JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY

Dual Action of Sulforaphane in the Regulation of Thioredoxin Reductase and Thioredoxin in Human HepG2 and Caco-2 Cells

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We have previously demonstrated that sulforaphane is a potent inducer for thioredoxin reductase in HepG2 and MCF-7 cells (Zhang et al. *Carcinogenesis* **2003**, *24*, 497−503; Wang et al. *J. Agric. Food Chem.* **2005**, *53*, 1417−1421). In this study, we have shown that sulforaphane is not only an inducer for thioredoxin reductase but also an inducer for its substrate, thioredoxin in HepG2, and undifferentiated Caco-2 cells. Sulforaphane acts at two levels in the regulation of thioredoxin reductase/thioredoxin system by the upregulation of the expression of both the enzyme and the substrate. In human hepatoma HepG2 cells, sulforaphane induced thioredoxin reductase mRNA and protein by 4- and 2-fold, respectively, whereas thioredoxin mRNA was induced 2.9-fold and thioredoxin protein was unchanged in whole cell extracts, but an increase in nuclear accumulation (1.8-fold) was observed. Moreover, the induction of thioredoxin reductase was found faster than that of thioredoxin. The effects of PI3K and MAPK kinase inhibitors, LY294002, PD98059, SP600125, and SB202190, have been investigated on the sulforaphane-induced expression of thioredoxin reductase and thioredoxin. PD98059 abrogates the sulforaphane-induced thioredoxin reductase at both mRNA and protein levels in HepG2 cells, although other inhibitors were found less effective. However, both PD98059 and LY294002 significantly decrease thioredoxin mRNA expression in HepG2 cells. None of the inhibitors tested were able to modulate the level of expression of either thioredoxin reductase mRNA or protein in Caco-2 cells suggesting that there are cell-specific responses to sulforaphane. In summary, the dietary isothiocyanate, sulforaphane, is important in the regulation of thioredoxin reductase/thioredoxin redox system in cells.

KEYWORDS: Isothiocyanate; sulforaphane; thioredoxin reductase; signaling; chemoprevention

INTRODUCTION

A higher consumption of cruciferous vegetables correlates with a decreased risk of many common cancers including lung, colon, and breast (*1*−*3*). Crucifers contain glucosinolates which can hydrolyze to produce isothiocyanates (ITCs) (*4*, *5*). Sulforaphane (SFN) is a well-studied ITC possessing bioactivities such as induction of phase II detoxification enzymes (*6*−*8*), inhibition of some isoforms of the phase I carcinogen activating enzymes (*9*, *10*), and induction of cell cycle arrest and apoptosis (*11*−*14*). In a human intervention study, following a single dose of 200 μmol broccoli sprout isothiocyanates, plasma ITC levels reached 0.94−2.27 μmol/L 1 h after feeding (*15*). In another study, plasma levels of SFN and its thiol conjugates reached more than 7 μmol/L in 2 h in human volunteers given a soup prepared with 100 g high-glucosinolate broccoli florets and more than 2 μmol/L in the same volunteers fed a similar soup prepared from a standard broccoli variety (*16*).

We have recently demonstrated that SFN can upregulate thioredoxin reductase 1 (TrxR1) expression in a dose-dependent manner in human hepatoma HepG2 and breast cancer MCF-7 cell lines (*17*, *18*). TrxR1 is a multifunctional selenoenzyme with a flavin adenine dinucleotide (FAD), a functional disulfide/dithiol, and a penultimate C-terminal selenocysteine residue (*19*, *20*). TrxR1 has broad substrate specificity, reducing many low molecular compounds such as hydrogen peroxide, lipid hydroperoxides, ascorbate and lipoic acid, and ubiquinone in addition to thioredoxin (Trx) (*21*). There is growing evidence that redox regulation by the TrxR system plays a crucial role in the biological response against oxidative stress (*22*) and in cell growth promotion and apoptosis (*23*).

Recent studies have indicated that both TrxR1/Trx have antioxidant responsive elements (AREs) in their promoter regions (*24*, *25*), therefore, they are potential targets for ITC modulation via Nrf2-ARE pathway. In this study, the effects of SFN in the upregulation of both TrxR1 and Trx expression have been examined, and signaling pathways that may be involved in the regulation of TrxR1/Trx have been dissected.

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10.1021/jf062398+ CCC: $37.00 © 2007 American Chemical Society
Published on Web 01/20/2007

MATERIALS AND METHODS

Materials. Human hepatoma HepG2 and human colon adenocarcinoma Caco-2 cell lines were obtained from European Collection of Cell Culture (Wiltshire, United Kingdom). Cell culture media and supplements were from Gibco, United Kingdom. Sulforaphane (4-methylsulfinylbutyl isothiocyanate, SFN, purity 97%) was purchased from LKT laboratories (St. Paul, MN). Signaling inhibitors PD98059, SP600125, SB202190, and LY294002 were purchased from Tocris Cookson (Bristol, United Kingdom). TaqMan primers and probes were purchased from Sigma-Genosys (Haverhill, United Kingdom). Equipment and reagents for electrophoresis and Western blotting supplies were obtained from Invitrogen (Paisley, United Kingdom) and GE Healthcare (Little Chalfont, United Kingdom), respectively. All other chemicals were purchased from Sigma (Poole, United Kingdom).

Cell Culture and Treatment. Cells were cultured in Eagle's minimum essential medium with fetal bovine serum (10%), penicillin (100 U/mL), and streptomycin (100 μg/mL) under 5% CO_2 in air at 37 °C. Cells were seeded in six-well plates at 2×10^4 cells/cm^2 and then were treated when confluence reached 70% (typically after 4 days). All compounds were added in dimethyl sulfoxide (DMSO) as carrier to a maximum final concentration of 0.1%. For time course experiments, treatments were made at appropriate intervals so that all treatments could be harvested at the same time. When signaling pathway inhibitors were used, they were added to cell culture media 1 h before subsequent coincubation with SFN for 24 or 48 h. Control cells were treated with equivalent concentrations of DMSO alone.

RNA Isolation. Total RNA was isolated from cells using the Genelute mammalian total RNA miniprep kit (Sigma) according to the manufacturer's instructions. Concentration and purity were determined by absorbance measurement at 260 and 280 nm. RNase inhibitor (20U) was added to each preparation before storage at −70 °C.

TrxR1 and Trx mRNA Quantification. TrxR1 and Trx mRNA were determined by real-time RT−PCR (TaqMan) using AB 7500 real-time PCR system. Forward and reverse primers and the fluorogenic TaqMan probes were designed using the ABI PRISM Primer Express Software (Applied Biosystems). Primer and probe sequences for the assays performed were as follows: TrxR1 forward primer, 5′-CCACTGGTGAAAGACCACGTT-3′; reverse primer, 5′-AGGAGA-AAAGATCATCACTGCTGAT-3′; probe, 5′-CAGTATTCTTTGT-CACCAGGGATGCCCA-3′; Trx forward primer, 5′-TGGTGTGGGC-CTTGCAA-3′; reverse primer, 5′-TTCAAGGAATATCACGTTG-GAATACTT-3′; probe, 5′-ATGATCAAGCCTTTCTTTCATTCCCTCT-CTGA-3′.

Probes were labeled with a 5′ reporter dye, FAM (6-carboxyfluorescein), and a 3′ quencher dye, TAMRA (6-carboxytetramethylthodamine). Reactions were carried out in a 96-well plate in a total volume of 25 μL/well consisting of TaqMan one-step RT−PCR reagents (Applied Biosystems, Warrington, United Kingdom), 100 nmol/L probe, 200 nmol/L forward primers, and 300 nmol/L (TrxR1) or 200 nmol/L (Trx) reverse primers and 10 ng total RNA. Reverse transcription was performed for 30 min at 48 °C, and then Amplitaq Gold activation was performed for 10 min at 95 °C, followed by 40 cycles PCR of denaturation for 15 s at 95 °C and annealing/extension for 1 min at 60 °C. Data were analyzed with Applied Biosystems Absolute Quantification software using a standard curve generated by a 2-fold serial dilution of total RNA from untreated cells. Data were normalized against an invariant endogenous control, 18S ribosomal RNA. 18S rRNA forward primer, 5′-GGCTCATTAAATCAGTTATG-GTTCCT-3′; reverse primer, 5′-GTATTAGCTCTAGAATTACCA-CAGTTATCCA-3′; probe, 5′-TGGTCGCTCGCTCCTCTCCCAC-3′.

Preparation of Cell Extracts. Adherent cells were washed three times with ice-cold PBS and then were lysed in situ on ice with 250 μL RIPA buffer (50 mmol/L Tris-HCl, pH 7.4, 150 mmol/L NaCl, 1% Triton X-100, 0.5% Na deoxycholate, 0.2% SDS, 1 mmol/L Na_3VO_4, 1 mmol/L NaF, complete miniprotease inhibitor cocktail tablet (Roche Diagnostics, Lewes, United Kingdom)) for 2 min. Lysates were transferred to an eppendorf and were incubated on ice with vortexing for a further 15 min and then were centrifuged at 13 000*g*, 4 °C for 10 min. Supernatants were collected and frozen at −70 °C. Protein concentration was determined (Bradford dye-binding assay) using bovine serum albumin as standard (*26*).

TrxR1 Protein (RIA Assay). TrxR1 protein levels were measured by an in-house RIA assay as described previously (*27*). Briefly, the tracer, ^{125}I-labeled human placental TrxR1, was prepared using Bolton-Hunter reagent (GE Healthcare). Standards were prepared using purified placental human TrxR1 diluted in fetal bovine serum (Gibco). Standards or cell extracts (100 μL) were added to 100 μL of ^{125}I-TrxR1 tracer (10 000 dpm; 50 pg/tube) and primary antibody (100 μL). After an overnight incubation at 4 °C, pre-precipitated second-antibody, donkey antirabbit reagent (100 μL) was added. After a further 1 h at room temperature with shaking, wash solution (0.05% Brij solution) was added to each tube, followed by centrifugation for 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. The ^{125}I radioactivity in the precipitate was counted in a multiwell γ-radiation counter, and results were interpolated using the LKB 1224-RIACalc RIA evaluation program.

Preparation of Nuclear Extracts. Nuclear extracts of HepG2 cells were prepared according to the method of Liu et al. (*28*). Briefly, adherent cells were washed twice with ice-cold PBS, were harvested by scraping, and were transferred in PBS into an eppendorf tube. The cells were pelleted and resuspended in 250 μL ice-cold buffer A (10 mmol/L HEPES pH 7.6, 0.1 mmol/L EDTA, 10 mmol/L KCl, 50 mmol/L NaF, 50 mmol/L β-glycerophosphate, 5% glycerol, 1 mmol/L DTT, complete miniprotease inhibitor cocktail tablet) and were incubated on ice for 15 min. A 1:20 volume of 10% nonidet p-40 was added and vortexed for 30 s followed by centrifugation for 30 s at 13 000*g*. The supernatant (cytosolic protein fraction) was removed completely and the pellet was washed once with a small volume of buffer A. The pellet was resuspended in 250 μL of ice-cold buffer B (20 mmol/L HEPES pH 7.6, 50 mmol/L KCl, 300 mmol/L NaCl, 0.1 mmol/L EDTA, 10% glycerol, 1 mmol/L DTT, 0.1 mmol/L PMSF, complete miniprotease inhibitor cocktail tablet) and was incubated on ice for 30 min with vortexing followed by centrifugation at 13 000*g*, 4 °C for 10 min. The supernatants were collected as nuclear protein fractions and were frozen at −70 °C. Protein concentration was determined (Bradford dye-binding assay) using bovine serum albumin as standard (*26*).

Trx Protein (Western Blotting). Equivalent aliquots of nuclear protein were mixed with ×4 SDS-PAGE sample buffer and DTT reducing agent (to 50 mmol/L) and were heated to 70 °C for 10 min. A volume of each reduced protein extract equivalent to 2 μg protein was then subjected to 10% SDS-polyacrylamide (NuPage Bis-Tris) gel electrophoresis in MES SDS running buffer. The resolved proteins were transferred to nitrocellulose membrane (Bio-Rad) using a semidry transfer system (XCell II Blot module). The membranes were blocked with 5% nonfat dry milk in TBST (Tris-buffered saline, pH 7.4, containing 0.1% Tween-20) for 1 h at room temperature, followed by incubation with 0.4 μg IgG/mL primary polyclonal antibodies (Santa Cruz, sc-20146) in TBST for 1 h. After washing three times with TBST, the membranes were incubated with 1:1000 antirabbit IgG peroxidase conjugate (Sigma) for 1 h. Then, the membranes were washed three times with TBST and the protein of interest was visualized with enhanced chemiluminescent (ECL) system. The membranes were than exposed to Kodak film for various times.

Statistical Analysis. Data are presented as the mean ± standard deviation (SD). The differences between the groups were examined using Student's *t*-test. A p value less than 0.05 was considered statistically significant.

RESULTS

Modulation of TrxR1 Expression by SFN. TrxR1 mRNA levels were induced by physiologically relevant doses of SFN in both HepG2 and Caco-2 cells. HepG2 cells were more sensitive to treatment with 10 μmol/L SFN. TrxR1 mRNA was induced 2.5-fold in 4-h treatment and was increased to 4-fold at 8 h and remained at that enhanced level until at least 48 h (**Figure 1A**). The induction of TrxR1 protein expression

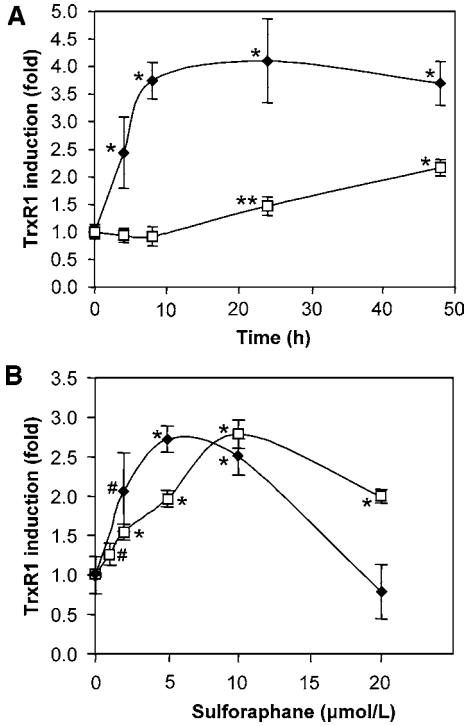

Figure 1. Effect of SFN treatment on the expression of TrxR1 mRNA (◆) and protein (□) in HepG2 cells. (**A**) Time course of treatment with 10 μmol/L SFN. (**B**) SFN dose response of mRNA (24 h) and protein (48 h). Mean ± SD of three replicate treatments of cells. Data normalized with mean level of expression in control cells = 1. Statistical significance from the control, *$p < 0.001$; **$p < 0.02$; #$p < 0.05$.

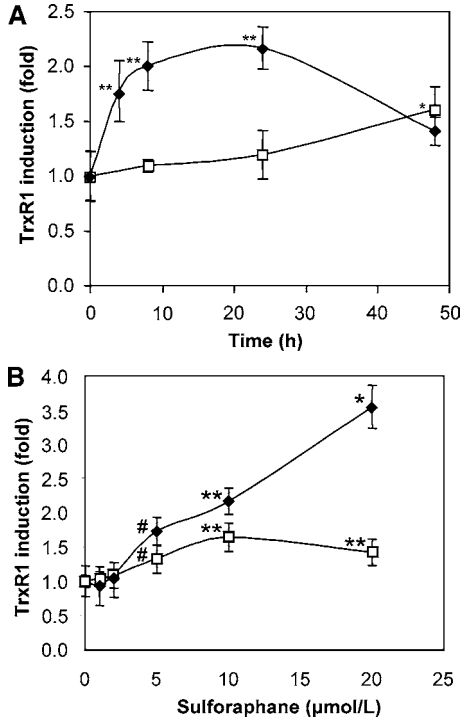

Figure 2. Effect of SFN treatment on the expression of TrxR1 mRNA (◆) and protein (□) in Caco-2 cells. (**A**) Time course of treatment with 10 μmol/L SFN. (**B**) SFN dose response of mRNA (24-h treatment) and protein (48-h treatment). Mean ± SD of three replicate treatments of cells. Data normalized with mean level of expression in control cells = 1. Statistical significance from the control, *$p < 0.002$; **$p < 0.02$; #$p < 0.05$.

followed a similar profile to mRNA but with a delay in terms of time required to produce an effect. Increases in TrxR1 protein were not observed until after 24-h treatment with 10 μmol/L SFN and continued to increase until at least 48 h (2.2-fold) (**Figure 1A**). In contrast, TrxR1 mRNA levels in Caco-2 cells only increased 2-fold under the same conditions and declined back to the control level at times over 24 h (**Figure 2A**). A significant increase in TrxR1 protein level (1.5-fold) was only observed in Caco-2 cells after 48-h treatment.

HepG2 cells were also more sensitive to lower doses of SFN with a 2-fold induction of mRNA observed at 2 μmol/L (**Figure 1B**), in contrast, 5 μmol/L was required to elicit a significant response in Caco-2 cells (**Figure 2B**). However, at higher doses, the level of TrxR1 mRNA expression in Caco-2 cells continued to rise (3.5-fold at 20 μmol/L SFN treatment) whereas the maximum induction in HepG2 cells was observed between 5 and 10 μmol/L but falling to below the control level at 20 μmol/L. This may be assigned to a cytotoxic effect. As observed with mRNA levels in HepG2 cells, a significant change in protein expression (1.5-fold) was observed following 2 μmol/L SFN treatment (in this case for 48 h) and this increase was maximal at 10 μmol/L and then decreased at higher concentrations (20 μmol/L) (**Figure 1B**). Caco-2 cells were again less sensitive to SFN with the maximum induction of TrxR1 protein observed of 1.5-fold following SFN treatment (10 μmol/L, 48 h) (**Figure 2A**). In contrast to mRNA expression, higher doses of SFN (20 μmol/L) did not induce TrxR1 protein levels further in Caco-2 cells (**Figure 2B**).

Modulation of Trx Expression by Sulforaphane. The pattern of induction of Trx mRNA by SFN in HepG2 cells closely followed the pattern of induction observed for TrxR1 mRNA although the responses were slower than that of TrxR induction. Following 10 μmol/L SFN treatment, the level rose to 2.1-fold at 8 h and was maintained with a 2.9-fold increase observed at 48 h (**Figure 3A**). A maximum response at 24-h treatment was observed between 5 and 10 μmol/L with levels again falling at higher concentrations (20 μmol/L) (**Figure 3B**). In Caco-2 cells, increases were observed in Trx mRNA with most SFN treatments but these were relatively small (not greater than a 1.5-fold increase) and in most cases cannot be regarded as significantly different to the untreated control.

In HepG2 cells, Trx protein expression in total cell extracts was unchanged as measured by Western blotting (data not shown), however, Trx protein levels in the nuclear fraction was significantly increased (see below).

Modulation of Induction TrxR1 mRNA and Protein by Signaling Pathway Inhibitors. Both HepG2 and Caco-2 cells were treated with signaling pathway inhibitors (PD98059 (MEK1 inhibitor), LY294002 (PI3K inhibitor), SB202190 (p38 inhibitor), and SP600125 (JNK inhibitor)) for 1 h followed by cotreatment with 10 μmol/L SFN. mRNA and protein were isolated at 24 and 48 h, respectively. Inhibitor concentrations were selected according to their reported optimal concentrations for maximal inhibition (i.e., a concentration ≥ IC_{50} values). All the inhibitors tested with the exception of SB202190 reduced both the basal levels of TrxR1 mRNA and reduced the induction by SFN (**Figure 4**). PD98059 was particularly effective, reducing basal expression to 56% of the control level and reducing the effect of SFN alone (3.4-fold induction) back to the control level (1.1-fold). The effects of signaling pathway inhibitors on TrxR1 protein levels in HepG2 cells, in general, mirrored the response seen in mRNA levels. PD98059 was again the most effective, reducing SFN induction from 2.8-fold to 1.1-fold with respect to the untreated control. None of the inhibitors added were able to reduce the protein level significantly below the basal level. In contrast, none of the inhibitors

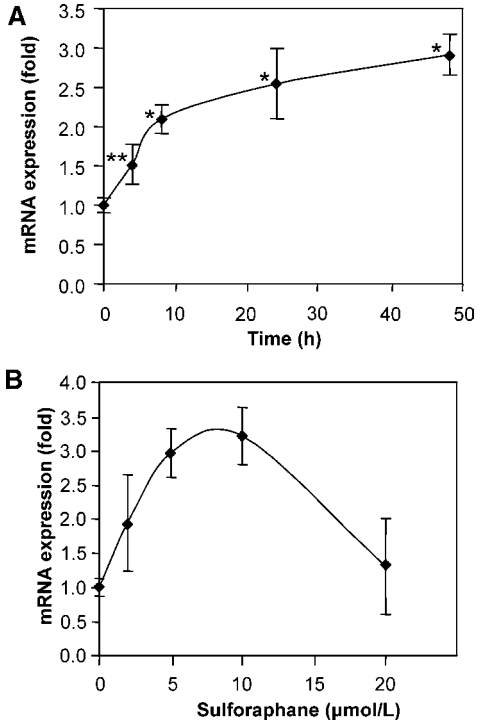

Figure 3. Effect of SFN treatment on the expression of Trx mRNA in HepG2 cells. (**A**) Time course of treatment with 10 μmol/L SFN. (**B**) Dose response after 24-h SFN treatment. Mean ± SD of three replicate treatments of cells. Data normalized with mean level of expression in control cells = 1. Statistical significance from the control, *$p < 0.001$; **$p < 0.05$.

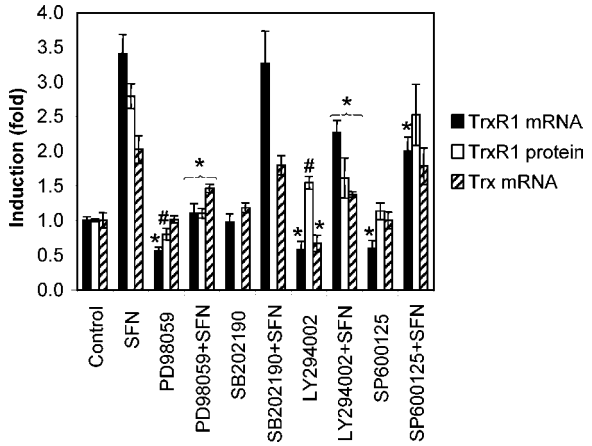

Figure 4. Effect of cotreatments of HepG2 cells with 10 μmol/L sulforaphane and signaling pathway inhibitors (20 μmol/L PD98059, 1 μmol/L SB202190, 10 μmol/L LY294002, 1 μmol/L SP600125) on TrxR1 and Trx mRNA expression (24 h) and TrxR1 protein expression (48 h). Mean ± SD of three replicate treatments of cells. Data normalized with mean level of expression in untreated (DMSO only) control cells = 1. Statistical significance from either the untreated control or 10 μmol/L SFN treated. *$p < 0.005$, #$p < 0.05$.

tested were able to modulate the level of expression of either TrxR1 mRNA or protein in Caco-2 cells, either in SFN treated or untreated cells (**Figure 5**).

Modulation of Induction Trx mRNA by Signaling Pathway Inhibitors. None of the inhibitors had any effect on the basal levels of Trx mRNA in HepG2 cells. However, PD98059 and LY294002 generated small but significant decreases (1.46- and 1.37-fold, respectively) in the level of induction by 10 μmol/L SFN (2.02-fold) (**Figure 4**). The effect of kinase inhibitors in Caco-2 cells was not measured as the induction of Trx mRNA by SFN is less than 2-fold.

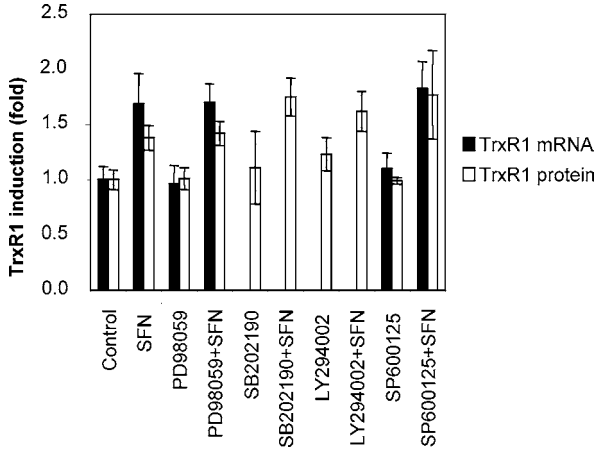

Figure 5. Effect of cotreatments of Caco-2 cells with 10 μmol/L sulforaphane and signaling pathway inhibitors (20 μmol/L PD98059, 1 μmol/L SB202190, 10 μmol/L LY294002, 1 μmol/L SP600125) on TrxR1 mRNA expression (24 h) and TR1 protein expression (48 h). Mean ± SD of three replicate treatments of cells. Data normalized with mean level of expression in untreated (DMSO only) control cells = 1. Signaling inhibitor treatments show no significant differences from the untreated control or 10 μmol/L SFN treated.

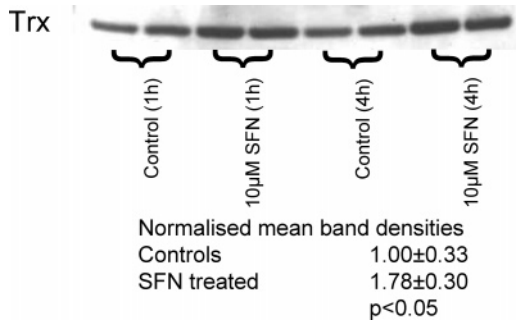

Figure 6. Effect of sulforaphane on nuclear translocation of Trx. HepG2 cells were treated with 10 μmol/L SFN for 1 or 4 h. Preparation of nuclear extracts and Western blotting were performed as described under Materials and Methods. All tracks are replicate treatments.

Nuclear Translocation of Trx by Sulforaphane. Short-term treatments (1 and 4 h) of HepG2 cells with SFN induced Trx translocation into the nuclei (1.8-fold) (**Figure 6**). No significant change was identified in Trx levels either in total cell extract or in the cytosolic fraction. This was consistent with our previous report that demonstrated a similar effect in Caco-2 cells (*29*).

DISCUSSION

Thioredoxin reductase (TrxR) in conjunction with thioredoxin (Trx) is a ubiquitous intracellular oxidoreductase system with antioxidant and redox regulatory roles. The properties of TrxR in combination with the functions of Trx position this system at the core of cellular thiol redox control and antioxidant defense.

Trx is a 12 kDa ubiquitous protein with a redox-active dithiol/disulfide at a highly conserved active site (-Cys-Gly-Pro-Cys-) in both prokaryotic and eukaryotic genomes. Trx catalyzes a protein disulfide reduction in combination with TrxR and nicotinamide adenine dinucleotide phosphate (NADPH) and is thought to be a strong scavenger of reactive oxygen species (ROS) (*30*). Trx can inhibit viral replication and is protective

in animal models of myocarditis (*31*). Overexpression of Trx in transgenic mice showed a protective function against postischemic reperfusion injury in brain in vivo (*32*). Recently, exogenous Trx has been shown to suppress airway hyperresponsiveness and airway inflammation in asthma (*33*). However, in some human tumors, the TrxR/Trx system is overexpressed and such tumors have a high proliferation capacity, a low apoptosis rate, and an elevated metastatic potential strongly implicating the involvement of the Trx system in the processes of tumorogenesis (*34*, *35*).

Trx stimulates the growth of normal and cancerous cells, and the mechanisms are multifaceted and include the provision of reducing equivalents for DNA synthesis, activation transcriptional factors that regulate cell growth, increased cell sensitivity to cytokines, and growth factors (*35*, *36*). The antiapoptotic effects of Trx may be related to its ability to bind to apoptosis-signal-regulating kinase 1 (ASK1). Because of its role in stimulating cancer cell growth and as an inhibitor of apoptosis, the TrxR/Trx system offers a target for the development of drugs to treat and prevent cancer (*37*−*39*).

Dietary ITCs are potent inducers for Nrf2-ARE-mediated upregulation of phase II carcinogen-detoxifying enzymes, including quinone reductase (QR), glutathione S-transferase (GST), UDP-glucuronosyl transferase (UGT), aldo-keto reductase (AKR), and γ-glutamylcystein synthetase (GCS) (*40*−*44*). However, there are reports that ITCs also induce phase I enzymes such as cytochrome P450 (CYP)1A1/2, CYP3A1/2, and CYP2E1 (*45*). Other adverse effects such as inductions of ROS, DNA, and mitochondrial damage have also been discussed recently (*44*, *47*).

There is a remarkable difference in the responses of HepG2 and undifferentiated Caco-2 cells to SFN treatment with regard to expression of TrxR1 mRNA and protein and their abrogation by signaling pathway inhibitors. This is in agreement with Zhang's report that ITCs can have tissue/cell type specific effects (*48*). In HepG2 cells, the induction of TrxR1 and Trx expression are mainly mediated via the ERK pathway since PD98059 decreased the induction of TrxR and Trx by SFN, whereas the mechanisms behind the lack of response of Caco-2 cells to PD98059 remain to be investigated.

In the light of knowledge that ITCs are potent inducers for TrxR (*17*, *18*, *49*, *50*) and Trx as shown in this study, we can clearly put forward the supposition that SFN has a double-edged effect in cells. Whether ITCs have beneficial or detrimental effects may not only depend on the dose of exposure but may also depend on the physiological conditions.

TrxR can be either a friend or a foe to humans (*51*). We fully support the view that more research is needed to establish a strategy to determine when to induce and augment TrxR/Trx and its practical application especially in human clinical trials. TrxR and Trx are antioxidants and ITCs can act as TrxR/Trx inducers, and thus we believe that ITCs are beneficial in protecting against oxidative stress and they may play a role in cancer prevention. On the other hand, ITCs, as activators for TrxR and Trx, may ultimately promote tumor growth, effectively outweighing any beneficial antioxidant properties. Recently, the potential role of TrxR in drug resistance has been reviewed and it has been suggested that inhibition of TrxR may contribute to a successful single, combinatory, or adjuvant cancer therapy (*52*). Therefore, before more in vivo study data becomes available, promotion of the consumption of high doses of ITCs should be approached with caution. Early diagnosed tumor patients should seek medical treatments rather than eat ITC-enriched cruciferous vegetables since ITCs at certain doses may promote the growth of tumor cells through the upregulation of TrxR and Trx expression. However, a relatively high dose might be beneficial if apoptosis of cancerous cells can be induced without harm to normal cells. Further studies are required to define the most beneficial dietary intake levels of ITCs for the right people and at the right time.

ABBREVIATIONS USED

ITCs, isothiocyanates; SFN, sulforaphane; Nrf2, nuclear factor E2-related factor 2; Trx, thioredoxin; TrxR1, thioredoxin reductase 1; ARE, antioxidant responsive element; DMSO, dimethyl sulfoxide; DTT, dithiothreitol; PMSF, phenylmethanesulfonyl fluoride; PBS, phosphate-buffered saline.

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Received for review August 21, 2006. Revised manuscript received December 12, 2006. Accepted December 15, 2006. This work was supported by a core strategic grant from the Biotechnology and Biological Sciences Research Council, U.K.

JF062398+