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Analytical Methods

Synergy between broccoli sprout extract and selenium in the upregulation of thioredoxin reductase in human hepatocytes

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Abstract

Dietary isothiocyanates and selenium (Se) can up-regulate thioredoxin reductase 1 (TR1) in cultured human HepG2 and MCF-7 cells [Zhang et al. (2003). Synergy between sulforaphane and selenium in the induction of thioredoxin reductase 1 requires both transcriptional and translational modulation. *Carcinogenesis*, *24*, 497–503; Wang et al. (2005). Sulforaphane, erucin and iberin up-regulate thioredoxin reductase expression in human MCF-7 cells. *Journal of Agricultural and Food Chemistry*, *53*, 1417–1421] at both the protein and mRNA levels. In this study, broccoli sprout extract (a rich source of the isothiocyanates sulforaphane and iberin) and Se interacted synergistically to induce TR1 in immortalised human hepatocytes. Broccoli sprout extracts containing 1.6, 4 and 8 μM isothiocyanates were tested for their ability to induce TR1 at the protein and mRNA level. Although induction of TR1 mRNA by broccoli sprout extract (1.6–8 μM) was only 1.7–2.2-fold, co-treatment with Se (0.2–1 μM) enhanced the expression of TR1 mRNA (3.0–3.3-fold). Moreover, broccoli sprout extract induced the cellular concentration of TR1 and TR enzymatic activity, an induction that was augmented by Se addition. Thus, broccoli sprout extract (8 μM) and Se induced cellular TR1 concentration and enzymatic activity 3.7- and 5-fold respectively, whereas, Se or broccoli sprout extract alone produced an induction of only approximately 2-fold. These data suggest that dietary isothiocyanates from broccoli sprouts and Se are important agents in the regulation of redox status in human liver cells. The synergistic effect between isothiocyanates and Se at physiologically-relevant concentrations on the induction of TR1 may play an important role in protection against oxidative stress.

Keywords: Isothiocyanates; Sulforaphane; Selenium; Thioredoxin reductase; Hepatocyte; Cruciferous vegetables

1. Introduction

A diet rich in cruciferous vegetables such as broccoli, brussels sprouts, cabbage and cauliflower correlates with a decreased risk of many common cancers, including those of the lung, colon, breast and bladder (Ambrosone et al., 2004; Verhoeven, Goldbohm, van Poppel, Verhagen, & van den Brandt, 1996; Zhao et al., 2007). Crucifers contain glucosinolates which can produce isothiocyanates (ITCs) under the action of myrosinase (Fenwick, Heaney, & Mullin, 1983; Rask et al., 2000). Broccoli sprouts are a particularly rich source of chemopreventive ITCs, which potently induce carcinogen-detoxifying phase II enzymes, including quinine reductase (NQO1), glutathione transferase (GST) (Fahey, Zhang, & Talalay, 1997; Zhang et al., 2006), and activate mitochondria-mediated apoptosis and arrest cell cycle progression in bladder cancer cells (Shan et al., 2006; Tang et al., 2006). Recently, a human intervention study has been carried out in Qidong County, China, using drinks made from hot water infusions of 3-day-old broccoli sprouts. The aim of the study was to reduce the risk of liver

Abbreviations: TR, thioredoxin reductase; ITCs, isothiocyanates; SFN, sulforaphane; Se, selenium; BSE, broccoli sprout extract.

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cancer in the population who are exposed to high levels of aflatoxin. The infusion increased urinary excretion of aflatoxin-DNA adducts (Kensler et al., 2005).

ITCs and selenium (Se) can up-regulate thioredoxin reductase 1 (TR1) in human hepatoma HepG2, breast cancer MCF-7 and endothelial EAhy926 cell lines (Campbell, Howie, Arthur, Nicol, & Beckett, 2007; Wang et al., 2005; Zhang et al., 2003). TR1 is a multifunctional selenoenzyme with a FAD, a functional disulfide/dithiol, and a penultimate C-terminal selenocysteine residue (Sandalova, Zhong, Lindqvist, Holmgren, & Schneider, 2001; Zhong & Holmgren, 2002); it exhibits broad substrate specificity, reducing many low molecular compounds such as hydrogen peroxide, lipid hydroperoxides, ascorbate, lipoic acid and ubiquinone in addition to thioredoxin (Trx) (Xia et al., 2003). Sulforaphane can upregulate both TR1 and Trx, suggesting that sulforaphane plays an important role in regulation of redox in cells (Bacon et al., 2007).

TR1 and Trx form a major redox control system involved in many central intracellular and extracellular processes, including cell proliferation, redox regulation of gene expression and signal transduction, protection against oxidative stress, anti-apoptotic functions and regulation of the redox state of the extracellular environment (Lincoln, Ali Emadi, Tonissen, & Clarke, 2003). The present study investigates the effects of broccoli sprout extract (BSE), which is rich in sulforaphane (SFN, 4-methylsulfinylbutyl isothiocyanate) and also contains minor amounts iberin (3-methylsulfinylbutyl isothiocyanate) and erucin (4-methylthiobutyl isothiocyanate) (Fahey et al., 1997), on TR1 induction in immortalised human hepatocytes. ITC-rich broccoli sprout extract is known to be a strong inducer of TR1 in cultured human immortalised hepatocytes; in addition, co-treatment with Se can significantly enhance expression of TR1 mRNA and protein.

2. Materials and methods

2.1. Reagents and broccoli sprout extract

Thioredoxin reductase (EC 1.6.4.5 from *Escherichia coli*, Catalogue number T7915), Trx (from *Spirulina* sp.), insulin, NADPH, 5,5′-dithiobis(2-nitrobenzoic acid) (DTNB), dimethyl sulfoxide (DMSO), phenylmethylsulphonyl fluoride (PMSF) and sodium selenite (purity 98%) were all purchased from Sigma (UK). Broccoli sprout extract (BSE) was kindly provided by Professor Yuesheng Zhang (Department of Chemoprevention, Roswell Park Cancer Institute, Buffalo, USA). Lyophilized SFN-rich broccoli sprout extract was prepared from 3-day-old fresh broccoli sprouts and stored at −80 °C before use. The preparation of the extract and the characterization of its ITC content have been previously described (Tang et al., 2006). In summary, broccoli sprouts, grown by Sprouters Northwest (Kent, WA) using seeds from an open-pollinated population based upon *Brassica oleracea* L. *var. italica* cultivars DeCicco and Calabrese, were added to boiling water, at a ratio of 100 kg sprouts to 300 L of water, in a stirred, steam-jacketed kettle. The sprouts were boiled for 30 min and filtered through a stainless-steel mesh screen. The filtrate contains the glucosinolate precursors of ITCs. Conversion of these glucosinolates to ITCs was effected by adding myrosinase obtained from daikon sprouts to the filtrates and incubating at 32–38 °C for 3.5 h. The mixture was transferred to trays and freeze-dried to yield 2.8 kg powder (from 100 kg broccoli sprouts). Each gram of extract contained 140 μmol ITCs comprising SFN, iberin and erucin (70%, 25% and 5%, respectively, as determined by HPLC). The freeze-dried BSE was dissolved in DMSO (8 mM), and stored at −20 °C in aliquots.

2.2. Cell culture

Immortalised human hepatocytes (defined as HHL-5) were kindly supplied by Dr. Arvind Patel, Medical Research Council (MRC) Virology Unit (Glasgow, UK). The primary hepatocytes were immortalised with Moloney's mouse leukaemia virus (Clayton et al., 2005) and contained markers of hepatocyte and biliary phenotype (cytokeratins 7, 8, 18 and 19), cytochrome P450 and albumin. HHL-5 contained active gap junctions and their cells respond to stimulation with IFN-alpha by upregulation of major histocompatibility complex (MHC)-I and -II.

Cells were cultured in DMEM supplemented with foetal bovine serum (FBS) (10%), 2 mM glutamine, penicillin (100 U/ml) and streptomycin (100 μg/ml) under 5% CO_2 in air at 37 °C. For studies of TR1 concentration and TR activity, cells ($1–2 \times 10^4/cm^2$) were seeded in 10-cm dish in triplicate. For mRNA quantification, cells ($2 \times 10^4/cm^2$) were cultured in 6-well plate in triplicate. When the cells achieved 70–80% confluence, they were exposed to various concentrations of BSE and Se treatments.

2.3. RNA isolation

Total RNA was isolated from human hepatocytes (HHL-5) using GenElute™ Total Mammalian RNA Kit (Sigma, UK) according to the manufacturer's instructions. RNA concentration and purity were determined by absorbance measurement at 260 and 280 nm using a nanodrop spectrophotometer (Labtech International, UK). After adding RNase inhibitor (20 U/preparation), the total RNA was stored at −80 °C.

2.4. TR1 mRNA quantification

TR1 mRNA was determined by TaqMan® real-time RT-PCR using the ABI 7500 Fast Real-Time PCR System. Primers and the fluorogenic TaqMan probes were designed using Primer Express according to human TR1 sequence. Primer/probe set for TR1 (GenBank Accession No. AF208018) is: forward primer: 5′-CCACTGGTGAAAGACCACGTT-3′. Reverse Primer: 5′-AGGAGAAAA-

GATCATCACTGCTGAT-3′. Probe: 5′-CAGTATTCTT-TGTCACCAGGGATGCCCA-3′.

The probes were labelled with a 5′ reporter dye, FAM (6-carboxyfluoroscein) and 3′ quencher dye, TAMRA (6-carboxytetramethylrhodamine). Real-time RT-PCR reactions were carried out in a 96-well plate in a total volume of 25 μl per well consisting of TaqMan one-step RT-PCR Master Mix Reagents (Applied Biosystems), 100 nM probe, 200 nM forward and 300 nM reverse primers and 10 ng of total RNA. A reverse transcription was performed for 30 min at 48 °C, then an AmpliTaq^{TM} gold activation for 10 min at 95 °C, followed by 40 PCR cycles of denaturation at 95 °C for 15 s and finally annealing/extension at 60 °C for 1 min. Reactions were carried out in triplicate. Data were normalized against an invariant endogenous control, 18S ribosomal RNA (Elliot, Bacon, & Bao, 2004). 18S rRNA forward primer: 5′-GGCTCATTAAATCAGTTATGGT-TCCT-3′, reverse primer 5′-GTATTAGCTCTAGAAT-TACCACAGTTATCCA-3′, probe: 5′-TGGTCGCTCGC-TCCTCTCCCAC-3′. TaqMan® threshold cycle number (C_t) was converted into fold of relative induction using the equation of $\Delta\Delta C_t$ method (Livak & Schmittgen, 2001).

2.5. Cell extract preparation

Human hepatocyes HHL-5 were washed twice with ice-cold PBS, harvested by scraping in 20 mM Tris–HCl (pH 8), 150 mM NaCl, 2 mM EDTA, 10% glycerol, 1% Nonidet P40 (NP-40) containing mini complete proteinase inhibitor (Roche Applied Science, UK) and 1 mM PMSF in an ice bath for 20 min and centrifuged at 12,000*g* for 15 min at 4 °C. Supernatant was collected and protein concentration determined by the Brilliant Blue G dye-binding assay of Bradford (1976) using bovine serum albumin as standard.

2.6. TR1 protein concentration

The concentration of TR1 was measured by an in-house RIA assay as described previously (Miller et al., 2001). The tracer was ^{125}I-labelled recombinant human TR1 (Lab Frontier Life Sciences, Seoul, Korea), prepared using Bolton-Hunter reagent (GE Healthcare, Little Chalfont, UK). Standards were prepared using purified placental human TR1 diluted in foetal bovine serum (Lonza, Wokingham, UK). Standard or sample (100 μl) was added with 100 μl of ^{125}I-TR1 tracer (10,000 dpm; 50 pg/tube) and primary antibody (100 μl). After overnight incubation at 4 °C, pre-precipitated second-antibody, donkey anti-rabbit reagent (100 μl) was added; and a further 1 h incubation at room temperature with shaking, 1.5 ml wash solution (0.05% Brij solution) was added to each tube, followed by centrifugation, 30 min at 1800*g* at 4 °C. The supernatant was decanted and the precipitate was washed with a further 1.5 ml of wash solution. ^{125}I radioactivity in the precipitate was counted in a multi-well g-radiation counter, and results were interpolated using the LKB 1224-RIACalc RIA evaluation program.

2.7. TR activity

TR activity was measured spectrophotometrically using insulin as a substrate (Holmgren & Bjornstedt, 1995). A stock reaction mixture was prepared by mixing 200 μl of 1.0 M, HEPES buffer (pH 7.6), 40 μl of NADPH (40 mg/ml), 40 μl of 0.2 M EDTA and 500 μl of insulin (10 mg/ml). To each test tube, 40 μl of the reaction mixture, 10 μl of 60 μM Trx, 40 μg protein sample, and water were added to a final volume of 120 μl. The samples were incubated at 37 °C for 20 min. The reaction was stopped by addition of 500 μl of 0.4 mg/ml DTNB/6 M guanidine hydrochloride in 0.2 M Tris–HCl (pH 8.0), and the absorbance at 412 nm was recorded. A sample of cell extract without addition of Trx was used as non-enzyme reaction control and the absorbance of the control was subtracted from that of the sample. TR activity was calculated from the standard curve prepared using pure TR (195 U/mg protein, Sigma) and results were expressed as U/mg protein.

2.8. Statistics

Data are presented as the mean ± SD. The differences between the groups were examined using one-way ANOVA test. A p value of less than 0.05 was considered statistically significant.

3. Results and discussion

The effects of broccoli sprout extract on TR1 expression were examined in human hepatocyes (HHL-5) cells over different times and doses of treatment. Induction of mRNA was observed at all doses and times tested. A significant induction of TR1 mRNA was achieved (2.2–2.5-fold) after 4 h treatment with BSE (8 μM) and maintained at this level at 24 h (Fig. 1a).

The induction of TR1 mRNA by BSE after 24 h treatment with ITCs of 1.6, 4 and 8 μM was 1.7–2.2-fold (Fig. 1b). Se (0.2–1 μM), had no synergistic effects on mRNA induction at lower levels of BSE treatment, but significantly augmented mRNA induction at 8 μM BSE exposure. Selenium addition alone did not induce TR1 mRNA, a finding consistent with previous observations on HepG2 and MCF-7 cells (Wang et al., 2005; Zhang et al., 2003). The housekeeping gene 18S showed no significant changes (<10%) under all treatments with either BSE or in combination with Se.

Selenium and sulforaphane have previously been shown to exert a synergistic effect on TR1 protein expression in human heptoma HepG2 cells (Zhang et al., 2003). The data presented here confirm that the induction observed in immortalised human hepatocytes was similar to that seen in tumor cell lines. Thus, Se alone at 0.2 μM or 1 μM induced TR1 protein significantly (1.2–1.25-fold) as did BSE when added alone at 4 and 8 μM (1.75-fold induction of TR1 protein). Co-addition of Se and BSE gave the greatest

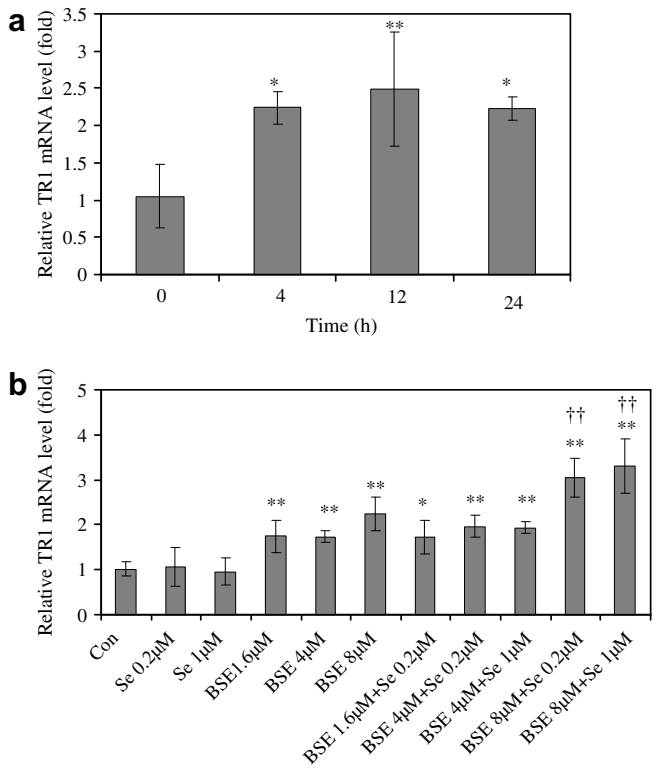

Fig. 1. Effect of BSE and/or Se on the expression of TR1 mRNA in HHL5 cells. (a) Time course of treatment with 8 μM BSE. (b) Dose and synergism response after 24 h BSE and/or Se treatment. Control cells were treated with DMSO (0.1%). Total RNA was isolated and TR1 mRNA was quantified as described in the Section 2. Data are presented as Mean ± SD of three replicate treatments. Data normalized with mean level of expression in control cells = 1. Statistical significance from the control, $^{*}p < 0.05$; $^{**}p < 0.01$. Statistical significance from Se or BSE treatment, †† $p < 0.01$.

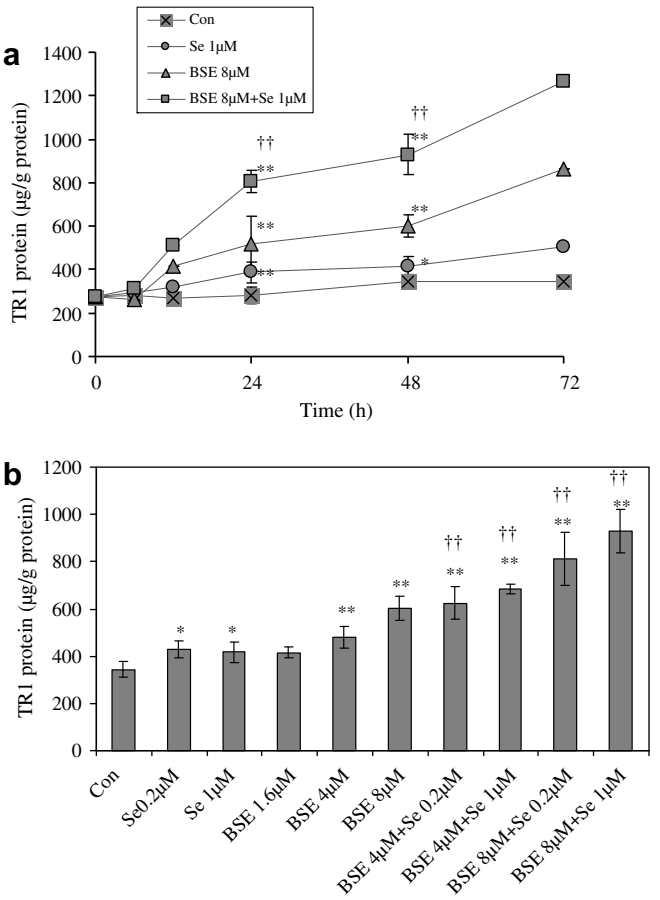

Fig. 2. Effect of BSE and/or Se on TR1 protein expression in HHL5 cells. (a) Time course of treatment with Se 1 μM and/or BSE 8 μM. Data are presented as Mean ± SD of three independent experiments for 24 and 48 h treatments; the remainders are from single dish studies and measured in duplicate. (b) Dose and synergism response after BSE and/or Se treatment (48 h). Control cells were treated with DMSO (0.1%). Protein was isolated and TR1 was determined as described in the Section 2. Data are presented as Mean ± SD (n = 3). Statistical significance from the control, $^{*}p < 0.05$; $^{**}p < 0.01$. Statistical significance from Se or BSE treatment, ††$p < 0.01$.

increase TR1 protein expression (up to 2.7-fold at 48 h, and 3.7-fold at 72 h) (Fig. 2a and b).

The effect of Se and BSE on inducing TR activity in these cells were consistent with the changes seen in TR1 protein. Thus the induction of TR activity by Se (0.2 μM, or 1 μM, 48 h treatment) was 1.6-fold; and the induction by BSE (4 and 8 μM) was 1.4–1.76 fold (the induction by 1.6 μM BSE was not significant). After co-treatment with Se and BSE (4 and 8 μM), TR activity was increased to 3- and 5-fold, respectively (Fig. 3b). Since SFN is a major ITC in BSE (70%), we treated HHL-5 cells with SFN (5 μM, 48 h), and found that SFN possessed similar activity as BSE with regard to the induction of TR1 protein and activity, i.e., SFN induced 1.7- and 1.9-fold. Co-treated with SFN and Se (1 μM) induced 2.4-fold of TR1 protein and 4.5-fold TR activity, respectively.

Epidemiological studies have suggested that both Se and cruciferous vegetables may play an important role in decreasing the risk of certain type of cancers (Keck & Finley, 2004; Whanger, 2004). Isothiocyanates appear to be dietary anticancer agents since they inhibit chemically-induced cancers in animal models, strongly induce detoxification enzymes (Talalay & Fahey, 2001), and regulate cell growth and apoptosis (Conaway, Yang, & Chung, 2002).

The interaction or synergism between dietary isothiocyanates and Se is an important area of research (Bao & Fenwick, 2004). A better understanding of the mechanism of interactions may form a basis for sound dietary advice on healthy eating and for prevention of chronic diseases using multiple dietary components. Se-enriched broccoli decreases intestinal tumorigenesis in multiple intestinal neoplasia mice (Davis, Zeng, & Finley, 2002), inhibits cell proliferation (Yeh et al., 2006,) and enhances the binding of transcriptional factors p53, NF-kB, AP-1 to their *cis*-acting elements (Zeng, Davis, & Finley, 2003). TR1 reduces thioredoxin which is then able to protect cells from oxidative stress. Redox control is a fundamental biological control mechanism and one of the major redox control systems is the thioredoxin system, comprised of Trx and TR1 (Lincoln et al., 2003.)

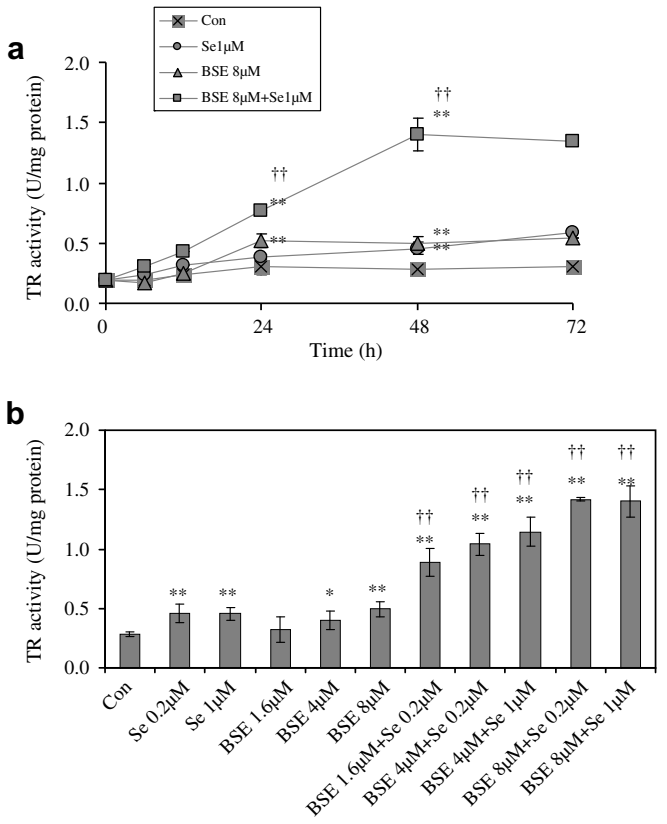

Fig. 3. Effect of BSE and/or Se on TR activity in HHL5 cells. (a) Time course of treatment with Se 1 μM and/or BSE 8 μM. Data are presented as Mean ± SD of three independent experiments for 24 and 48 h; the remainder are from single dish studies and measured in duplicate. (b) Dose-dependent and synergistic effect of BSE and/or Se (48 h). Control cells were treated with DMSO (0.1%). Protein was isolated and TR activity was determined as described in the Section 2. Data are presented as Mean ± SD of three independent experiments. Statistical significance from the control, $^{*}p < 0.05$; $^{**}p < 0.01$. Statistical significance from Se or BSE treatment, ††$p < 0.01$.

There is growing evidence that redox regulation by the TR system plays a crucial role in the biological response against oxidative stress (Tanaka et al., 2001), and in cell growth promotion and apoptosis (Soderberg, Sahaf, & Rosen, 2000). The level of total ITCs in human plasma is obviously dependent on the intake of cruciferous vegetables. In a human intervention study, a single oral dose of 200 μmol broccoli sprout isothiocyanates, allowed plasma ITC levels to reach 0.94–2.27 μM 1 h after feeding (Ye et al., 2002). In a subsequent study, plasma levels of SFN and its thiol conjugates reached 2.2 or 7.3 μM after 2 h in human volunteers given a soup prepared with 100 g of standard or high-glucosinolate broccoli florets (Gasper et al., 2005). The data presented here show TR1 can be up- regulated by BSE at relatively low concentrations (1.6–8 μM) in human hepatocytes. These concentrations can be achieved in human plasma by consumption of normal cruciferous vegetables or ITC-rich vegetables (Faulkner, Mithen, & Williamson, 1998). A recent screening study of sulforaphane levels in European *Brassica* sp. revealed that the highest level of sulforaphane was 2.21 mg/g dry weight in San Martino 3-day old seedlings of *B. oleracea* L. (Sivakumar, Aliboni, & Bacchetta, 2007).

The liver is a key organ in xenobiotic metabolism and plays an important role in detoxification of food born-mutagens. Previous work has demonstrated that SFN can protect against a heterocyclic amine PhIP-induced DNA adduct formation in human hepatocytes (Bacon et al., 2003). There are also reports that accumulation of Se in broccoli enhances the anticarcinogenic potential of broccoli in the chemical-induced rat aberrant crypt model (Davis et al., 2002; Keck & Finley, 2004). It is clear that ITCs from crucifers up-regulate selenoprotein TR1 transcription, and Se is essential for the activity of TR1. Co-treatment with ITCs and Se results in a synergistic induction of TR-1 expression integrated from both transcriptional and translational co-operations in the cells.

In summary, broccoli sprout extract is a potent inducer of TR1 and selenium can enhance the TR1 expression at mRNA, protein and activity levels. This study indicates that an ITC-rich diet may play an important role in modulation of antioxidant enzyme TR1 expression and play a role in maintaining the redox and homeostasis in cells. Another significant implication from these data is that the requirement for ITCs or Se in the induction of antioxidant enzyme TR could be much less when used in combination than used alone. It should also be recognized that both ITCs and Se are toxic at higher levels and may possess adverse effects. Induction of antioxidant enzymes such as TR1 in human hepatocytes suggests that ITC-rich vegetables and Se play a significant role in protection against oxidative stress and related degenerative diseases.

Acknowledgments

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References

Ambrosone, C. B., McCann, S. E., Freudenheim, J. L., Marshall, J. R., Zhang, Y., & Shields, P. G. (2004). Breast cancer risk in premenopausal women is inversely associated with consumption of broccoli, a source of isothiocyanates, but is not modified by GST genotype. *Journal of Nutrition, 134*, 1134–1138.

Bacon, J., Plumb, G., Howie, A. F., Beckett, G. J., Wang, W., & Bao, Y. P. (2007). The dual-action of sulforaphane in the regulation of thioredoxin reductase and thioredoxin in human HepG2 and Caco-2 cells. *Journal of Agricultural and Food Chemistry, 55*, 1170–1176.

Bacon, J., Williamson, G., Garner, C., Lappin, G., Langouet, S., & Bao, Y. P. (2003). Sulforaphane and quercetin modulate PhIP-DNA adduct formation in human Hep G2 and hepatocytes. *Carcinogenesis, 24*, 1903–1911.

Bao, Y. P., & Fenwick, R. (2004). Future perspectives in phytochemicals and health research. In Y. P. Bao & R. Fenwick (Eds.), *Phytochemicals in health and disease* (pp. 331–342). New York: Marcel Dekker Inc.

Bradford, M. M. (1976). A rapid and sensitive method for the quantitation of microgram quantities of protein, utilizing the principle of protein-dye binding. *Analytical Biochemistry, 72*, 248–254.

Campbell, L., Howie, F., Arthur, J., Nicol, G., & Beckett, G. (2007). Selenium and sulforaphane modify the expression of selenoenzymes in the human endothelial cell line EAhy926 and protect cells from oxidative damage. *Nutrition, 23*, 138–144.

Clayton, R. F., Rinaldi, A., Kandyba, E. E., Edward, M., Willberg, C., Klenerman, P., et al. (2005). Liver cell lines for the study of hepatocyte functions and immunological response. *Liver International, 25*(2), 389–402.

Conaway, C. C., Yang, Y. M., & Chung, F. L. (2002). Isothiocyanates as cancer chemopreventive agents: their biological activities and metabolism in rodents and humans. *Current Drug Metabolism, 3*, 233–255.

Davis, C. D., Zeng, H., & Finley, J. W. (2002). Selenium-enriched broccoli decreases intestinal tumorigenesis in multiple intestinal neoplasia mice. *Journal of Nutrition, 132*, 307–309.

Elliot, R., Bacon, J., & Bao, Y. P. (2004). Nutritional genomics. In Y. P. Bao & R. Fenwick (Eds.), *Phytochemicals in health and disease* (pp. 1–25). New York: Marcel Dekker Inc.

Fahey, J. W., Zhang, Y., & Talalay, P. (1997). Broccoli sprouts: an exceptionally rich source of inducers of enzymes that protect against chemical carcinogens. *Proceedings of the National Academy of Sciences of the United States of America, 94*, 10367–10372.

Faulkner, K., Mithen, R., & Williamson, G. (1998). Selective increase of the potential anticarcinogen 4-methylsulphinylbutyl glucosinolate in broccoli. *Carcinogenesis, 19*, 605–609.

Fenwick, G. R., Heaney, R. K., & Mullin, W. J. (1983). Glucosinolates and their breakdown products in food and food plants. *Critical Reviews in Food Science and Nutrition, 18*(2), 123–201.

Gasper, A. V., Al-Janobi, A., Smith, J. A., Bacon, J. R., Fortun, P., Atherton, C., et al. (2005). Glutathione *S*-transferase M1 polymorphism and metabolism of sulforaphane from standard and high-glucosinolate broccoli. *The American Journal of Clinical Nutrition, 82*(6), 1283–1291.

Holmgren, A., & Bjornstedt, M. (1995). Thioredoxin and thioredoxin reductase. *Methods in Enzymology, 252*, 199–208.

Keck, A. S., & Finley, J. W. (2004). Cruciferous vegetables: cancer protective mechanisms of glucosinolate hydrolysis products and selenium. *Integrative Cancer Therapies, 3*, 5–12.

Kensler, T. W., Chen, J. G., Egner, P. A., Fahey, J. W., Jacobson, L. P., Stephenson, K. K., et al. (2005). Effects of glucosinolate-rich broccoli sprouts on urinary levels of aflatoxin-DNA adducts and phenanthrene tetraols in a randomized clinical trial in He Zuo township, Qidong, People's Republic of China. *Cancer Epidemiology, Biomarkers & Prevention, 14*, 2605–2613.

Lincoln, D. T., Ali Emadi, E. M., Tonissen, K. F., & Clarke, F. M. (2003). The thioredoxin-thioredoxin reductase system: over-expression in human cancer. *Anticancer Research, 23*, 2425–2433.

Livak, K. J., & Schmittgen, T. D. (2001). Analysis of relative gene expression data using real-time quantitative PCR and the 2(−Delta Delta C(T)) method. *Methods, 25*(4), 402–408.

Miller, S., Walker, S. W., Arthur, J. R., Nicol, F., Pickard, K., Lewin, M. H., et al. (2001). Selenite protects human endothelial cells from oxidative damage and induces thioredoxin reductase. *Clinical Science, 100*, 543–550.

Rask, L., Andreasson, E., Ekbom, B., Eriksson, S., Pontoppidan, B., & Meijer, J. (2000). Myrosinase: gene family evolution and herbivore defence in Brassicaceae. *Plant Molecular Biology, 42*, 93–113.

Sandalova, T., Zhong, L., Lindqvist, Y., Holmgren, A., & Schneider, G. (2001). Three-dimensional structure of a mammalian thioredoxin reductase: implications for mechanism and evolution of a selenocysteine-dependent enzyme. *Proceedings of the National Academy of Sciences of the United States of America, 98*, 9533–9538.

Shan, Y., Sun, C., Zhao, X., Wu, K., Cassidy, A., & Bao, Y. P. (2006). Effect of sulforaphane on cell growth, G0/G1 phase cell progression and apoptosis in human bladder cancer T24 cells. *International Journal of Oncology, 29*, 883–888.

Sivakumar, G., Aliboni, A., & Bacchetta, L. (2007). HPLC screening of anti-cancer sulforaphane from important European Brassica Species. *Food Chemistry, 104*, 1761–1764.

Soderberg, A., Sahaf, B., & Rosen, A. (2000). Thioredoxin reductase, a redox-active selenoprotein, is secreted by normal and neoplastic cells: presence in human plasma. *Cancer Research, 60*, 2281–2289.

Tanaka, T., Nakamura, H., Nishiyama, A., Hosoi, F., Masutani, H., Wada, H., et al. (2001). Redox regulation by thioredoxin superfamily; protection against oxidative stress and aging. *Free Radical Research, 33*, 851–855.

Talalay, P., & Fahey, J. W. (2001). Phytochemicals from cruciferous plants protect against cancer by modulating carcinogen metabolism. *Journal of Nutrition, 131*(Suppl.), 3027S–3033S.

Tang, L., Zhang, Y., Jobson, H. E., Li, J., Stephenson, K. K., Wade, K. L., et al. (2006). Potent activation of mitochondria-mediated apoptosis and arrest in S and M phases of cancer cells by a broccoli sprout extract. *Molecular Cancer Therapeutics, 5*(4), 935–944.

Verhoeven, D. T., Goldbohm, R. A., van Poppel, G., Verhagen, H., & van den Brandt, P. A. (1996). Epidemiological studies on brassica vegetables and cancer risk. *Cancer Epidemiology, Biomarkers & Prevention, 5*, 733–748.

Wang, W., Wang, S. A., Howie, F., Beckett, G., Mithen, R., & Bao, Y. P. (2005). Sulforaphane, erucin and iberin up-regulate thioredoxin reductase expression in human MCF-7 cells. *Journal of Agricultural and Food Chemistry, 53*, 1417–1421.

Whanger, P. D. (2004). Selenium and its relationship to cancer: an update dagger. *The British Journal of Nutrition, 91*, 11–28.

Xia, L., Nordman, T., Olsson, M., Damdimopoulos, A., Bjorkhem-Bergman, L., Nalvarte, I., et al. (2003). The mammalian cytosolic selenoenzyme thioredoxin reductase reduces ubiquinone. A novel mechanism for defense against oxidative stress. *The Journal of Biological Chemistry, 278*, 2141–2146.

Ye, L., Dinkova-Kostova, A. T., Wade, K. L., Zhang, Y., Shapiro, T. A., & Talalay, P. (2002). Quantitative determination of dithiocarbamates in human plasma, serum, erythrocytes and urine: pharmacokinetics of broccoli sprout isothiocyanates in humans. *Clinica Chimica Acta, 316*, 43–53.

Yeh, J. Y., Ou, B. R., Liang, Y. C., Burchfiel, J., Butler, J. A., Forsberg, N. E., et al. (2006). Mechanism for proliferation inhibition by various selenium compounds and selenium-enriched broccoli extract in rat glial cells. *Biometals, 19*, 611–621.

Zeng, H., Davis, C. D., & Finley, J. W. (2003). Effect of selenium-enriched broccoli diet on differential gene expression in min mouse liver. *The Journal of Nutritional Biochemistry, 14*, 227–231.

Zhang, J. S., Svehlikova, V., Bao, Y. P., Howie, A. F., Beckett, G. J., & Williamson, G. (2003). Synergy between sulforaphane and selenium in the induction of thioredoxin reductase 1 requires both transcriptional and translational modulation. *Carcinogenesis, 24*, 497–503.

Zhang, Y., Munday, R., Jobson, H. E., Munday, C. M., Lister, C., Wilson, P., et al. (2006). Induction of GST and NQO1 in cultured bladder cells and in the urinary bladders of rats by an extract of broccoli (*Brassica oleracea* L. var. *italica*) sprouts. *Journal of Agricultural and Food Chemistry, 54*(25), 9370–9376.

Zhao, H., Lin, J., Grossman, H. B., Hernandez, L. M., Dinney, C. P., & Wu, X. (2007). Dietary isothiocyanates, GSTM1, GSTT1, NAT2 polymorphisms and bladder cancer risk. *International Journal of Cancer, 120*(10), 2208–2213.

Zhong, L., & Holmgren, A. (2002). Mammalian thioredoxin reductases as hydroperoxide reductases. *Methods Enzymology, 347*, 236–243.