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Mechanism for proliferation inhibition by various selenium compounds and selenium-enriched broccoli extract in rat glial cells

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Abstract

The objective of this study was to investigate the differential effects of various selenium (Se) compounds and Se-enriched broccoli extracts on cell proliferation and the possible mechanism responsible for the Se-induced growth inhibition. C6 rat glial cells were incubated with graded concentrations up to 1000 nM of selenite, selenate, selenomethionine (SeM), Se-methyl-selenocysteine (SeMCys), high-Se broccoli (H-SeB) extract or low-Se broccoli (L-SeB) extract for 24 and 48 h. MTT results indicated that all Se sources and levels examined inhibited C6 cell proliferation at 48 h. The results from cell cycle progression and apoptosis analysis indicated that SeM, SeMCys, H-SeB or L-SeB treatments at the concentration of 1000 nM reduced the cell population in G_0/G_1 phase, but induced G_2/M phase arrest and increased apoptosis and secondary necrosis in C6 cells at 24 h. The populations of apoptotic cells and secondary necrotic cells were increased by all Se sources examined. The COMET assay indicated that there was no significant DNA single-strand break found for all Se treatments in C6 cells for 48 h. In addition, the Se-induced proliferation inhibition may involve a hydrogen peroxide (H_2O_2)-dependent mechanism with elevated cellular glutathione peroxidase (cGPX) activity. Both H-SeB and L-SeB inhibited C6 cell proliferation but H-SeB was less inhibitory than L-SeB. The proliferation inhibition by H-SeB in C6 cells is apparently related to the increased H_2O_2 with the elevated cGPX activity, but the inhibition by L-SeB was H_2O_2-independent without change in cGPX activity.

Introduction

Selenium (Se) is an essential trace element for humans and animals and is known to suppress carcinogenesis nonspecifically in animals (Whanger 2004). After oral supplementation with 200 μg Se/day as enriched yeast for 4.5 years the cancer incidence of lung, colon and prostate cancers was significantly reduced (Clark *et al.* 1996, 1998). This is consistent with other human trials that indicated that Se supplementation reduced the incidence of certain cancers (Whanger 2004).

The form and the concentration of Se used are critical for cancer prevention (Sinha & El-Bayoumy 2004). Various Se compounds influence cellular responses differently in various tumor models (Tapiero *et al.* 2003). Most of the cancer prevention studies have used selenite or selenomethionine (SeM) as the primary Se compounds (Sinha & El-Bayoumy 2004). Se-methylselenocysteine (SeMCys)

is reported to be the most effective selenocompound against carcinogen-induced mammary tumors in rats (Ip 1998), and selenite and selenate reduced the formation of aberrant crypts (AC) in rats (Feng *et al*. 1999). With the knowledge of Se as an anticarcinogenic agent, delivery of this protective element through the food systems is a natural and harmless method to provide enrichment for humans. Since plants with high sulfur content tend to take up high levels of Se (Terry *et al*. 2000), broccoli (Finley *et al*. 2001), garlic (Ip *et al*. 1992), onion (Cai *et al*. 1995) and ramps (Whanger *et al*. 2000) have been enriched with Se for possible oral supplementation of Se through dietary consumption. The primary form of Se in Se-enriched broccoli, garlic, onions and ramps is SeMCys (Cai *et al*. 1995; Whanger *et al*. 2000). Plants apparently produce this selenocompound as a defense mechanism against Se toxicity, but this is in turn beneficial to the animal with respect to tumor inhibition. In the mammary tumor model, SeMCys has been shown to be the most effective one for tumor reduction (Whanger 2002). Recent studies indicated that colon carcinogenesis was inhibited effectively in rats fed high-Se broccoli whereas SeM did not significantly reduce AC (Finley *et al*. 2000; Finley & Davis 2001).

The addition of Se-enriched garlic or Se-enriched broccoli to the rat diets significantly reduced the mammary tumor incidence from 83 to 33% (Ip *et al*. 1992) or 90 to 37% (Finley *et al*. 2001), respectively. In addition, Se-enriched broccoli florets (Finley *et al*. 2000; Finley & Davis, 2001) and sprouts (Finley *et al*. 2001) significantly reduced colon tumors in rats. Similar to broccoli (Finley *et al*. 2001), Se-enriched ramps significantly reduced the incidence of mammary tumors (Whanger *et al*. 2000).

Inhibition of cell proliferation, induction of apoptosis and elevation of antioxidative ability are important cellular responses that may be involved in the anticarcinogenic effect of Se (Sinha & El-Bayoumy 2004). Se exerts many of its effects through modulating the functions of intracellular proteins related to cell proliferation to regulate cell cycle events in several human tumor cell lines (Ip 1998; Venkateswaran *et al*. 2002). Selenite-induced apoptosis has been shown to be mediated by DNA damage involving DNA topoisomerase II (Zhou *et al*. 2003). Dietary Se supplementation with SeM or high-Se yeast at 3 or 6 μg/kg body weight/day for 7 months showed the DNA damage and apoptosis in the aging canine prostate (Waters *et al*. 2003). However, SeM was reported to protect human fibroblasts and keratinocytes from DNA damage (Seo *et al*. 2002; Rafferty *et al*. 2003).

Se also acts as an antioxidant with anticancer effect and is a key component in selenoproteins that incorporate Se as selenocysteine translationally into the amino acid sequence, such as glutathione peroxidase (GPX) family (Whanger 2004). GPXs are antioxidative-related enzymes with different substrate specificities and they collaborate to provide the main defense system against oxidative stress in mammalian cells for detoxification of intracellular oxidative stress (Gladyshev & Hatfield 1999).

The objective of this study was to investigate the differential effects of various Se compounds and Se-enriched broccoli extracts on cell proliferation by using C6 rat glial cells as the experimental model and further investigate the mechanism for the Se-induced growth effect.

Materials and methods

Materials

MTT (3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyl tetrazolium bromide), xylenol orange (3, 3′-bis[*N*,*N*-dicarboxymethyl-aminomethyl]-*o*-cresolsulfone-phthalein, sodium salt), sodium selenite, sodium selenate, SeM, SeMCys, Triton X-100, RNase A, propidium iodide, reduced glutathione, glutathione reductase and reduced beta-nicotinamide adenine dinucleotide phosphate (β-NADPH) were purchased from Sigma Chemical Co. (St. Louis, Missouri USA). Fetal bovine serum (FBS) and the media and reagents for cell culture were obtained from Hyclone (Logan, Utah USA) and GIBCO Life Technologies (Grand Island, New York USA). Annexin V-FITC kit was purchased from Beckman Coulter International Corporation (Miami, Florida USA).

Cell culture

C6 rat glial cell line was used as the experimental material. The cells were maintained routinely and passed in the culture medium (DMEM supplemented with 10% FBS) under a humidified

atmosphere of 5% CO_2 and 95% air at 37 °C. The culture medium was changed every 2–3 days until the cells reached confluence and the experiments were performed when the cells reached 80% confluence.

In vitro *digestion of broccoli extract*

Broccoli extracts containing low and high levels of Se were kindly provided by Dr. John Finley (Human Nutrition Laboratory, USDA, Grand Forks, ND). Briefly, sodium selenate solutions were added to the growth media for broccoli (Finley *et al*. 2000) and the broccoli extracts were prepared by in vitro enzymatic digestion of broccoli powder as previously described with slight modifications (Gangloff *et al*. 1996). The mixture of broccoli powder with ascorbic and citric acids was adjusted to pH 2 and incubated with pepsin (40 mg/ml) in 0.1 HCl for 1 h. Then 2.5 ml of pancreatin (2 mg/ml) and bile (12 mg/ml) in 0.1 M sodium bicarbonate were added and incubated for another 2 h. The digested mixture was then dialyzed against distilled water overnight using dialysis bags with 12,000–14,000 MW cut off. The high-Se (H-SeB) and low-Se (L-SeB) broccoli extracts were analyzed for Se according to the previously published method (Beilstein & Whanger 1986) with modifications and found to contain 1.08×10^{-4} M Se and 2.50×10^{-7} M Se, respectively.

Cell proliferation assay

The cells were seeded onto 96-well plates at a density of 1×10^4 cells/well. After overnight culture, the medium was replaced with the fresh medium containing graded concentrations (0, 62.5, 125, 250, 500 and 1000 nM) of sodium selenite, sodium selenate, SeM, SeMCys or H-SeB. The culture plates were then incubated at 37 °C for 48 h. To compare the possible difference between treatments of H-SeB and L-SeB, graded dilutions from 108× (equal to 1000 nM and 2.31 nM Se for H-SeB and L-SeB, respectively) to 1728× dilution were used to treat the cells for 48 h instead. MTT assay was performed to measure cell proliferation with modifications (Yeh *et al*. 2002). This assay is based on the cleavage of the yellow tetrazolium salt MTT by mitochondrial dehydrogenases of metabolically active cells to purple formazan crystals which are then solubilized and spectrophotometrically quantified at 590 nm (Mosmann 1983).

Cell treatments

The C6 cells were cultured on 10-cm tissue culture plates. To investigate the Se-induced cellular effects, 1000 nM of sodium selenite, sodium selenate, SeM, SeMCys or H-SeB, were added to cells and the culture plates were incubated at 37 °C for 24 h to determine cell cycle progression and apoptosis, and for 48 h to assay the possible DNA damage, intracellular H_2O_2 content and cGPX activity. At the end of the experiments, the cells were either collected immediately by trypsinization and subjected to further cell cycle progression, apoptosis analysis and DNA damage assay or frozen at −80 °C for analyses of H_2O_2 content and cGPX activity in cell cytosols. The protein content of cell cytosols was determined by using bovine serum albumin as standards (Bradford 1976).

Flow cytometric analysis and Annexin V-FITC/PI dual stain

Both detached and adherent cells were harvested from tissue culture plates and washed with ice-cold phosphate buffered saline (PBS). To examine the effect of various Se compounds on cell cycle progression, the cell pellets were resuspended in ice-cold 70% ethanol to fix overnight at −20 °C. Centrifugation at 300× *g* for 5 min at 4 °C was performed the next day and 0.5% Triton X-100 and RNase A were added to resuspend the cell pellets. The mixture was incubated at 37 °C for 30 min and the supernatant was discarded following centrifugation at 300×*g* for 5 min at 4 °C. Then propidium iodide (PI) was added and the mixture was incubated for 10 min on ice (Yeh *et al*. 2003). To determine the apoptosis in Se-induced C6 cells, the cell pellets were resuspended and incubated with Annexin V-FITC and PI according to the manufacturer's protocol. The mixture for cell cycle progression or Annexin V-FITC/PI stain was applied to the Coulter Epics XL-MCL Flow Cytometer (Beckman Coulter International Corporation, Miami, Florida USA) for analysis (approximately 10,000 events). The $FITC^+/PI^-$ cells represent early apoptosis whereas $FITC^+/PI^+$ cells represent secondary necrosis or late apoptosis.

COMET assay

The frosted microscopic slides were prepared for COMET assay by dipping into the 60 °C normal melting point agarose (1.5% in Ca^{2+}- and Mg^{2+}-free PBS). After wiping off the remaining agarose at the bottom side of the slides, the slides were dried. The cells from various Se treatments were collected, mixed with low melting point agarose, and added to the agarose-covered slides. The slides were then covered by glass coverslips to give the even layers of cell suspension and were allowed to solidify on ice. After removing the glass coverslips, the slides were placed in the ice-cold lysis buffer at 4 °C overnight. The slides were then incubated in a horizontal gel electrophoresis apparatus filled with fresh, cold electrophoresis buffer for 30 min at 4 °C to unwind the DNA completely prior to electrophoresis. After electrophoresis, the slides were transferred to the neutralization buffer for 15 min to remove alkali and detergents and then dehydrated in ethyl alcohol several times. The slides were stained with ethidium bromide and covered with coverslips. A total of 50 cells from each slide were scored for image analysis (Singh *et al.* 1988).

Intracellular hydrogen peroxide level

The intracellular H_2O_2 content was determined by modification of a prior method (Zhou *et al.* 1997). The measurement was based on the oxidation of ferrous ions to ferric ions by H_2O_2 under acidic conditions to form a stable colored complex with xylenol orange that can be measured spectrophotometrically at 560 nm at room temperature.

Cellular glutathione peroxidase activity

Glutathione peroxidase activity was determined by an enzyme-coupled method with glutathione reductase, utilizing H_2O_2 as substrate (Paglia & Valentine 1967) with a DU Series 60 Spectrophotometer (Beckman Instruments, Fullerton, CA USA) at 30 °C. The rate of decrease in the NADPH concentration was followed at 340 nm over the 3-min period at 30-s intervals.

Statistical analysis

Data were examined for equal variance and normal distribution prior to statistical analysis. Mean values were compared by analysis of variance (ANOVA) with Fisher's least significant difference (LSD) method for comparing groups (Steel & Torrie 1980). A significance level of 5% was adopted for all comparisons.

Results

Effect of Se on C6 cell proliferation

MTT assay was used to determine the influence of various Se compounds and Se-enriched broccoli extract on C6 cell proliferation. All the Se compounds examined in this study showed significant ($P < 0.05$) growth inhibition of C6 cells at the concentration examined as low as 62.5 nM for 48 h, and the increase in Se concentration did not further inhibit C6 cell proliferation (Figure 1A). There is no significant difference ($P > 0.05$) in growth inhibition among various Se compounds and H-SeB treatments. Different dilutions (108–1728× dilutions) of H-SeB and L-SeB were applied to C6 cells to determine the effect of Se-enriched broccoli extract alone on cell proliferation. H-SeB and L-SeB regardless of Se content resulted ($P < 0.05$) in C6 growth inhibition at the dilution as low as 1728× after 48 h treatment (Figure 1B). However, at the graded dilutions of 108–432×, L-SeB (containing 0.58–2.31 nMSe) resulted in greater inhibition ($P < 0.05$) than H-SeB (containing 250–1000 nMSe) did.

Effect of Se on C6 cell cycle progression, apoptosis and DNA damage

Cell cycle progression was determined with PI staining and the apoptosis induction was determined by Annexin V/PI dual stain by flow cytometry. The result showed that SeM, SeMCys or H-SeB at 1000 nM significantly reduced ($P < 0.05$) the cell population in G_0/G_1 phase and induced ($P < 0.05$) G_2/M phase arrest at 24 h (Figure 2A). H-SeB or L-SeB (at the 108× dilution) significantly reduced ($P < 0.05$) cell

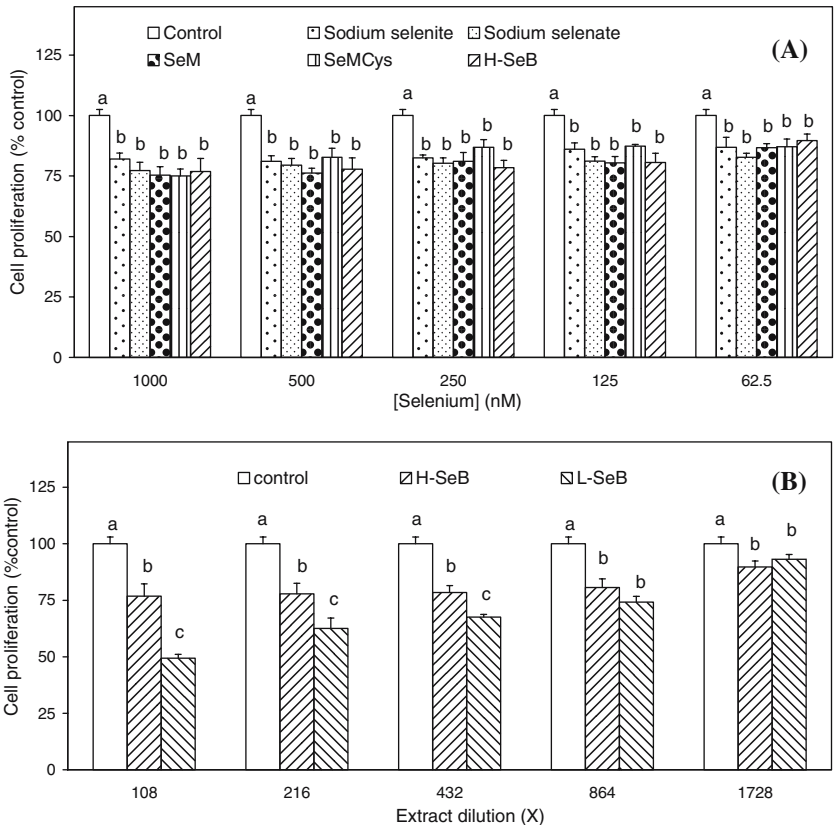

Figure 1. (A) Inhibition on C6 cell proliferation by various Se sources for 48 hr. Different letters (a–b) indicate significant difference ($P < 0.05$) among treatments at the same concentration. (B) Inhibition on C6 cell proliferation by broccoli extracts for 48 hr. Different letters (a–c) indicate significant difference ($P < 0.05$) among treatments at the same dilution. Each data point represents the mean ± SEM from three independent experiments.

population in G_0/G_1 phase with increased ($P < 0.05$) G_2/M phase arrest compared to those in control group (Figure 2B). Compared to the control group, all the Se compounds and Se-enriched broccoli extract induced significant increase ($P < 0.05$) of apoptosis and late apoptosis/secondary necrosis (Figure 3A). L-SeB (at the 108× dilution) induced ($P < 0.05$) larger apoptotic cells than that induced by H-SeB with the same dilution (Figure 3B). COMET assay was used to evaluate DNA single-strand break in C6 cells. There was no significant difference ($P > 0.05$) in tail intensity, tail moment or tail migration after treatment with various Se compounds and H-SeB for 48 h (Figure 4A) although both H-SeB and L-SeB tended to nonsignificantly reduce ($P > 0.05$) the tail migration compared to that in the control group (Figure 4B).

Effect of Se on cellular oxidative status in C6 cells

To investigate the oxidative status influenced by various Se compounds and H-SeB, intracellular hydrogen peroxide content and cGPX activity were determined. The cellular H_2O_2 content and GPX activity were increased significantly ($P < 0.05$) in all Se-treated cells (Figure 5A) at 1000 nM for 48 h. Selenite and SeMCys treatments increased cGPX activity to the greatest extent, followed by selenate and H-SeB. SeM induced the smallest increase in cGPX activity in C6 cells. In addition, L-SeB (108× dilution containing 2.31 nM Se) did not affect the cellular H_2O_2 content or cGPX activity in C6 cells (Figure 5B). The ratio of cGPX activity/H_2O_2 content did not change ($P > 0.05$) among any of the Se compounds and broccoli extracts (Figure 5A), but the ratio in the H-SeB group

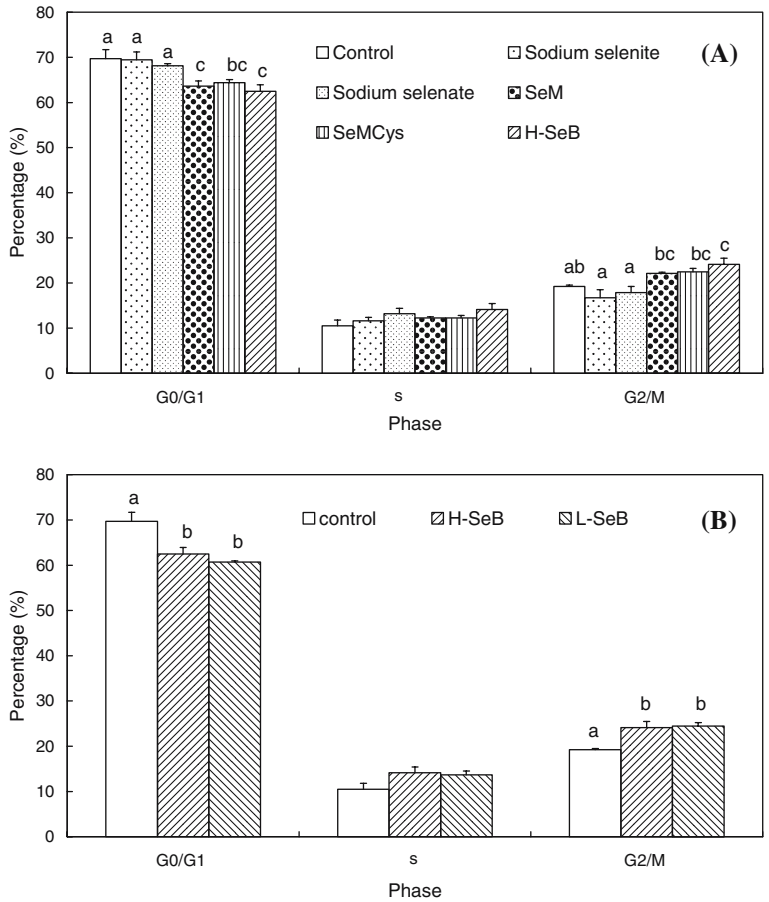

Figure 2. (A) Influence on C6 cell cycle progression by various Se sources at 1000 nM for 24 hr. Different letters (a–c) indicate significant difference ($P < 0.05$) among treatments in each cell cycle phase. (B) Influence on C6 cell cycle progression by broccoli extracts at 108× dilution for 24 h. Different letters (a–b) indicate significant difference ($P < 0.05$) among treatments in each cell cycle phase. Each data point represents the mean ± SEM from three independent experiments.

was higher ($P < 0.05$) than that in the L-SeB group (Figure 5B).

Discussion

Different forms of Se compounds inhibited C6 cell proliferation, but showed no difference in proliferation inhibition pattern among the various Se compounds. SeMCys is the primary Se form in Se-enriched plants such as broccoli, onions, garlic and ramps (Whanger 2002). Since SeMCys and H-SeB showed similar inhibitory effect on C6 cell proliferation (Figure 1A), SeMCys in Se-enriched broccoli probably accounts for the inhibitory effect of H-SeB on cellular response in C6 cells.

Both H-SeB and L-SeB resulted in inhibition on C6 cell proliferation. At the 864× and 1728× dilutions, H-SeB (containing 125 and 62.5 nM Se, respectively) and L-SeB (containing 0.29 and 0.14 nM Se, respectively) regardless of Se content inhibited C6 cell proliferation at 48 h, but there is no Se effect found between H-SeB and L-SeB because the inhibition pattern found between either treatment was similar (Figure 1B). However, there was Se-induced proliferation inhibition by H-SeB at the graded dilutions (108–432× dilutions containing 1000–250 nM Se) in C6 cells compared to that observed by L-SeB (containing 2.31–0.58 nM Se) treatment. The different proliferation inhibition between H-SeB and L-SeB was not due to the broccoli extract alone, since H-SeB and L-SeB were included with the cells at the same dilution

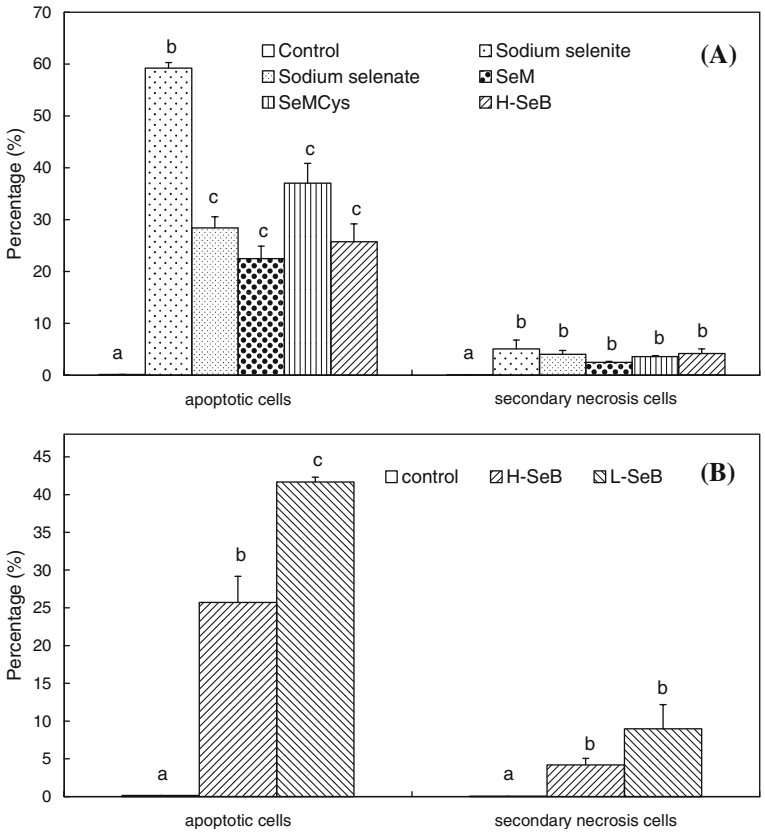

Figure 3. (A) Effect on C6 apoptosis by various Se sources at 1000 nM for 24 hr. Different letters (a–c) indicate significant difference ($P < 0.05$) among treatments in each cell cycle phase. (B) Effect on C6 apoptosis by broccoli extracts at 108× dilution for 24 hr. Different letters (a–b) indicate significant difference ($P < 0.05$) among treatments in each cell cycle phase. Each data point represents the mean ± SEM from three independent experiments.

(Figure 1B). The higher Se content in H-SeB appeared to exert more protective effect by reducing proliferation inhibition in C6 cells. Therefore, the difference between H-SeB and L-SeB is postulated to be due to the Se in the Se-enriched broccoli.

The mechanisms responsible for Se-induced proliferation inhibition is proposed to involve the disturbance of cell cycle progression, influence on apoptosis, induction of DNA damage and/or effect on cellular oxidative status (Sinha & El-Bayoumy 2004). Several studies have demonstrated the influence on cell cycle and apoptosis by selenite (Zhong & Oberley 2001), SeM (Redman *et al*. 1998) and SeMCys (Yeo *et al*. 2002). In addition, selenite pretreatment as low as 1 nM was reported to reduce 20 μM arsenic trioxide-induced cytotoxicity, but not apoptosis in porcine aortic endothelial cells (Yeh *et al*. 2003). The results in this study indicated that the populations of apoptotic cells and late apoptotic (secondary necrotic) cells were increased by all Se sources at 1000 nM for 24 h. Selenite or selenate treatment promoted most of the C6 cells to the G_0/G_1 phase and into apoptosis as well as late apoptosis (secondary necrosis). In contrast, SeM, SeMCys and H-SeB (108× dilution) treatment reduced the cell population at the G_0/G_1 phase with the induction of G_2/M phase arrest, causing the increase in apoptosis and late apoptosis (secondary necrosis) at 24 h (Figure 2A; Figure 3A). All Se sources examined in this study at the concentration as high as 1000 nM for 48 h did not result in the DNA single-strand break in C6 cells (Figure 4A). Most other studies observed Se effect mediated by DNA damage due to the toxic concentration used, such as 500 μM selenite for 30 min (Zhou *et al*. 2003) and 126.6 μM selenite and 316.6 μM SeM for 24 h (Shen *et al*. 2001). The highest Se concentration used in our study was far less than other studies and this Se level was not toxic enough to induce

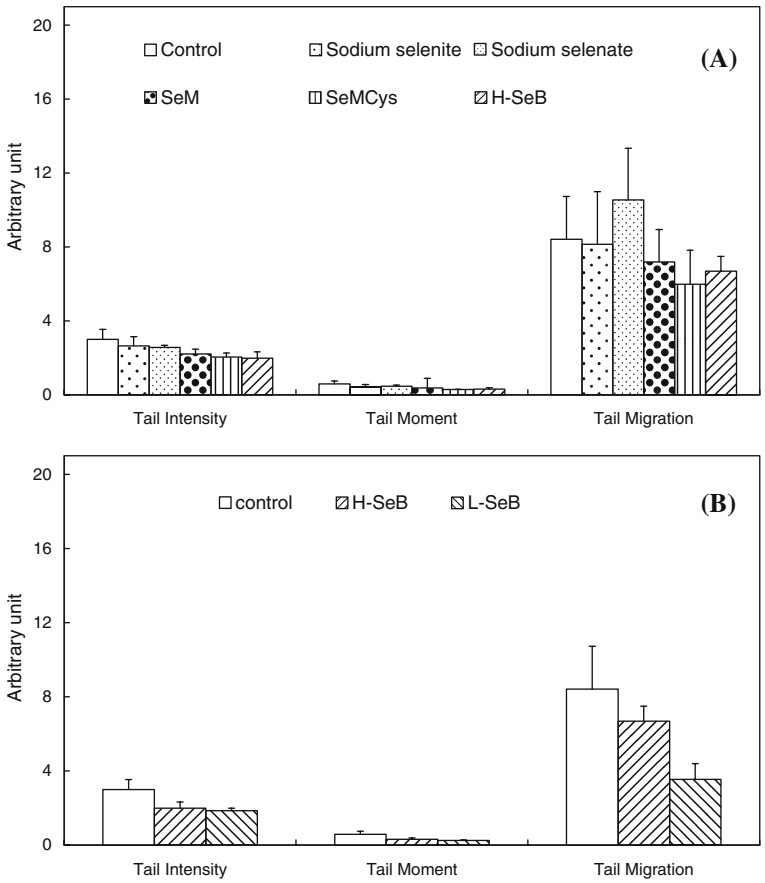

Figure 4. DNA damage assay in C6 cells by (A) various Se sources at 1000 nM or (B) broccoli extracts at 108× dilution for 48 hr. Each data point represents the mean ± SEM from three independent experiments.

the DNA damage in the cells. In addition, the study investigating SeMCys and methylseleninic acid effects on apoptosis and DNA damage in mouse mammary hyperplastic epithelial cell line also showed that treatment with SeMCys (25 or 50 μM) or methylseleninic acid (2.5 or 5 μM) increased apoptosis in cells and these effects were independent of DNA damage determined by COMET assay (Ip *et al*. 2000). However, different selenium compounds do regulate cellular responses specifically in different cell types.

The results in this study suggest that the C6 proliferation inhibition induced by the various Se compounds and H-SeB examined in this study is postulated to be due to the Se-induced increase at the G_2/M phase arrest and/or apoptosis without the involvement of the DNA damage pathway. There was Se-induced protective effect observed on apoptosis between H-SeB and L-SeB because L-SeB induced larger apoptotic cells than did H-SeB (Figure 3B) with the same dilution (108× dilution).

The interactions of bioactive components/compounds in a food are complicated. According to the published literature, the content of other compounds in Se-enriched broccoli was altered due to the increase in Se content in the broccoli, especially for sulforaphane (Sf) that was decreased by more than 80% (Charron *et al*. 2001; Finley *et al*. 2005; Robbins *et al*. 2005). Sf affects cell cycle and induces apoptosis in cancer cells (Parnaud *et al*. 2004). Therefore, the higher apoptotic effect in the cells treated with L-SeB may be due to the high Sf/Se ratio compared to that in H-SeB.

The cell cycle in mammalian cells is controlled by a series of complex interactions involving multiple cell cycle proteins. Se had been reported to modulate the functions of intracellular proteins involved in cell proliferation and cell cycle and induces apoptosis in several human tumor cell

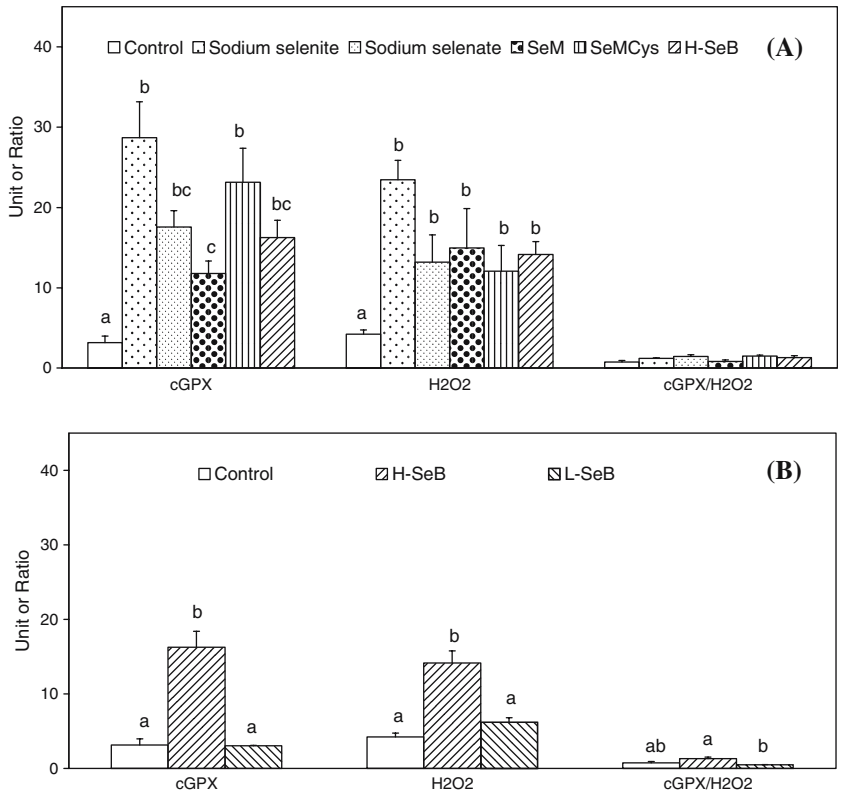

Figure 5. (A) cGPX activity, H_2O_2 content and the ratio of cGPX activity to H_2O_2 content in C6 cells by various Se sources at 1000 nM for 48 hr. Different letters (a–c) indicate significant difference ($P < 0.05$) among treatments. (B) cGPX activity, H_2O_2 content and the ratio of cGPX activity to H_2O_2 content in C6 cells by broccoli extracts at 108× dilution for 48 hr. Different letters (a–b) indicate significant difference ($P < 0.05$) among treatments. Each data point represents the mean ± SEM from three independent experiments. cGPX activity is expressed as nmol NADPH oxidized/min/mg protein, whereas H_2O_2 content is expressed as nmol/mg protein.

lines (Zhu *et al*. 1996; Ip 1998; Park *et al*. 2000). Various forms of Se and Se-enriched broccoli extract examined in this study appear to affect cell cycle by inducing G_2/M phase arrest and/or apoptosis and the mechanisms responsible for the cellular response may be related to the differential regulation of cell cycle-related proteins, such as caspase 3 and p53 (Cummings *et al*. 2002). The detailed mechanism involving the regulation of cell cycle proteins by various Se forms and H-SeB is currently under investigation.

Se is likely to exert the protective effect through modulations of antioxidative-related selenoproteins. Selenite pretreatment modulates arsenic trioxide-induced cytotoxicity through cGPX modulation in porcine aortic endothelial cells (Yeh *et al*. 2003). Other reports also suggest that Se may exert its protective effect against lipid hydroperoxide damage in human endothelial cell lines and cholesterol oxide-induced vascular damage in rat through regulation of selenoproteins (Huang *et al*. 2002; Lewin *et al*. 2002). A study investigating the cytotoxic oxysterol effect indicated that selenoproteins protect the rat vascular smooth muscle cells from apoptosis and cellular damage (Tang & Huang 2004). The Se-induced C6 proliferation inhibition in this study involved the H_2O_2-dependent mechanism with elevated cGPX activity (Figure 5A, B). The C6 growth inhibition induced by all Se sources examined appeared to be associated with the increased cellular H_2O_2 level. The H_2O_2 content and cGPX activity in H-SeB-treated C6 cells were higher than those in L-SeB-treated cells, and L-SeB treatment alone did not result in the increase in H_2O_2 content or the change in cGPX activity. The higher cGPX activity in H-SeB than L-SeB is no doubt due to the higher Se content (Figure 5B). Since the Se-enriched broccoli was grown by adding sodium

selenate solutions to the growth media for broccoli, there might be substantial amounts of selenate existing in H-SeB. Based on the results in this study, there is no significant difference between sodium selenate and H-SeB groups, therefore sodium selenate in H-SeB may contribute in part to the finding of concurrent high GPX and high H_2O_2 in cells treated with H-SeB. However, other forms of Se in broccoli extract may also be involved in the effect by H-SeB.

cGPX is one of the important antioxidative enzymes that play crucial roles in the decomposition of H_2O_2. Although the ratio of cGPX activity/H_2O_2 content did not change among any of the Se compounds and extracts, the higher ratio of cGPX activity to H_2O_2 content in H-SeB-treated cells suggests that Se exerted its protective effect by increasing the H_2O_2 content (Figure 5B). This resulted in further induced increase in cGPX activity which resulted in elevation of the antioxidative defense system in C6 cells. The growth inhibition induced by H-SeB appeared to be H_2O_2-dependent and was associated with cGPX, whereas that induced by L-SeB was H_2O_2-independent and not related to cGPX.

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