

行政院國家科學委員會專題研究計畫 成果報告

高硒青花椰菜萃取液對砷去毒機制之探討(第2年) 研究成果報告(完整版)

計畫類別：個別型
計畫編號：NSC 95-2313-B-468-002-MY2
執行期間：96年08月01日至97年07月31日
執行單位：亞洲大學生物科技學系

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處理方式：本計畫可公開查詢

中華民國 97年11月01日

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INTRODUCTION

As toxicity and its detoxification mechanisms

Some heavy metals are industrial and environmental contaminants to which human beings could be chronically exposed (Goyer, 1986). Arsenic (As) is a ubiquitous metal in the environment and the accumulation of arsenic in ground water and plants poses a health risk to both humans and animals (Abernathy et al., 1999). Inorganic As compounds, especially the trivalent compounds, appear to be more toxic than the organic As compounds. Arsenic trioxide (As_2O_3) is used as a therapeutic drug in treating acute promyelocytic leukemia patients, therefore most arsenic studies have focused on the anti-leukemic effect of As_2O_3 in cancer cells (Gianni et al., 1998; McCabe et al., 2000; Roboz et al., 2000; Ling et al., 2002). The toxic effects of As on animals and humans are important issues for investigation since it is a potential carcinogen (Salnikow and Cohen, 2002). Exposure to As compounds in animals and humans is reported to cause various health effects, such as Blackfoot disease, ischemic heart disease, vascular diseases, hypertension, diabetes, hepatic damages and cancers (Smith et al., 1992; Chen et al., 1995; Chiou et al., 1997; Hopenhayn et al., 1998; Santra et al., 2000; Tseng et al., 2000).

The As detoxification mechanistic paths involve antioxidative defense system and multidrug resistance transporters. Glutathione (L-r-glutamyl-L-cysteinylglycine; GSH) released continuously from liver and heart of animals is a biologically active thiol tripeptide and the most abundant thiol in living cells that plays an important role in detoxification of exogenous compounds and oxidative insults (Reed, 1990). It is an important antioxidant in various tissues to protect the cells from oxidative injury (Walker et al., 1995). Glutathione exists as two forms in the cells, reduced form (GSH) and oxidized form (GSSG). Several antioxidative enzymes are involved in the detoxification of As compounds, such as glutathione peroxidase (GPX) family, superoxide dismutases (SODs), GSH reductase (GR) and glutathione S-transferase (GST). The GPX family is a group of selenoproteins that incorporate selenium (Se) as selenocysteine (SeCys) into the amino acid sequence. It prevents the formation of reactive oxygen species by catalyzing H_2O_2 decomposition with the concomitant oxidation of GSH to GSSG (Wendel, 1980). 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 and Hatfield, 1999). SODs are ubiquitous metalloproteins that play a major protective role in living organisms by catalyzing the dismutation of superoxide into hydrogen peroxide and molecular oxygen. GSH reductase is the important enzyme responsible for the availability of GSH in GSH redox cycle by catalyzing the reduction of GSSG to GSH utilizing nicotinamide adenine dinucleotide phosphate (NADPH) (Meister, 1994). GSTs involve in metabolic detoxification by catalyzing the reaction between GSH and electrophilic substrates and prevent toxic damage in several tissues (Keen et al., 1976; Habig and Jakoby, 1981).

Drug pumps are an important part of cell defense system against toxin or carcinostatic drug. Several drug pumps are found in both mammalian cells and bacteria and these multidrug

resistance transporters are members of adenosine triphosphate (ATP)-binding cassette superfamily (Ambudkar et al., 1999; Putman et al., 2000). Two kinds of multidrug resistance pumps have been identified, P-glycoprotein transporter and Multidrug resistance protein (MRP) family. P-glycoprotein transporter is a phosphoglycoprotein encoded by a gene family that comprises MDR genes (MDR1 and MDR 3) in humans and *mdr* genes (*mdr* 1a, *mdr* 1b and *mdr* 2) in rodents, and functions as an ATP-dependent efflux pump on the cell membrane (Gottesman and Pastnan, 1993; Shapiro and Ling, 1995). The expression of human MDR1 as well as rodent *mdr1a* and *mdr1b* appears to confer multidrug resistance selectively. Active P-glycoprotein transporter is present in tumor and non-tumor tissues (Sleeman et al., 2000).

Multidrug resistance protein (MRP) family is a superfamily of transmembrane glycoproteins that mediate extracellular efflux of variety of xenobiotics and heavy metals (Keppler et al., 2000). The MRPs are organic anion transporters and they transport anionic drug and neutral drugs conjugated to acidic ligands, such as GSH, glucuronate or sulfate (Borst et al., 2000). The MRPs express in human tissues and cells with differential amounts (Cole et al., 1992; Zaman et al., 1993). The MRP family is composed of seven members (MRP 1-7) at least (Borst et al., 2000). MRPs 1-3 share overlapping and similar substrate specificities, but their kinetic properties differ markedly (Keppler et al., 2000). MRP1 is the ATP-dependent transporter responsible for the efflux of GSH-conjugating complex out of the cell during cellular detoxification process and mediates the transport of negatively charged conjugated hydrophilic compounds with a large hydrophobic moiety such as glucuronide, sulfate and glutathione S-conjugates of drugs. MRP1 is expressed ubiquitously with a high level in kidney, lung, intestine and brain while low expression in liver (Cherrington et al., 2002). MRP2, expressed in liver, kidney and intestine, is the ATP-binding cassette protein that also known as the canalicular multispecific organic anion transporter. Treatment of As was reported to up-regulate MRP2 gene expression transcriptionally in primary rat and human hepatocytes and this regulation was related to cellular GSH level (Vernhet et al., 2001). MRP3 is also an organic anion transporter with the preference of glucuronate conjugates as substrates over GSH conjugates (Hirohashi et al., 1999). MRP3 is expressed in pancreas and kidney with a high expression in intestines and adrenal cortex, but very low expression in liver (Cherrington et al., 2002). With the location in the basolateral membrane of hepatocyte, MRP3 may allow efflux of organic anions from the liver into the blood when secretion into bile is blocked (Kool et al., 1999a). MRP4 is suggested to be an organic anion transporter specific for phosphate conjugates. It functions as a cellular efflux pump for nucleotide analogue (Schuetz et al., 1999). MRP4 is expressed in muscle, prostate, lung, pancreas, testis, ovary, bladder and gallbladder (Borst et al., 2000). MRP5, expressed ubiquitously, is located on the basolateral membrane and is a multispecific organic anion transporter for nucleotide analogs (Wijnholds, et al., 2000). MRP6 gene is located on chromosome 16 which immediately next to MRP1. It is highly expressed in liver and kidney and to a low or very low extent in a few other tissues (Kool et al., 1999b). MRP7 is a recent addition to the MRP family (Borst et al., 2000).

Hydrogen peroxide (H₂O₂) production is associated with arsenic toxicity (Jing et al., 1999) and can damage vascular endothelium (Li and Lau, 1993). Research data indicated that

As-induced oxidative stress, such as H₂O₂ formation and lipid peroxidation, is associated with the change in intracellular GSH level and cellular regulations of antioxidative enzyme system (Han et al., 1996; Dai et al., 1999; Yeh et al., 2002). The elevations of cellular GSH level and antioxidative enzymes exert the protective effect in counteracting oxidative stress (Meister, 1991). As was also reported to cause growth inhibition and affect cell cycle by inducing G₂/M phase arrest and apoptosis through regulation of cell cycle-related proteins in a variety of human tumor cell lines (Smith and Fornace, 1996; Akao et al., 1999; Huang et al., 2000b; Park et al., 2001; Vega et al., 2001; Vogt and Rossman, 2001; Yih and Lee, 2001; Cumming et al., 2002; Ling et al., 2002; Yeh et al., 2003). Some metals or toxins are taken up or removed from cells by GSH-mediated pathways (Ballatori, 1994). GSH and As form complex and are exported outside the cells to reduce metal toxicity (Rosen, 1999). The trivalent As compounds was reported to increase cellular GST activity (Yeh et al., 2002). GSTs are involved in the conjugation of GSH with As, thus increased GST activity by trivalent arsenic compounds suggests that the elevated intracellular GSH level in response to the oxidative stress may be used to conjugate arsenic in PAECs and facilitate the efflux of arsenic (Yeh et al., 2002). Research results from embryonic stem cells indicated that the activity of multidrug resistance transporters was associated with the change in intracellular GSH level and involved in the GSH efflux system (Rappa et al., 1997). Thus, the As detoxification mechanism may also be mediated in part by multidrug resistance transporters. The living organisms are very complicated and the As-induced cellular responses observed during As toxicity are expected to be the combination effects for different detoxification mechanisms.

Protective effect of selenium on As-induced toxicity

Selenium (Se) is an essential trace element for humans and animals. Se deficiency results in skeletal muscle dystrophy in livestock and Keshan Disease in humans (Schubert et al., 1961; Chen et al., 1980). Se 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, selenoprotein W, selenoprotein P, thioredoxin reductase, iodothyronine deiodinase and other selenoproteins (Behne and Kyriakopoulos, 2001; Whanger, 2004). Se is known to suppress carcinogenesis nonspecifically in animals (Whanger, 2004). After oral supplementation with 200 ug 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). 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 and 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). Se as selenomethionine was reported to protect human fibroblasts and keratinocytes from DNA damage (Seo et al., 2002; Rafferty et al., 2003). Pretreatment of Se as selenite suppresses apoptosis in UVB-exposed human embryonic kidney cells (Park et al., 2000a).

Arsenic-induced toxicity is associated with increased oxidative stress through regulation of antioxidative-related enzymes and cell cycle progression (Barchowsky et al., 1996; Jing et al., 1999; Yeh et al., 2002). Se may exert its protective effect on As-induced toxicity through modulations of cell cycle-related protein and antioxidative-related selenoproteins. Recent reports indicated that Se exerts its protective effect against oxidative stress and vascular damage through regulation of selenoproteins both in vitro and in vivo (Huang et al., 2002a; Lewin et al., 2002). Se pretreatment as selenite reduced As₂O₃-induced cytotoxicity through GPX modulation (Yeh et al., 2003).

Potential effect of Se-enriched broccoli extract

Cancer is the leading cause for death in human health and may be prevented through improving physiological antioxidative system by consuming protective compounds or avoiding mutagen exposure (DeFlora, 1998). Natural plant extracts have gained much attention recently due to the antioxidative and antimutagenic abilities to defense the dangerous effects caused by toxins or mutagens (Bronzetti, 1994). The form and the concentration of Se used are critical for cancer prevention (Sinha and El-Bayoumy, 2004). Various Se compounds influence cellular responses differently in various cell types (Tapiero et al., 2003). 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 addition of Se-enriched broccoli (SeB) to the rat diets significantly reduced the mammary tumor incidence from 90% to 37% (Finley et al., 2001). In addition, SeB florets (Finley et al., 2000; Finley and Davis, 2001) and sprouts (Finley et al., 2001) significantly reduced colon tumors in rats.

A study investigating the differential effects of SeB extract on cell proliferation by using C6 rat glial cells indicated that the proliferation inhibition by 1000 nM Se as SeB extract was apparently related to the H₂O₂-dependent mechanism with elevated cellular glutathione peroxidase (cGPX) activity (Yeh et al., 2005). SeB extract also involved in the modulation of cell cycle progression and apoptosis (Yeh et al., 2005).

Effect of Se on multidrug resistance

Se was shown to inhibit the growth of drug-resistance cell lines (Caffrey and Frenkel, 1997; Bjorkhem-Bergman et al., 2002) and to prevent the development of drug resistance (Caffrey et al., 1998). However, no available information can be found on the interaction of selenium with multidrug resistance transporters.

OBJECTIVES

Arsenic is a known endothelial toxin and has a direct effect on endothelial cells causing vascular disorders and endothelial cell dysfunction (Chang et al., 1991; Tsai et al., 2001). The vascular endothelial cells are considered to be one of the primary targets for As toxicity and are used extensively as the experimental model to examine cellular responses induced by As (Engel and Smith, 1994; Barchowsky et al., 1996). Previous in vitro studies indicated that P-glycoprotein is present and functionally active in primary cultures of rat, porcine and bovine cerebral endothelial cells (Greenwood, 1992; Huwyler et al., 1996; Fenart et al., 1998). Whereas, there were evidences indicated that the MRP expressed in primary cultures of bovine brain microvessel endothelial cells (Huai-Yun et al., 1998). It was reported that P-glycoprotein transporter and the MRP family expressed in porcine oocyte and the high homology of these multidrug resistance transporters between porcine and human genes was observed (Yashi et al., 2001). We propose to use the primary culture of porcine aortic endothelial cells (PAECs) continuously as the experimental materials in this proposed study not only for the high homology of multidrug resistance transporters observed between porcine and human gene (Yashi et al., 2001), but also for the possibly differential responses between normal and tumor cells.

The primary objective of this proposed study was to investigate the possible mechanism responsible for the protective effect of SeB extract on As-induced toxicity. The activities of cGPX and GST as well as the cell cycle progression in primary culture of porcine aortic endothelial cells (PAECs) will be investigated. The regulation of specific multidrug resistance transporters expression by Se-enriched broccoli extract was also investigated.

MATERIALS AND METHODS

Cell culture

Porcine aortic endothelial cells (PAECs) were isolated and identified previously from our laboratory (Yeh et al., 2002). The primary culture of isolated PAECs was used as the experimental materials in this study. The cells were maintained routinely and passed in sterile basal medium (M199, 100 U penicillin/mL, 100 µg streptomycin/mL, 2.2 g sodium bicarbonate/L media, pH 7.4) supplemented with 10% FBS under a humidified atmosphere of 5% CO₂ and 95% air at 37°C. The culture medium was changed every 2-3 days and the microscopic examination was used to observe the cell morphology and monitor cell growth. All experiments were performed when the cells reach 80-90% confluence.

In vitro digestion of Se-enriched broccoli (SeB) extract

Broccoli extracts containing low and high levels of Se was provided by Dr. John Finley (Human Nutrition Laboratory, USDA, Grand Forks, ND, USA). Briefly, sodium selenate solutions were added to the growth media for broccoli and the broccoli extracts were prepared by in vitro enzymatic digestion of broccoli powder. 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 hr. 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 hr. The digested mixture was then dialyzed against distilled water overnight using dialysis bags with 12,000 to 14,000 MW cut off. The high-Se (H-SeB) and low-Se (L-SeB) broccoli extracts were analyzed to contain 1.08×10^{-4} M Se and 2.50×10^{-7} M Se, respectively (Yeh et al., 2005).

MTT assay

MTT assay was used to measure cell proliferation and cytotoxicity. 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 (Mosmann, 1983). PAECs were seeded in 96-well tissue culture microplates at a concentration of 1×10^4 cells per well. Various experiments were performed after the cells reach 80-90% confluence. Prior to investigate the protective effect of SeB on As₂O₃-induced cytotoxicity, the effect of various Se compounds and SeB extracts on cell proliferation in PAECs was determined. The different dilutions of H-SeB and L-SeB were applied to PAECs to determine the effect of SeB extracts alone and the possible contribution of broccoli extraction buffer on cell proliferation. The different concentrations of SeB (H-SeB or L-SeB) will be added to the culture media and the plates were incubated at 37°C up to 24 hr. An appropriate concentration of SeB extracts was determined for later pretreatment experiments To investigate the protective effect of SeB extract on As₂O₃-induced cytotoxicity, PAECs were pretreated with or without an appropriate concentration of SeB extract or Na₂SeO₃ (as positive control) at 37°C for 24 hr. The media from one set of the plates was then changed to the culture media containing 20 µM of As₂O₃, while the media from the other set of the plates was changed to the culture

media containing 20 μM of As_2O_3 with addition of SeB extract or Na_2SeO_3 . The plates were incubated at 37°C for additional 24 hr. At the end of the experiments, 10 μL of MTT stock solution (final MTT concentration: 0.5 mg/mL) was added to each well (100 μL culture media/well) and the microplates were then incubated at 37°C for additional 4 hr. After adding 100 μL of solubilization solution (10% SDS in 0.01M HCl) to each well to dissolve the purple formazan crystals, the plates were read on a microplate reader at the wavelength of 590 nm.

Cell treatments for cell cycle progression, enzymatic assays and RT-PCR analysis

PAECs were cultured on 10-cm tissue culture plates, then were pretreated with or without an appropriate concentration of various selenocompounds or SeB extracts at 37°C for 24 hr. To investigate the protective effect of Se pretreatment on As_2O_3 -induced cell cycle progression and enzyme activities, the media from one set of the plates was changed to the regular culture media (DMEM+10% FBS), while the media from the other set of the plates were changed to the culture media containing 20 μM of As_2O_3 . The plates were then incubated at 37°C for additional 24 hr. At the end of the experiments, the plates were subjected to the following procedure for determination of cell cycle progression or frozen in -80°C after three washes with ice-cold PBS for further enzymatic analysis or RT-PCR analysis.

Cell cycle progression analysis

To determine the cell cycle phase-distribution, both detached and adherent cells were harvested from the plates at the end of experiments, washed with ice-cold PBS and resuspended in 8 mL of ice-cold 70% ethanol to fix overnight at -20°C. Centrifugation was performed at 300 x g for 5 min at 4°C the next day and 0.5 mL of 0.5% Triton X-100 and 2 μL of RNase A was added to resuspend the cell pellets. The mixture was incubated in a waterbath at 37°C for 30 min, and the supernatant was discarded following centrifugation at 300 x g for 5 min at 4°C. Then 0.5 mL of propidium iodide stock solution (20 $\mu\text{g}/\text{mL}$ in PBS) was added to the pellet and this mixture was incubated for 10 min on ice. The mixture was then applied to the Flow Cytometer for analysis (approximately 10000 events).

Intracellular antioxidative enzyme activities

To prepare cell cytosols, an appropriate amount of homogenate buffer (20 mM Tris [pH 7.5], 0.25 M sucrose, 1mM EGTA, 5mM EDTA, 1mM PMSF, 50 mM beta-mercaptoethanol and 25 $\mu\text{g}/\text{ml}$ leupeptin; pH 7.4) was added to each frozen plate and the cells were scraped into centrifuge tubes by rubber policeman. After sonication for 10 sec on ice, the mixtures were centrifuged at 17000 x g for 10 min at 4°C. Protein concentration of cell cytosols was determined by the Bradford assay (Bradford, 1976) using bovine serum albumin as standards. The cell cytosols were then be used for measurement of cGPX and GST activities.

Cellular glutathione peroxidase activity was determined by an enzyme-coupled method with glutathione reductase, utilizing hydrogen peroxide as substrate (Paglia and 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. Glutathione-S-transferase (GST) activity was determined spectrophotometrically at 340 nm by monitoring the formation of the conjugated glutathione and CDNB at 25°C (Habig and Jakoby, 1981). The rate of increase in the absorbance at 340 nm was monitored over the 3-min period at 30-s intervals.

Total RNA extraction and RT-PCR analysis of multidrug resistance transporters

Due to the low expression level for multidrug resistance transporters, reverse transcription-polymerase chain reaction (RT-PCR) assay was used to identify the differential expression of multidrug resistance transporters in PAECs. The total RNA was extracted from PAECs using Trizol reagent (Invitrogen). The RNA was quantitated by spectrophotometer at a wavelength of 260/280 nm prior to RT-PCR analysis. RT reaction was performed using the commercial RT kit (TAKARA). Subsequent PCR reaction was performed using various specific primers for multidrug resistance transporters. The specific primers for various multidrug resistance transporters were designed from the published literatures and the sequences in GenBank (Liu et al., 2001; Vernhet et al., 2001; Yashi et al., 2001). The GAPDH primers will be used as the internal control.

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 and Torrie, 1980). A significance level of 5% was adopted for all comparisons.

RESULTS

1. Based on the MTT results, various selenocompounds as well as Se-enriched broccoli extracts exhibited higher protection in As₂O₃-induced toxicity at the concentration of 1 X 10⁻⁶M for 24 hr (Fig. 1) than at 1 X 10⁻⁷M (Fig 2). Thus, the concentration for Se pretreatment was determined to be 1 X 10⁻⁶M.
2. The protective effect observed with HSeB pretreatment for 24 hr in As₂O₃-induced toxicity is due to the high selenium content in HSeB and may be related to the sulforaphane (SF) content in HSeB (Figs. 3-4).
3. Pretreatment with various selenocompounds alone for 24 hr did not affect SOD (superoxide dismutase) and CAT (catalase) activities in PAECs. The GST activity in PAECs was increased by HSeB pretreatment for 24 hr and the cGPX activity was increased by selenate pretreatment (Fig. 5). Pretreatment of various selenocompounds did not affect the GST activity in As₂O₃-induced PAECs, whereas the As₂O₃-induced increase in cGPX activity was further increased by selenate pretreatment (Fig. 6).
4. The increased in GST activity in PAECs induced by HSeB pretreatment may be due to SF effect (Fig. 7).
5. Pretreatment with selenite or SeM (selenomethionine) for 24 hr resulted in an increase in cell population in G₀/G₁ phase (Fig. 8). There is no effect by Se pretreatment in cell population in S and G₂/M phase. Se pretreatment did not influence the As₂O₃-induced cell cycle progression (Fig. 9).
6. The specific primers used for multidrug resistance associated transporters in RT-PCR analysis are listed in Table 1. The mRNAs of GST2, MDR1, MRP1 and MRP5 were expressed in PAECs (Fig. 10). Using GAPDH mRNA as internal control (Fig. 11), the results from RT-PCR analysis indicated that there is no difference among pretreatment of Se-enriched broccoli extracts in As₂O₃-induced gene expression of multidrug resistance transporters (Figs. 12-15).

SUMMARY

The results in this study indicated that Se-enriched broccoli extracts protected porcine aortic endothelial cells (PAECs) from arsenic-induced toxicity mainly through modulation of glutathione S-transferase (GST) activity, but not mRNA expression. Multidrug resistance associated transporters, MDR1, MRP1 and MRP5 were not involved in selenium protective mechanism for arsenic toxicity. However, other multidrug resistance associated transporters may be involved in the possible mechanism. Therefore, further investigation of expression of other multidrug resistance associated transporters in As-induced toxicity is needed.

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SELF-EVALUATION

The results from this study are promising. Further investigation of regulation of other specific multidrug resistance transporters by Se-enriched broccoli extract will help to understand the mechanism responsible for the protective effect of Se-enriched broccoli extracts on arsenic-induced toxicity.

Table 1. Specific primers used for RT-PCR analysis

Primer	Sequence (5'→3')	PCR product
MDR1-F	GCCTGGCAGCTGGAAGACAAATACACAAAATT	283 bp
MDR1-R	CAGACAGCAGCTGACAGTCCAAGAACAGGACT	
MRP1-F	CACACTGAATGGCATCACCTTC	317 bp
MRP1-R	CCTTCTCGCCAATCTCTGTCC	
MRP5-F	ACATCACGTCCAGCATAACAGG	594 bp
MRP5-R	TGCAGCCTCCAGATAACTCC	
GST 2-F	GATGGGGTGGAGGACCTTCGATGC	272 bp
GST 2-R	CTGAGGCGAGCCACATAGGCAGAG	
GAPDH-F	AGTCGGAGTCAACGGATTTG	305 bp
GAPDH-R	TCTCCATGGTGGTGAAGACA	

Figure 1. Pretreatment of various selenocompounds (1X10⁻⁶M Se) for 24 hr followed by 20 uM As₂O₃ treatment for 24 hr

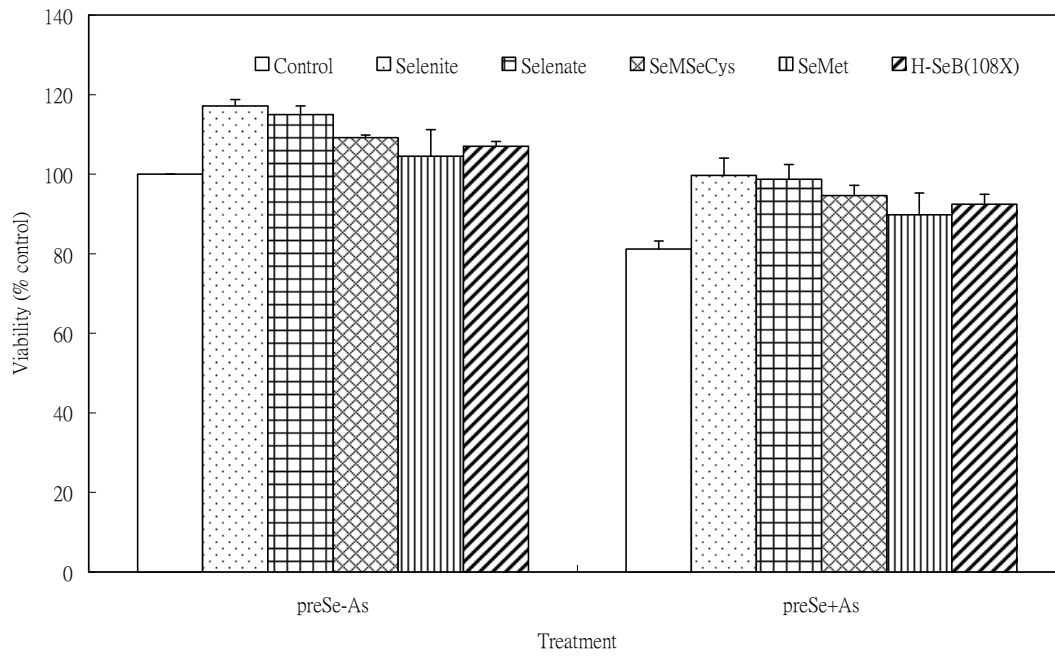


Figure 2. Pretreatment of various selenocompounds (1X10⁻⁷M Se) for 24 hr followed by 20 uM As₂O₃ treatment for 24 hr

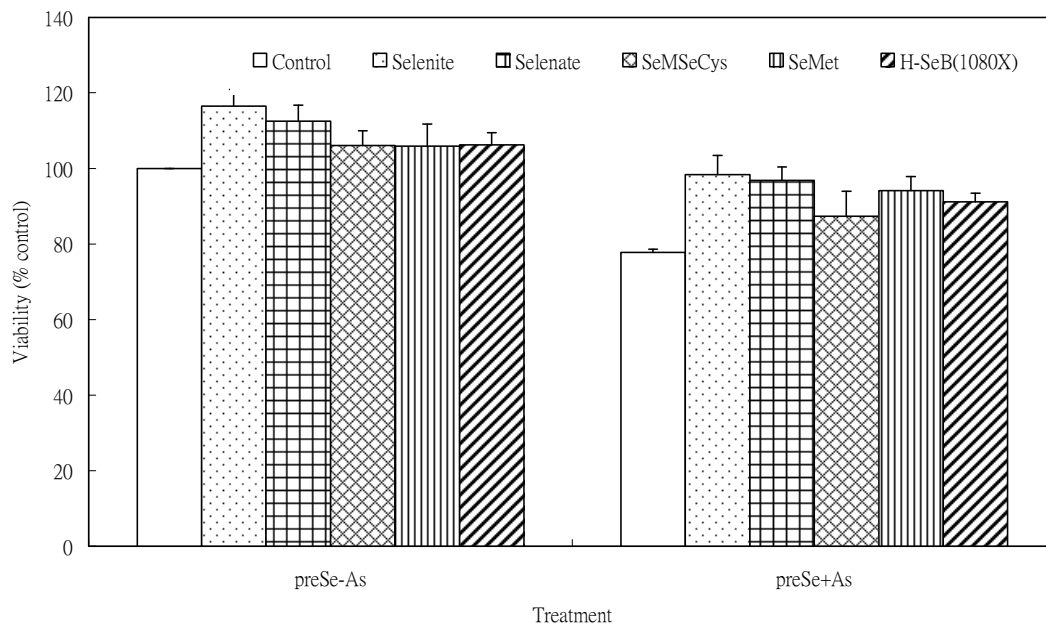


Figure 3. Pretreatment of Se-enriched broccoli extracts (108X dilution) and sulforaphane for 24 hr followed by 20 μ M As₂O₃ treatment for 24 hr

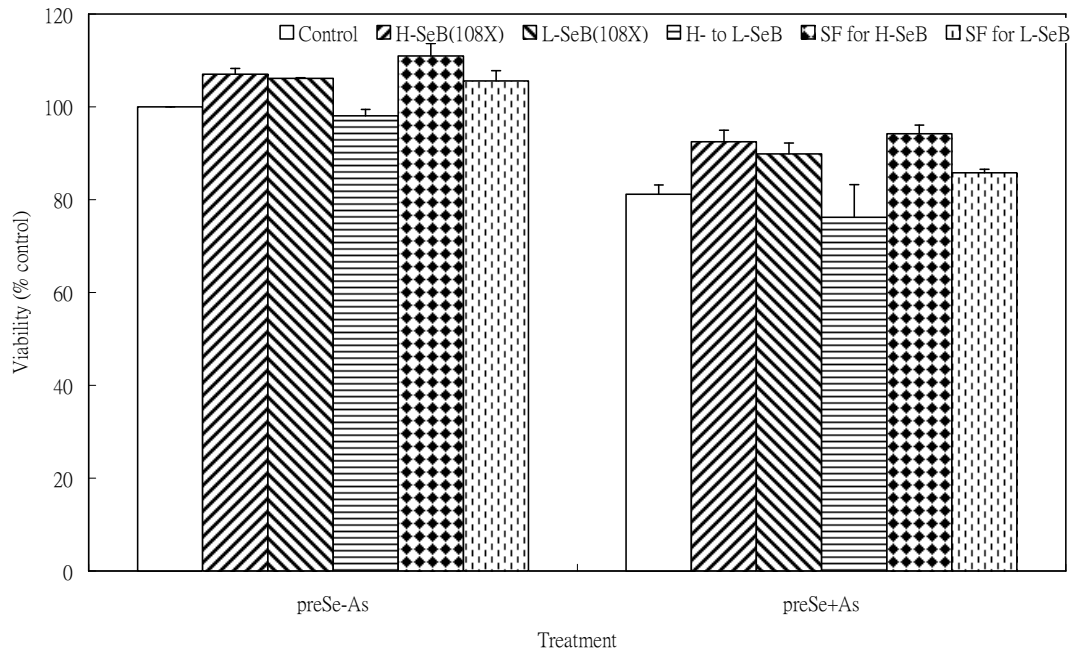


Figure 4. Pretreatment of Se-enriched broccoli extracts (1080X dilution) and sulforaphane for 24 hr followed by 20 μ M As₂O₃ treatment for 24 hr

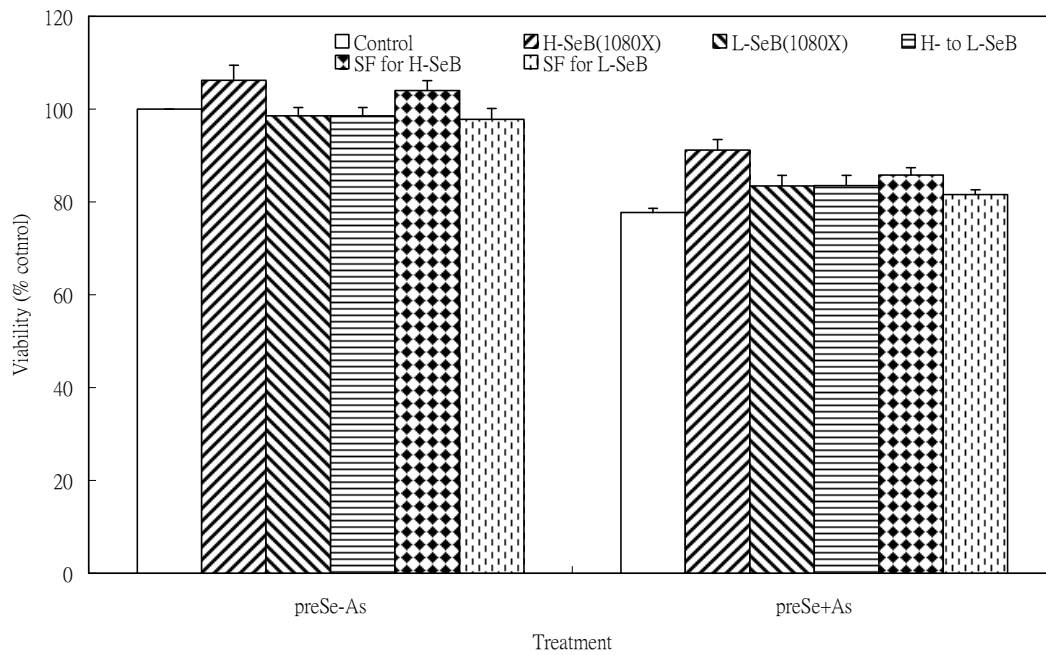


Figure 5. Pretreatment effect of various Se compounds (1 X 10⁻⁶M) for 24 hr on enzyme activities in PAECs

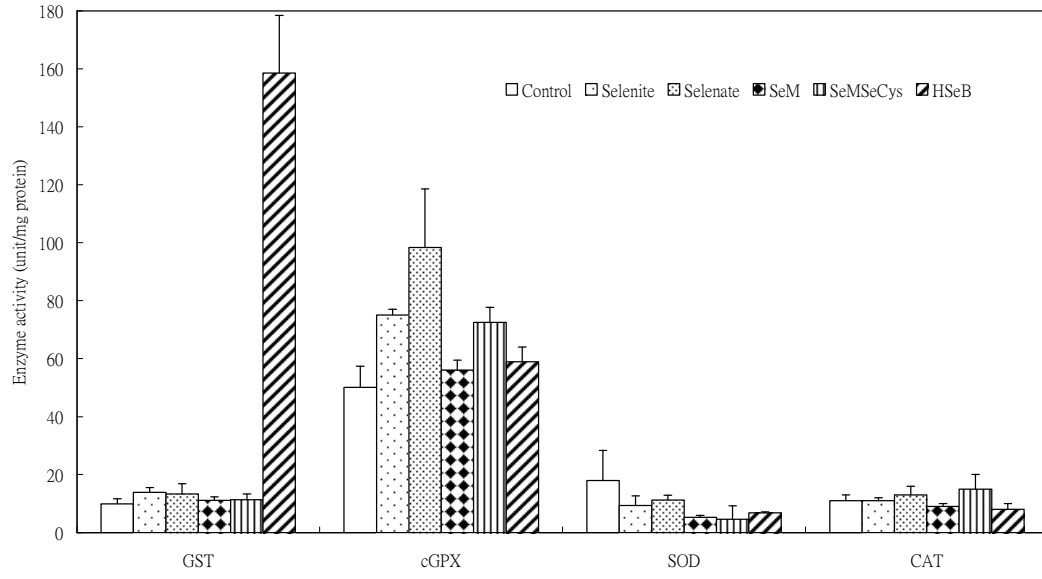


Figure 6. Pretreatment effect of various Se compounds (1 X 10⁻⁶M) for 24 hr on As₂O₃-induced enzyme activities in PAECs

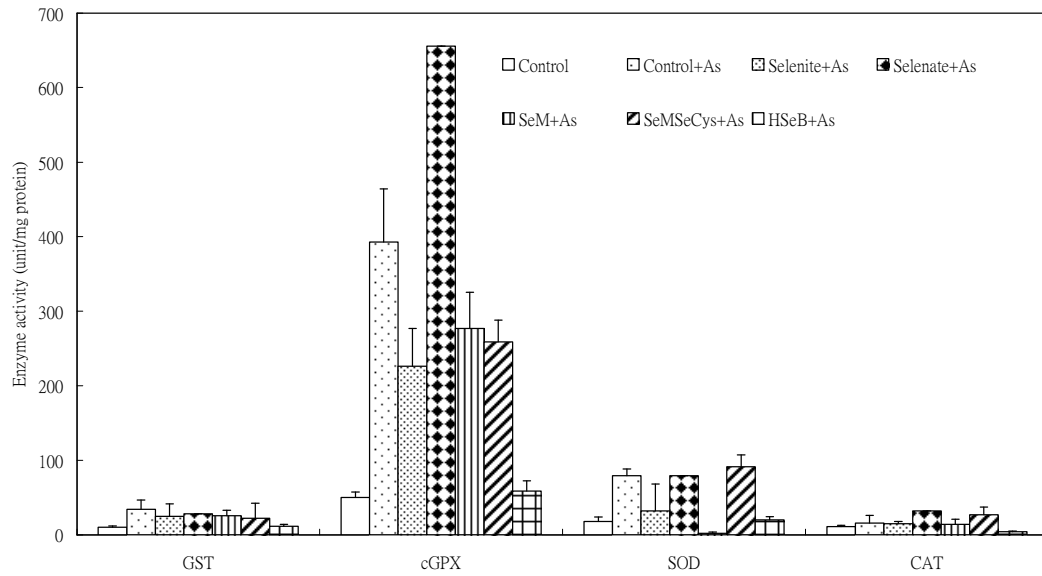


Figure 7. Pretreatment effect of Se-enriched broccoli extracts and sulforaphane for 24 hr on enzyme activities in PAECs

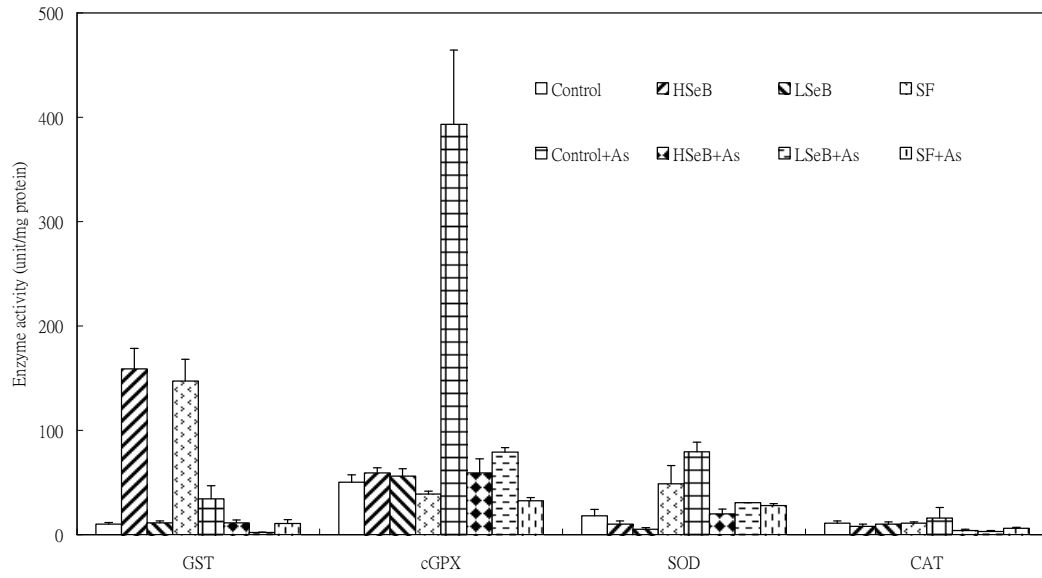


Figure 8. Pretreatment effect of various Se compounds (1 X 10⁻⁶M) for 24 hr on cell cycle progression in PAECs

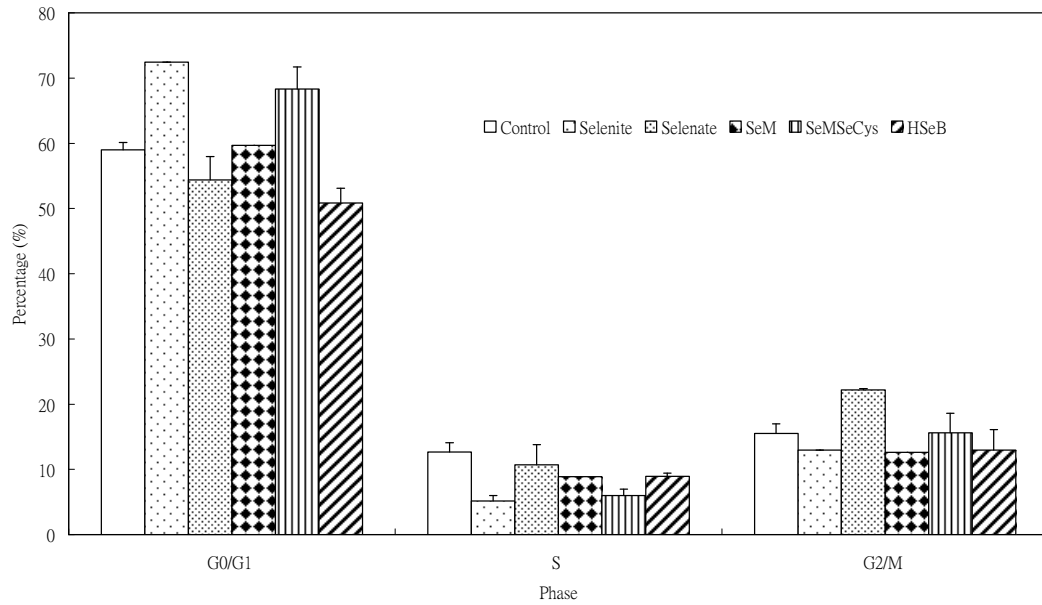
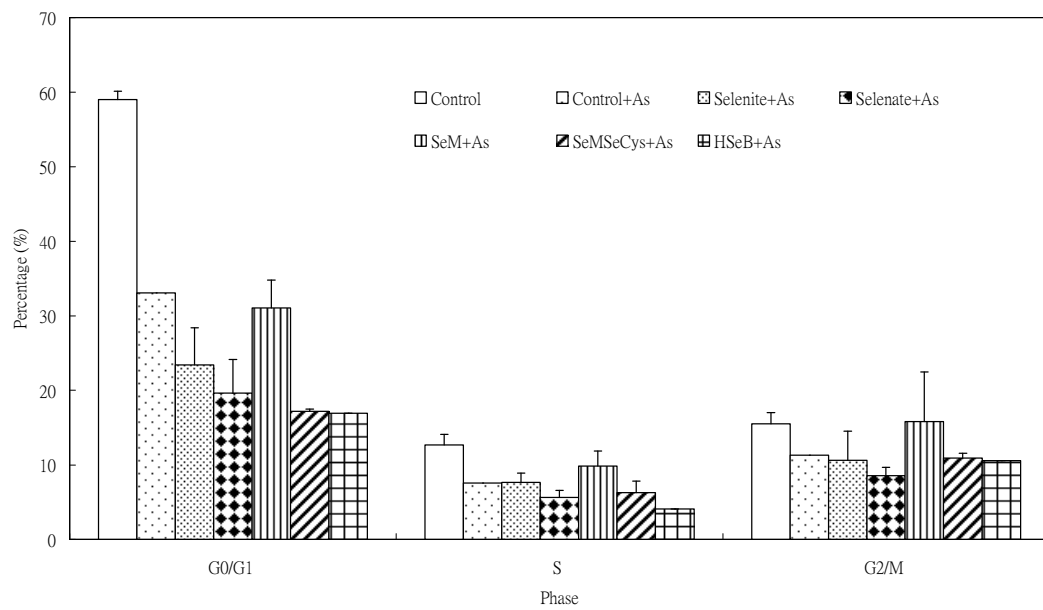


Figure 9. Pretreatment effect of various Se compounds (1 X 10⁻⁶M) for 24 hr on As₂O₃-induced cell cycle progression in PAECs



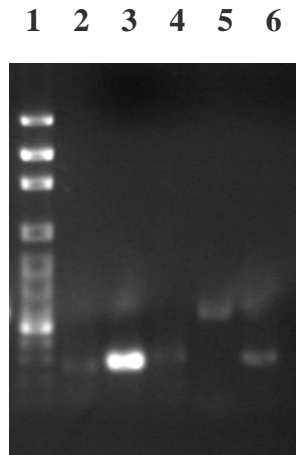


Figure 10. The mRNA Expression of GST2, MDR1, MRP1, MRP5 and GAPDH in PAECs. Lane 1: 100bp marker; Lane 2: GST 2; Lane 3: MDR1; Lane 4: MRP1; Lane 5: MRP5 and Lane 6: GAPDH.

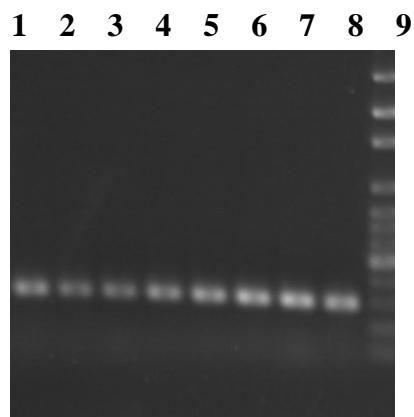


Figure 11. GAPDH mRNA expression by Se-enriched broccoli extracts and sulforaphane (SF) pretreatments for 24 hr in As_2O_3 -induced PAECs. Lane 1: HSeB; Lane 2: LSeB; Lane 3: SF; Lane 4: control+As; Lane 5: HSeB+As; Lane 6: LSeB+As; Lane 7: SF+As; Lane 8: control and Lane 9: 100 bp marker.

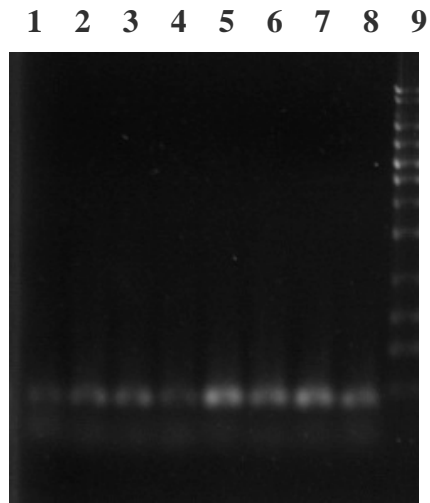


Figure 12. GST2 mRNA expression by Se-enriched broccoli extracts and sulforaphane (SF) pretreatments for 24 hr in As_2O_3 -induced PAECs. Lane 1: control; Lane 2: HSeB; Lane 3: LSeB; Lane 4: SF; Lane 5: control+As; Lane 6: HSeB+As; Lane 7: LSeB+As; Lane 8: SF+As and Lane 9: 1 kb marker.

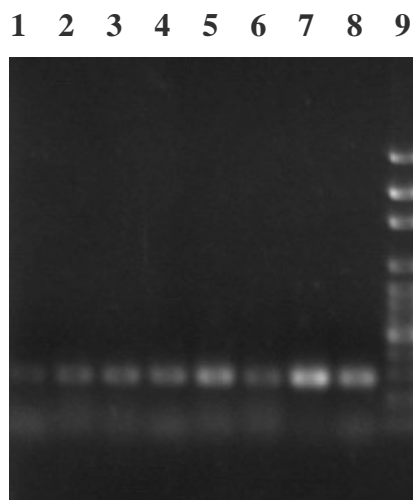


Figure 13. MDR1 mRNA expression by Se-enriched broccoli extracts and sulforaphane (SF) pretreatments for 24 hr in As_2O_3 -induced PAECs. Lane 1: control; Lane 2: HSeB; Lane 3: LSeB; Lane 4: SF; Lane 5: control+As; Lane 6: HSeB+As; Lane 7: LSeB+As; Lane 8: SF+As and Lane 9: 100 bp marker.

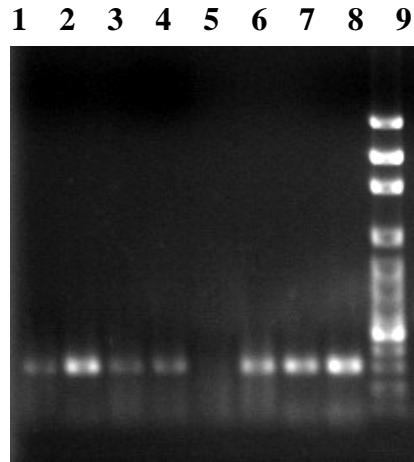


Figure 14. MRP1 mRNA expression by Se-enriched broccoli extracts and sulforaphane (SF) pretreatments for 24 hr in As_2O_3 -induced PAECs. Lane 1: control; Lane 2: HSeB; Lane 3: LSeB; Lane 4: SF; Lane 5: control+As; Lane 6: HSeB+As; Lane 7: LSeB+As; Lane 8: SF+As and Lane 9: 100 bp marker.

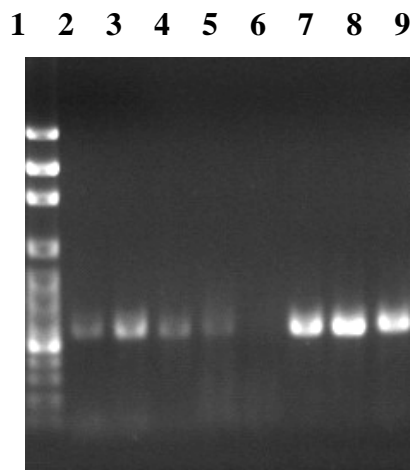


Figure 15. MRP5 mRNA expression by Se-enriched broccoli extracts and sulforaphane (SF) pretreatments for 24 hr in As_2O_3 -induced PAECs. Lane 1: 100 bp marker; Lane 2: control; Lane 3: HSeB; Lane 4: LSeB; Lane 5: SF; Lane 6: control+As; Lane 7: HSeB+As; Lane 8: LSeB+As and Lane 9: SF+As.