehouse School of Medicine Department of Pharmacology and Toxicology 720 Westview Dr. S.W. Atlanta, GA 30310. Academic Institution: Morehouse School of Medicine, Atlanta, GA 30310

with 52,180 deaths in 2007. Colon cancer has a long latency period preceding malignancy; therefore, one approach to control colon cancer is chemopreventive intervention [1,2]. Dietary phytochemicals are a promising group of chemopreventive agents because of their low toxicity and their health benefits associated with other chronic diseases [3]. Previous research shows that the use of dietary phytochemicals as cancer chemopreventive agents to block or slow the onset of premalignant tumors such as colon carcinomas has been widely accepted [4]. Furthermore, research studies have shown dietary phytochemicals to induce apoptosis, decrease cell proliferation and induce cell cycle arrest.

The ability of chemopreventive or chemotherapeutic agents to suppress the growth of cancer cells is also associated with blocking the cell cycle progression at G2/M checkpoint [5]. Cell cycle check points and apoptosis play critical roles in the molecular pathogenesis of cancer and can influence the outcome of chemotherapy and radiotherapy [6]. For example, Shen and colleagues [7] reported that sulforaphane, an isothiocyanate found in broccoli, inhibits cell growth and serum-stimulated reinitiation of cell cycle in serum-deprived HT-29 cells. Therefore, induction of cell cycle arrest and apoptosis by chemopreventive agents could be an effective approach to check uncontrolled cell proliferation and survival in tumor cells [2]. Because carcinogenesis is a complex process, finding effective therapies often relies on new discoveries about the underlying cellular mechanisms [8]. Therefore, researchers have focused attention on understanding the mechanisms in which dietary phytochemicals prevent the proliferation of cancer cells. Previously, we have shown that a common effect of dietary phytochemicals investigated is to oxidize the intracellular glutathione pool [9]. Glutathione (GSH), which is primarily in its reduced form within the cell, plays a key role in cellular resistance against oxidative damage [9] and has been associated with regulation of cell proliferation [8,10,11]. Therefore, the objective of this study was to evaluate whether changes in the GSH redox potential in response to select dietary phytochemicals was related to their induction of cell cycle arrest.

Materials and methods

Cell culture and reagents

Human adenocarcinoma colon cells (HT29) were purchased from American Type Culture Collection (Manassas, VA) and cultured under recommended conditions in McCoy's 5A medium (Sigma-Aldrich Chemical Co., St. Louis, MO) supplemented with 10% fetal bovine serum (FBS, Invitrogen, Carlsbad, CA) at 37°C in 5% CO₂. Allyl disulfide (ADS), benzyl isothiocyanate (BIT), buthionebuthione sulfoxamine (BSO), dimethyl fumarate (DMF), lycopene (LYC), N-acetylcysteine (NAC), and sodium butyrate (NaB) were purchased from Sigma-Aldrich.

Cell Proliferation

HT29 cells were plated at 2.5, 5, 10 × 10⁴ cells per well, in 96-well plates. After cell attachment, the concentration of FBS in the medium was gradually reduced from 10% to 5%, 1% and 0% with 24 hrs at each concentration, then replaced with medium containing 10% FBS to stimulate proliferation, 6 hrs prior to phytochemical exposure. As indicated in Figure 1, FACS analysis of cells deprived of serum, showed distribution within the cell cycle at G0/G1: 86 ± 3%, S: 11 ± 2 % G2/M: 3 ± 1 %. Six hr after addition of 10% FBS, these proportions shifted to an average distribution of G0/G1: 59 ± 3 %, S 26 ± 2 %, G2/M 15 ± 1 %. Cells were treated with (dimethylsulfoxide, DMSO, 0.2%) as controls or with one of the phytochemicals ADS, BIT, BSO, DMF, LYC, NaB, and NAC. Except for NAC and NaB, phytochemicals were dissolved in DMSO as 500x stock solutions then added to cell culture medium, such that DMSO concentrations were 0.2% of culture volume. NAC and NaB were also added from 500x stock but dissolved in medium. After 16 hrs of phytochemical treatment, [³H]-thymidine (2 µCi in

10 μ l medium) was added to each of the 96 wells and after 6 hrs cells were harvested (Skatron Cell Harvester, Sterling VA) onto filters and the radioactivity incorporated into DNA, determined by scintillation counting, was taken as a relative measure of cell proliferation and expressed as percent of control. Aliquots of treated cells were tested for cell viability, determined as the percentage of cells that excluded 0.2% (wt/vol) trypan blue. Following exposure to phytochemicals, there was no difference in viability (> 90%) between treated cells and controls.

Analysis of GSH and GSSG and Redox Potential Calculation

HT29 cells were seeded in 6-well plates and grown to 70% confluency. Following phytochemical treatment, medium was aspirated and 300 μ l of 10% perchloric acid (4°C) added. Thiols were derivatized with iodoacetic acid and treated with dansyl chloride for fluorescence detection following HPLC separation. From the stoichiometry for $\text{GSSG} + 2\text{e}^- \rightleftharpoons 2\text{H}^+ + 2\text{GSH}$, E_h values were calculated from the Nernst equation 9):

$$E_h = E_o + \frac{RT \ln [\text{GSSG}]}{2F [\text{GSH}]^2}, \text{ which is: } E_h = -240 \text{ mV} + 30 \left(\log \left(\frac{[\text{GSSG}]}{[\text{GSH}]^2} \right) \right)$$

Cell cycle analysis

HT29 cells were seeded in 6-well plates in McCoy's 5A medium supplemented with 10% FBS and grown to 70% confluency, then deprived of serum as described above. Following serum-stimulation, cells were treated with or without N-acetylcysteine (NAC) for 6 hours. Following NAC pretreatment cells were treated for 16 hours with DMSO (control), BSO (positive control), BIT, NaB, DADS, DMF or LYC. Cells were harvested, medium removed by centrifugation and washed with phosphate buffered saline, then fixed in 70% ethanol and stored at 4°C overnight. The ethanol was aspirated prior to staining with propidium iodide solution (30 min incubation, 4°C). Flow cytometry analysis was performed for cell cycle analysis on the treated and control cells, using a Becton Dickinson FACS Calibur and data analyzed using ModFit LT software.

Statistical Analysis

Tests for statistically significant differences ($p \leq 0.05$) were performed by analysis of variance (ANOVA) and Dunnett's multiple range tests, with all treatments compared to control values or Newman-Keul's test for individual comparisons between treatments (Graphpad Software, Inc. San Diego, CA). Data are given as mean $\pm$ SEM for three to five separate experiments.

Results

Cell proliferation decrease by phytochemicals and reversal by N-acetylcysteine

To determine equipotent concentrations for the inhibition of cell proliferation, HT29 cells were treated for 16 h with a range of concentrations of each phytochemical. As indicated in Figure 2, there was a linear decrease in [^3H]-thymidine incorporation in response to increasing phytochemical concentrations. As indicated on the figure, treatments resulting in a 50% decrease in proliferation (ED_{50}) ranged from 0.4 μM for LYC to 1280 μM for NaB. These decreases in cell proliferation were readily reversed in a dose-dependent manner by pretreatment with NAC (1–5 mM, 8 h) for ADS, BIT, and DMF, but not for LYC or NaB (Figure 3). Similarly, treatments with BSO, an inhibitor of GSH synthesis, decreased cell proliferation. The effect of 50 μM BSO was reversed by subsequent treatment with NAC in a concentration dependent manner (Figure 4). However, cell proliferation decreases in response to lower concentrations of BSO were not affected by NAC treatment.

Redox potential oxidation in response to phytochemicals

To determine the relative effect of phytochemical treatment on glutathione oxidation, cells were treated as above, harvested in perchloric acid and analyzed by HPLC. Measurements of GSH and GSSG concentrations and calculations of E_h (8) indicated that in response to phytochemical treatments at concentrations within 1-2 fold of the ED_{50} , the GSH redox potential was oxidized 10 - 20%, except for LYC (Figure 5). These oxidations represent approximately a 10 to 20-fold shift in the ratio of oxidized to reduced GSH and occurred within the first 2 h of treatment, was sustained through 16 hr (Figure 5, other time points not shown) before equilibrating back to the normal E_h of -240 mV within 24 h of treatment.

Cell cycle arrest and reversal by N-acetylcysteine

To evaluate whether changes in glutathione redox potential were associated with cell cycle arrest, flow cytometry analysis was performed on cells isolated after 16 h of BSO or phytochemical treatment (BIT, NaB, DADS, DMF or LYC). From the cell cycle frequency distribution, treatment of HT29 cells with benzyl isothiocyanate or sodium butyrate resulted in induction of cell cycle arrest at G2/M (increase in distribution of cells, Figure 6) with no significant affect on G1 or S when compared to control treatments (DMSO, 0.2% or medium alone). Pre-treatment with the antioxidant, N-acetylcysteine, resulted in a partial reversal of the increased distribution of cells at the G2/M phase of the cell cycle (Figure 6). Treatments with the dietary phytochemicals diallyl disulfide, dimethyl fumarate, and lycopene did not induce cell cycle arrest at any phase of the cell cycle (data not shown).

Discussion

Cancer is a multi-step disease that may take years to progress from cancer initiation to metastasis [12]. As a consequence, there are multiple steps at which cancer prevention may block the neoplastic progression. Recognition of diet as a primary causative factor for cancer risk has directed much research attention towards the chemical compounds present in foods. A major preventive role of certain cancers has also been found for dietary phytochemicals [13,14]. Proposed mechanisms of cancer chemoprevention include inhibition of cell growth, initiation of cell cycle arrest and induction of apoptosis. The phytochemicals tested in this study decreased cell proliferation of HT29 cells, with benzyl isothiocyanate and sodium butyrate inducing G2/M cell cycle arrest. These results are similar to findings of Zhang and colleagues in Capan-2 human pancreatic cancer cells treated with varying concentrations of BIT for 24h. This resulted in increased accumulation of the cells in G2/M phase that was readily reversed by pretreatment with N-acetylcysteine [15]. Lung carcinoma cells in response to diallyl disulfide had an increase in cells at G2/M arrest and undergoing apoptosis; both of these effects were reversed by pretreatment with NAC [16]. The results from these studies and ours in colon cells in which a reversal of the effects on cell growth, using a glutathione precursor, combined with our demonstration of the oxidation of GSH to GSSG in response to the phytochemicals indicates that a shift in the intracellular glutathione redox couple is a possible signaling mechanism involved in cell cycle arrest.

Organosulfur compounds reported to protect against cancer [17] occur as glucosinolates in a variety of cruciferous vegetables. Isothiocyanates, such as BIT, are phytochemicals found in broccoli, cabbage, watercress and cauliflower. These isothiocyanates, synthesized and stored in plant cells as glucosinolates, are released when plant cells are injured [18] during the process of crushing and chewing [7]. It has been proposed that BIT inhibits tumor development by suppressing the harmful effects of oxidative stress [19]. Additionally, isothiocyanates are potent inducers of NAD(P)H:quinine oxidoreductase and glutathione S-transferase activity, enzymes that reduce the carcinogenicity of a variety of environmental chemicals [8,17,20,

21]. Thus, isothiocyanates may prevent both the initiation phase of the carcinogenesis process as well as inhibiting the promotion phase by inducing cell cycle arrest in cancer cells [13,15].

Sodium butyrate is a short chain fatty acid that is produced by bacterial fermentation of dietary fiber. In a recent study, butyrate treatment of human colon cancer cells, LS174T inhibited cell proliferation in a dose-dependent manner and promoted cell cycle arrest [22]. NaB treatment of SAS tongue cancer cells, at low concentrations (2-8 mM) induced G0/G1 arrest, whereas higher concentrations (8-24 mM) led to G2/M arrest [23].

Cell cycle checkpoints are critical in molecular pathogenesis and may influence the effectiveness of cancer chemotherapy [24]. Moreover, cancer progression has been suggested to involve the loss of cell cycle checkpoint controls that regulate the passage through the cell cycle [25]. The findings in the present study indicate that the distribution of cells at phases of the cell cycle were influenced by intracellular oxidation. It can also be concluded from these results that inhibition of cancer cell growth by modulation of the intracellular redox environment is a potential mechanism for cancer prevention by dietary phytochemicals.

Previous studies have indicated that the increase in G2/M cell cycle arrest in the HT29 cells may be due to modulation of p21 and p27 protein expression. The p21 protein is a cyclin-dependent kinase (CDK) inhibitor which is responsible for inhibiting CDK 1 and inducing G2/M phase arrest [26]. A study conducted by Takagaki et al. [27], indicated that treatment with apigenin of HT-29 and MG63 human osteosarcoma cell lines (both having mutated, non-functional p53), led to G2/M phase arrest, and a rapid induction of p21^{WAF1} in a p53-independent manner. Thus, the p53 point mutation in HT29 cells doesn't prevent treatments from elevating p21 expression and slowing cell proliferation. From our data, HT29 cells, in the absence of growth factors contained in serum, still arrest at G0/G1, indicating that there are additional mechanisms to p53 that are involved in this arrest. Upon serum stimulation, a portion of cells re-enter the cell cycle, which can be slowed by phytochemical treatments. Recent studies have shown HT29 cells to arrest at G1 [28] and G1/S [29], this latter effect possibly involving AP-1 activation, which is known to be affected by oxidative stress.

The results from this study confirmed that oxidative changes in glutathione redox potential were associated with cell cycle arrest, in response to all of the phytochemicals except lycopene. Although lycopene did slow HT29 cell proliferation, this effect was not reversed by NAC, nor was there a significant oxidation of GSH:GSSG or effect on distribution of cells at G2/M arrest. This indicates that although glutathione redox regulation is involved in the effects of some phytochemicals on cell proliferation, lycopene appears to work through a different mechanism. NaB treatment resulted in decreased cell proliferation that was not readily reversed by NAC; however, NaB did oxidize the GSH redox couple and its effect of an increase in cells arrested at G2/M was reversed by NAC pretreatment. Thus the relationship between phytochemical treatment, cell proliferation, cell cycle regulation and glutathione redox potential is not consistent with all phytochemicals involved in slowing of cell proliferation. The strongest evidence for such a regulatory role for glutathione in cell cycle progression and cell proliferation is indicated by allyl disulfide, benzyl isothiocyanate, and dimethyl fumarate. All three of these phytochemicals shift the intracellular equilibrium from reduced to oxidized glutathione, slow HT29 cell proliferation and increase the proportion of cells at the G2/M phase of the cell cycle. Both of these latter effects were readily reversed by N-acetylcysteine in a dose-dependent manner. An increased understanding of this relationship between GSH and cancer cell proliferation may provide valuable insight into the mechanisms of dietary phytochemicals in cancer prevention.

Acknowledgements

This research was supported by grants from the National Institutes of Health:

S06GM008248 and RCMI RR03032. We appreciate the support and expertise of Dr. Mark Maloney. Portions of these experimental results were presented at the American Association for Cancer Research 2008 annual meeting, San Diego, CA.

Research support: National Institutes of Health - S06GM008248, RCMI RR03032

References

1. Fenton JJ, Hord NG. Stage matters: choosing relevant model systems to address hypotheses in diet and cancer chemoprevention research. *Carcinogenesis* 2006;27:893–902. [PubMed: 16474168]
2. Kaur M, Singh RP, Gu M, Agarwal R, Agarwal C. Grape seed extract inhibits in vitro and in vivo growth of human colorectal carcinoma cells. *Clin Cancer Res* 2006;12(20):6194–6202. [PubMed: 17062697]
3. Lim DY, Tyner AL, Park JB, Lee JY, Choi YH, Park JHY. Inhibition of colon cancer cell proliferation by the dietary compound conjugated linoleic acid is mediated by the CDK inhibitor p21^{CIP1/WAF1}. *J Cell Physiol* 2005;205:107–113.
4. Reddy BS, The Fourth DeWitt S. Goodman lecture. Novel approaches to the prevention of colon cancer by nutritional manipulation and chemoprevention. *Cancer Epidemiol Biomarkers Prev* 2000;9:239–247. [PubMed: 10750661]
5. Agarwal C, Singh RP, Dhanalakshmi S, Tyagi AK, Tecklenburg M, Sclafani RA, Agarwal R. Silibinin upregulates the expression of cyclin-dependent kinase inhibitors and causes cell cycle arrest and apoptosis in human colon carcinoma HT-29 cells. *Oncogene* 2003;22:8271–8282. [PubMed: 14614451]
6. Lim do Y, Jeong Y, Tyner AL, Park JHY. Induction of cell cycle arrest and apoptosis in HT-29 human colon cancer cells by the dietary compound luteolin. *Am J. Physiol Gastrointest Liver Physiol* 2007;292:G66–G75. [PubMed: 16901994]
7. Shen G, Xu C, Chen C, Hebbar V, Kong AT. p53-independent G1 cell cycle arrest of human carcinoma cells HT-29 by sulforaphane is associated with induction of p21^{CIP1} and inhibition of expression of cyclin D1. *Cancer Chemother Pharmacol* 2006;57:317–327. [PubMed: 16170570]
8. Conney AH. Tailoring cancer chemoprevention regimens to the individual. *J Cell Biochem* 2004;91:277–286. [PubMed: 14743388]
9. Kirilin WG, Cai J, Thompson SA, Diaz D, Kavanagh TJ, Jones DP. Glutathione Redox Potential in Response to Differentiation and Enzyme Inducers. *Free Radic Biol Med* 1999;27:1208–1218. [PubMed: 10641713]
10. Shan XQ, Aw TY, Jones DP. Glutathione-Dependent Protection Against Oxidative Injury. *Pharmacol Ther* 1990;47:61–71. [PubMed: 2195557]
11. Attene-Ramos MS, Kitiphongspattana K, Ishii-Schrade K, Gaskins HR. Temporal Changes Of Multiple Redox Couples From Proliferation To Growth Arrest In IEC-6 Intestinal Epithelial Cells. *Am J Physiol Cell Physiol* 2005;289:C1220–1228. [PubMed: 15958525]
12. E.R. Fearon ER, Vogelstein B. A genetic model for colorectal carcinogenesis. *Cell* 1990;61:759–767. [PubMed: 2188735]
13. Surh YJ. Cancer chemoprevention with dietary phytochemicals. *Nat Rev Cancer* 2003;3:768–780. [PubMed: 14570043]
14. Moreno DA, Carvajal M, López-Berenguer, García-Viguera C. Chemical and biological characterization of nutraceutical compounds of broccoli. *J. Pharmaceutical and Biomedical Analysis*. 2006
15. Zhang R, Loganathan S, Humphreys I, Srivastava S. Benzyl isothiocyanate-induced DNA damage causes G2/M cell cycle arrest and apoptosis in human pancreatic cancer cells. *J Nutr* 2006;136:2728–2734. [PubMed: 17056792]

16. Wu XJ, Kassie F, Mersch-Sundermann V. The role of reactive oxygen species (ROS) production on diallyl disulfide (DADS) induced apoptosis and cell cycle arrest in human A549 lung carcinoma cells. *Mut Res* 2005;579:115–124. [PubMed: 16024053]
17. Juge N, Mithen RF, Traka M. Molecular basis for chemoprevention by sulforaphane: a comprehensive review. *Cell Mol Life Sci* 2007;64:1105–1127. [PubMed: 17396224]
18. Zhang Y, Tang L, Gonzalez V. Selected isothiocyanates rapidly induce growth inhibition of cancer cells. *Mol Cancer Ther* 2003;2:1045–1052. [PubMed: 14578469]
19. Yang YM, Conaway CC, Chiao JW, Wang CX, Amin S, Whysner J, Dai W, Reinhardt J, Chung FL. Inhibition of benzo(a)pyrene-induced lung tumorigenesis in A/J mice dietary N-acetylcysteine conjugates of benzyl and phenethyl isothiocyanates during the postinitiation phase is associated with activation of mitogen-activated protein kinases and p53 activity and induction of apoptosis. *Cancer Res* 2002;62:2–7. [PubMed: 11782348]
20. Bonnesen C, Eggleston IM, Hayes JD. Dietary indoles and isothiocyanates that are generated from cruciferous vegetables can both stimulate apoptosis and confer protection against DNA damage in human colon cell lines. *Cancer Res* 2001;61(16):6120–30. [PubMed: 11507062]
21. Nakamura Y, Ohigashi H, Masuda S, Murakami A, Morimitsu Y, Kawamoto Y, Osawa T, Imagawa M, Uchida K. Redox regulation of glutathione S-transferase induction by benzyl isothiocyanate: correlation of enzyme induction with the formation of reactive oxygen intermediates. *Cancer Res* 2000;60:219–225. [PubMed: 10667562]
22. Hatayama H, Iwashita J, Kuwajima A, Abe T. The short chain fatty acid, butyrate, stimulates MUC2 mucin production in the human colon cancer cell line LS174T. *Biochem Biophys Res Comm* 2007;356:599–603. [PubMed: 17374366]
23. Jeng H, Kuo MY, Lee PH, Wang YJ, Lee MY, Lee JJ, Lin BR, Tai TF, Chang MC. Toxic and metabolic effect of sodium butyrate on SAS tongue cancer cells: of cell cycle deregulation and redox changes. *Toxicol* 2006;223:235–247.
24. Bai S, Goodrich DW. Different DNA lesions trigger distinct cell death responses in HCT116 colon carcinoma cells. *Mol Cancer Ther* 2004;3:613–619. [PubMed: 15141019]
25. Srivastava SK, Singh S. Cell cycle arrest, apoptosis induction and inhibition of nuclear factor kappa B activation in anti-proliferation activity of benzyl isothiocyanate against human pancreatic cancer cells. *Carcinogenesis* 2004;25:1701–1709. [PubMed: 15117814]
26. Bhonde MR, Hanski ML, Notter M, Gillisen BF, Daniel PT, Zeitz M, Hanski C. Equivalent effect of DNA damage-induced apoptotic cell death or long-term cell cycle arrest on colon carcinoma cell proliferation and tumor growth. *Oncogene* 2006;25:165–175. [PubMed: 16170360]
27. Takagaki N, Sowa Y, Oki T, Nakanishi R, Yogosawa S, Sakai T. Apigenin induces cell cycle arrest and p21/WAF1 expression in a p53-independent pathway. *Intl J Onco* 2005;26:185–189.
28. Cheung KL, Khor TO, Yu S, Kong AN. PEITC induces G1 cell cycle arrest on HT-29 cells through the activation of p38 MAPK signaling pathway. *AAPS J*. 2008Epub, May 13, 2008
29. Rakitina TV, Vasilevskaya IA, O'Dwyer PJ. Inhibition of G1/S transition potentiates oxaliplatin-induced cell death in colon cancer cell lines. *Biochem Pharmacol* 2007;73:1715–1726. [PubMed: 17343830]

Figure 1.

Effect of serum deprivation and re-exposure to FBS on cell cycle distribution. Phytochemical treatments were performed on cells gradually deprived of FBS and then serum-stimulated prior to phytochemical treatment. Cell distribution by FACS analysis under conditions of deprivation and serum stimulation are shown in the upper panel. The percentage of cells at different cell cycle phases under these growth conditions is indicated in the bottom panel. Significant difference from control (confluent, 0% FBS) is indicated as ** $p < 0.01$, *** $p < 0.001$.

Figure 2. Inhibitory effects of phytochemical treatments on HT29 cell proliferation as measured by [³H]-thymidine incorporation. Insert table indicates ED₅₀ as estimated from linear regression of phytochemical concentrations versus radiolabel incorporation compared to control treatments.

Figure 3.

N-acetylcysteine reversal of redox-mediated decreases in cell proliferation mediated by ADS, BIT, DMF, LYC, and NaB. Cells were pretreated with NAC at concentrations of 0, 1mM, 2.5mM and 5 mM, prior to phytochemical treatments. Significant difference from control is indicated as ‡ p<0.05; significant difference from treated in response to NAC reversal * p<0.05; ** p<0.01.

Figure 4.

N-Acetylcysteine reversal of redox-mediated decreases in cell proliferation mediated by buthionine sulfoximine. Cells were pretreated with NAC at concentrations of 0, 1mM, 2.5mM and 5 mM, prior to oxidativeoxidative treatments. Significant difference from control is indicated as † $p < 0.05$; significant difference from treated in response to NAC reversal * $p < 0.05$; ** $p < 0.01$.

Figure 5. Phytochemical effects on GSH redox potential of HT29 cells after 16 hour exposure. Allyl disulfide, benzyl isothiocyanate, dimethyl fumarate and sodium butyrate treatments resulted in significant oxidations of GSH redox potential compared to control.

Figure 6.

Effect on G1, S and G2/M cell cycle distributions in HT29 human colon cancer cells, relative to untreated control cells, after treatment with (a) BSO (50 μ M, positive control) and the dietary phytochemicals: (b) BIT (25 μ M), and (c) NaB (5mM) at concentrations known to cause oxidation of the GSH:GSSG pool. Results indicate no substantial change in proportion of cells in G1 or S-phase of the cell cycle. Pretreatment with N-acetylcysteine did cause a slight increase in proportion of cells in G1 in response to BSO, BIT, and NaB and a slight decrease in cells in S-phase compared to BSO, BIT, or NaB, alone (no effects were significantly different from control, arbitrarily set at 100%). However, the greater than 2-fold increase in number of cells in G2/M in response to BIT was almost completely reversed by pretreatment with NAC and the 2-fold increase in cells in G2/M in response to NaB was similarly reversed by NAC pretreatment. * $p < 0.05$; ** $p < 0.01$. NAC treatment alone had no effect on the phase distribution of cells, similar to data in Fig 2.