Transcription Regulation of Rat Glutathione S-Transferase Ya Subunit Gene Expression by Chemopreventive Agents[1]

Peiwen Fei,[2] George A. Matwyshyn,[3] Thomas H. Rushmore,[4] and Ah-Ng Tony Kong[2,3,5]

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Purpose. To study the transcription regulation of rat glutathione S-transferase Ya (rGSTya) subunit gene expression by chemopreventive agents.

Methods. The effects of chemopreventive agents; tamoxifen, genistein, oltipraz, indole-3-carbinol, and various isothiocyanates—sulforaphane, PMITC, PEITC, PBITC, and PPITC, on the transcriptional activation of rGSTya were investigated in cell culture. These were accomplished with a stable human hepatoma Hep G2 cell line transfected with a 1.6 kilobase (kb) 5′-flanking region of the rGSTya fused with the chloramphenicol acetyltransferase (CAT) reporter gene. Concentration-effect relationship and the kinetics of gene activation following treatments of the cells with different chemopreventive agents were carried out by quantitating CAT reporter protein using ELISA. Northern blot analysis of total RNA on the expression of CAT mRNA as well as potential transcription factors such as c-Jun, c-Fos, and LFR-1 were performed.

Results. Treatment of the cells with increasing concentrations of different chemopreventive agents resulted in corresponding increases in the gene expression of CAT reporter protein. Kinetically, induction of CAT protein was seen as early as 3 hr and peaked at about 20 hr. Northern blot analysis revealed an increase in CAT mRNA transcripts and these mRNA inductions in general were in agreement with those quantitated by the production of CAT reporter protein. Induction of the transcription factor, c-Jun mRNA was observed with sulforaphane.

Conclusions. These results show that different chemopreventive agents transcriptionally activate rGSTya CAT in a time and dose-dependent fashion.

KEY WORDS: glutathione S-transferase; GST; GSTya; gene regulation; drug metabolism; Hep G2, antioxidants; chemopreventive agents.

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[2] Division of Clinical Pharmacology, Thomas Jefferson University, Philadelphia, Pennsylvania.

[3] Center for Pharmaceutical Biotechnology, Department of Pharmaceutics and Pharmacodynamics, College of Pharmacy, University of Illinois, Chicago, Illinois.

[4] Department of Drug Metabolism, Merck Research Laboratories, West Point, Pennsylvania.

[5] To whom correspondence should be addressed.

ABBREVIATIONS: $rGSTy_a$, rat glutathione S-transferase Ya subunit; BHA, 3-(2)-*tert*-butyl-4-hydroxyanisole; tBHQ, 3-(2)-*tert*-butyl-4-hydroxyquinone; TAM, tamoxifen; I3C, indole-3-carbinol; SUL, sulforaphane; OLT, oltipraz, GEN, genistein; PMITC, benzyl isothiocyanate; PEITC, phenethyl isothiocyanate; PBITC, phenyl butyl isothiocyanate; PPITC, phenyl propyl isothiocyanate; ARE, antioxidant responsive element; XRE, xenobiotic responsive element; GRE, glucocorticoid responsive element; AP-1, activator protein-1; NF-κB, nuclear factor-kappa B.

INTRODUCTION

The glutathione *S*-transferases (GST) (EC 2.5.1.18) are a super family of enzymes that play a major role in the detoxification and metabolism of a variety of drugs and xenobiotics (1,2). The GSTs catalyze the conjugation of glutathione (GSH) to various electrophiles, and excreted in the urine or bile *via* the mercapturic pathway (2). In addition, they bind with high affinity to a variety of hydrophobic compounds, including heme, bilirubin, polycyclic hydrocarbons, and glucocorticoids and may also act as transport proteins (2). Based on the nucleotide and amino acid sequence similarities, mammalian cytosolic GSTs can be classified into four subfamilies designated Alpha, Mu, Pi and Theta, which form homodimers or heterodimers and are composed of at least 13 subunits (2). In rat liver, there are at least 7 GST isozymes that are constitutively expressed at relatively high and inducible levels (2). The $rGSTy_a$ subunit gene had been demonstrated to be transcriptionally elevated in rat liver by a variety of inducing agents including phenobarbital (PB), 3-methylcholantrene (3MC), and the antioxidant, 3-(2)-*tert*-butyl-4-hydroxyanisole (BHA) (2). Induction of GST activity by BHA antioxidant had been implicated in the protection of animals from potent carcinogens induced tumors (3). From drug metabolism perspective, regulation of gene expression of drug metabolizing enzymes including GST may play a role in the biotransformation of some pharmaceutical compounds as well as in toxicology of many environmental pollutants and carcinogens.

Many naturally occurring compounds found in vegetables and fruits can act as chemopreventive agents in experimental models of cancer in animals. These chemicals are postulated to prevent the development or retard the progression of carcinogenesis either by preventing carcinogens from reaching or reacting with cellular targets [a process termed initiation]; or by suppressing the expression of neoplasia in cells exposed to carcinogens [a process termed promotion] (3). The former process has been attributed to involve the induction of phase 2 detoxifying enzymes such as GSTs, quinone reductases, epoxide hydrolases, and UDP-glucuronosyltransferases (4). To date, more than 500 chemicals have been shown to have potential cancer chemopreventive action (5). However, the molecular events that lead to phase 2 enzymes gene activation in response to chemopreventive agents remain unclear. Our main objective in this study is to evaluate the transcription regulation of gene expression by several potential chemopreventive agents, such as, oltipraz, tamoxifen, genistein, sulforaphane, as well as a series of phenyl isothiocyanates with different alkyl (carbon) chain lengths. The model that we have chosen involves a 1.6 kilobase (kb) 5′-flanking region of the rat GSTya subunit gene fused to a reporter chloramphenicol acetyltransferase (CAT) [rGSTya-1.6-CAT] stably expressed in a human hepatoma Hep G2 cell line (2). The *cis*-acting regulatory elements of this region have been identified and characterized to contain at least 3 drug inducible elements; (1) GRE—glucocorticoid-responsive element; (2) XRE—xenobiotic-responsive element or AhRE—aromatic hydrocarbon responsive element; and (3) ARE—antioxidant responsive element or EpRE—electrophile responsive element (2). By evaluating the transcriptional activation of rGSTya gene expression in a cell line, insights would be gained as to the potential biological mechanisms in the

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regulation of phase 2 enzymes gene expression by these diverse class of potentially beneficial cancer protecting agents.

EXPERIMENTAL

Materials

Tamoxifen (TAM), indole-3-carbinol (I3C) and genistein (GEN) were purchased from Sigma Chemical Co. (St. Louis, MO). Benzyl isothiocyanate (PMITC) and phenethyl isothiocyanate (PEITC) were purchased from Aldrich Chemical Co. (Milwaukee, WI). Sulforaphane and phenyl butyl isothiocyanate (PBITC) were purchased from LKT Laboratories (St. Paul, MN), and phenyl propyl isothiocyanate (PPITC) was a gift of Dr. Fung-Lung Chung (American Health Foundation, Valahalla, NY). Oltipraz was kindly provided by Drs. Vernon E. Steele and Ronald A. Lubet (National Cancer Institute, NIH). A hepatoma cell line (HepG2) stably expressed the cDNA plasmid *rGSTya-1.6-CAT,* was grown in Dulbecco's modified Eagle's medium (DMEM) with 10% fetal calf serum as described previously (6,7).

Induction

Sixteen to twenty hr after splitting the cells in 6-well plates, the following dose-response and kinetics experiments were performed. Dose-response or concentration-effect studies were carried out by incubation of cells for 22 hr with different concentrations of the inducing agents, control (DMSO vehicle alone <0.1% v/v—a concentration which did not affect the CAT gene expression), TAM (1–15 μM); OLT (10–75 μM); SUL (0.5–20 μM); I3C (25–500 μM); GEN (50–200 μM); PMITC (1–8 μM); PEITC (0.25–15 μM); PBITC (1–15 μM); and PPITC (2–15 μM). Kinetic studies were performed by incubation of cells at O, 3, 6, 10, 12, 16 and 20 hr with 20 μM of SUL. The concentration was chosen to represent near maximum induction of CAT protein as shown in the dose-response studies. All experiments were performed in triplicate unless indicated otherwise.

Collection of Cell Lysates and CAT Assays

After treatments, the cells were washed with ice-cold phosphate buffered saline pH 7.4 (PBS) and were lysed with buffers that were provided in the CAT ELISA Kit (Boehringer Mannbeim Biochemica, Indianapolis, IN). Cellular CAT protein were quantitated with ELISA according to protocols provided by the manufacturer. Measurement of CAT protein by ELISA was identical to that performed with ^{14}C-CAT assay (unpublished observation), however, the ELISA assay had the advantage of not using the long half-life ^{14}C radioactive substances. Protein concentrations were determined by the BCA method (Pierce Chemical Co., Rockford, IL) with bovine serum albumin as the standard.

Isolation of Total RNA and 'Northern Blot' Analysis

Total RNA from the cells was isolated by the guanidine-thiocyanate method as described previously (6) using the RNA-gents Total RNA Isolation Kit (Promega, Madison, WI). The RNA was subjected to electrophoresis in denaturing formaldehyde/1% agarose gel and transferred onto GeneScreen nylon membranes (NEN-Dupont, Boston, MA). These membranes were prehybridized, then hybridized with a [^{32}P]-labelled *rGSTya-1.6-CAT* (7), *c-Jun, c-Fos, LFR-1* and human β-actin (ATCC). *c-Jun* and *c-Fos* cDNAs were provided by Drs. T.K. Kerppola and T. Curran (Roche Institute of Molecular Biology, Nutley, NJ). *LRF-1* cDNA was provided by Dr. Rebecca A. Taub (Howard Hughes Medical Institute, University of Pennsylvania, Philadelphia, PA). Filters were washed and autoradiographed at −70°C. The Northern blotting hybridization and washing conditions were described previously (6). The density of the bands on the Northern blots were quantitated by Bioimagine Whole Band Equalizer (Milipore, Bedford, MA).

RESULTS

Effect of Different Concentrations of Chemopreventive Agents on the Regulation of rGSTy$_a$

To assess the effects of chemopreventive agents on the activation of the 1.6-kb of the 5′ flanking region of rGSTy$_a$ in mediating the induction of CAT reporter gene expression, a human hepatoma HepG2 cell line transfected with the rGSTya-1.6-CAT plasmid, was incubated for 22 hr in the medium with different concentrations of chemopreventive agents. Figure 1 shows the folds of induction (mean ± SEM) of CAT protein over control treatment after the addition of different concentrations of the chemopreventive agents to the cells. There were corresponding increases in the fold of induction of CAT protein with increasing concentrations of the compounds. At higher concentrations, the induction of CAT protein started to decrease, suggesting the possibility of toxic effects and/or inhibitory effects of the compounds on the cells (data not shown). The induction of CAT protein by most compounds ranged between two to six folds.

Dose Response and Time Course of Induction of rGSTy$_a$ by Sulforaphane

Figure 2A shows the concentration-effect relationship of SUL in the induction of CAT protein. Compared to other compounds (Figure 1), SUL displayed much greater induction of CAT protein. Figure 2B shows the effect of time course of induction of CAT protein after the 20 μM of SUL at 0, 3, 6, 10, 16, and 20 hr. Induction of CAT protein was seen as early as 3 hr, peaked at 10 hr and sustained up to 20 hr. Other compounds displayed similar kinetics of induction of CAT protein (data not shown).

Northern Blot Analysis

Figure 3 shows the Northern blot analysis of total RNA isolated from the stable rGSTya-1.6-CAT Hep G2 cells after treatment for 8 hr with the following chemopreventive agents; control (lane 1), 8 μM PMITC (lane 2), 6 μM PBITC (lane 3), 15 μM PEITC (lane 4), 15 μM PPITC (lane 5), 100 μM GEN (lane 6), 20 μM SUL (lane 7), 100 μM I3C (lane 8), 75 μM OLT (lane 9) and 15 μM TAM (lane 10). The blot was first hybridized to *rGSTya-1.6-CAT* cDNA probe revealed a prominent mRNA transcript (Figure 3 top panel). Hybridization of the same blot to human β-actin cDNA probe showed even loading (Figure 3 bottom panel). After normalization by human β-actin, the level of CAT mRNA transcript was 8.8, 7.5, 5.2,

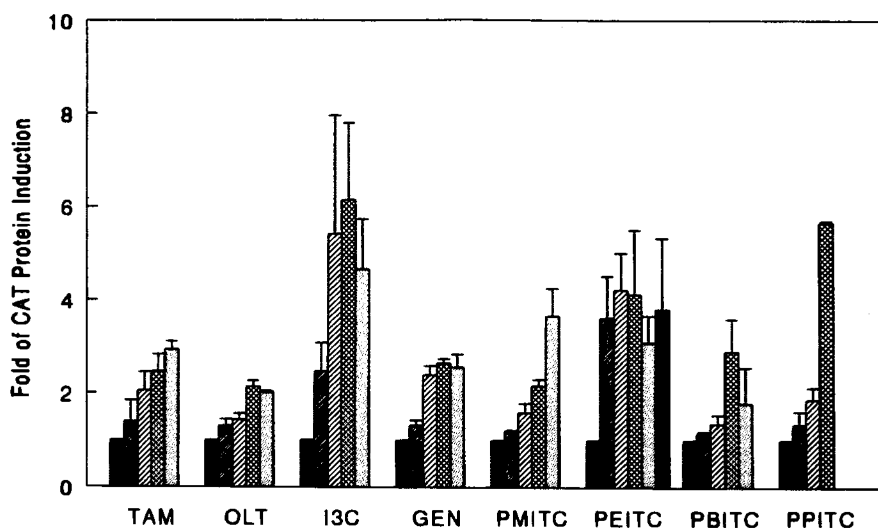

Fig. 1. Effect of different concentrations of chemopreventive agents on the regulation of rGSTya by quantitating the induction of CAT reporter protein in a stable HepG2 cell line containing the 1.6 kb 5′-flanking region of the gene (1.6-CAT). Results are expressed as fold of induction over control with n = 3 (mean ± SEM). The chemicals with the increasing order of concentrations (in μM) were as follows: TAM (0, 1, 5, 10, 15); OLT (0, 1, 10, 25, 50); I3C (0, 25, 50, 100, 250); GEN (0, 50, 100, 150, 200); PMITC (0, 1, 2, 4, 8); PEITC (0, 0.25, 0.5, 2, 6, 15); PBITC (0, 1, 2, 6, 15); and PPITC (0, 2, 6, 15).

0.84, 8.0, 25.1, 6.3, 8.3 and 2.4 times of that of control following treatments with PMITC, PBITC, PEITC, PPITC, GEN, SUL, I3C, OLT and TAM, respectively. The levels of induction of CAT mRNA, were in good agreement with that measured by the productions of CAT reporter protein as shown in Figures 1 and 2, with the exception of PPITC, where induction of CAT mRNA was less than that of CAT protein, the reason for this will be elaborated later.

Figure 4 shows the Northern blot analysis of the kinetics of mRNA induction of the proto-oncogene transcription factor, *c-Jun*, after SUL (20 μM) treatment of the rGSTya-1.6-CAT HepG2 cell line at; 0 (lane 1), 0.5 (lane 2), 1 (lane 3), 2 (lane

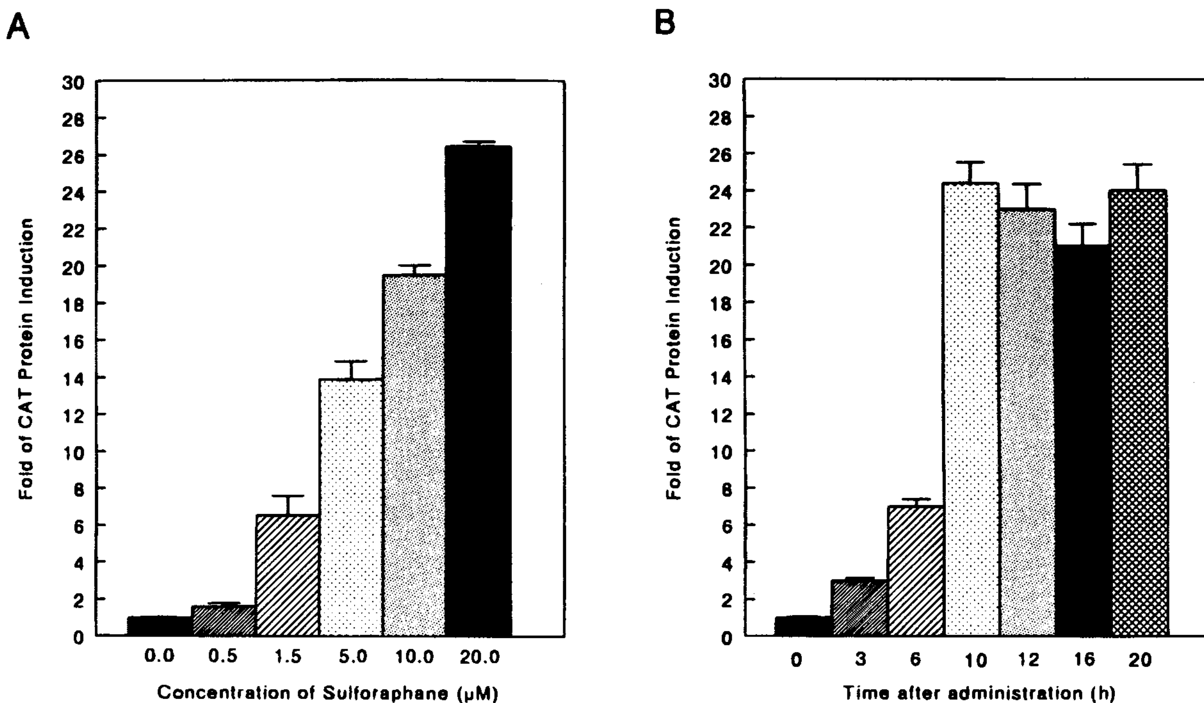

Fig. 2. (A) Effect of different concentrations of SUL (0, 0.5, 1.5, 5, 10, 20 μM), and (B) the time course of 20 μM of SUL on the regulation of *rGSTya*. Fold of induction of CAT reporter protein in the 1.6-CAT HepG2 cell line.

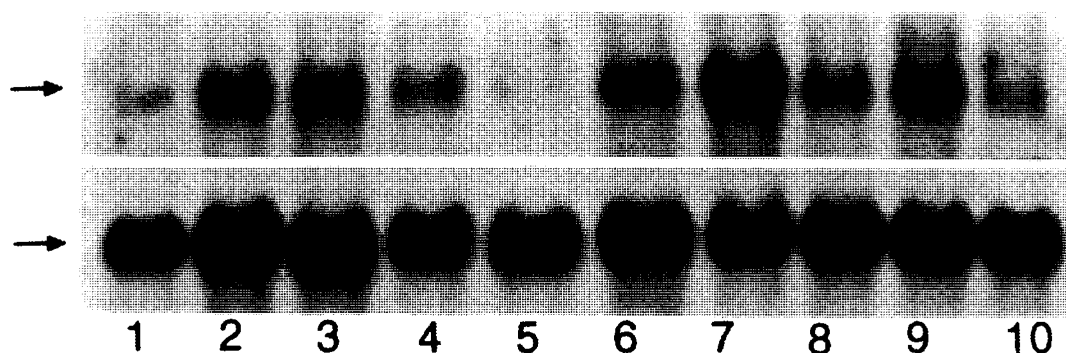

Fig. 3. Northern blot analysis of total RNA (about 15 μg) prepared from the 1.6-CAT HepG2 cell line treated for 8 hr with control (lane 1), 8 μM PMITC (lane 2), 6μM PBITC (lane 3), 15 μM PEITC (lane 4), 15 μM PPITC (lane 5), 100 μM genistein (lane 6), 20 μM SUL (lane 7), 100 μM 13C (lane 8), 75 μM OLT (lane 9) and 15 μM TAM (lane 10). The blot was hybridized to cDNA of *rGSTya-CAT* and human β-actin.

4), 4 (lane 5), and 6 (lane 6) hr. The blot was hybridized to cDNA of *c-Jun* cDNA probe (Figure 4 top panel) and human β-actin (Figure 4 bottom panel). *c-Jun* mRNA was induced as early as 2 hr (lane 4) and peaked at 6 hr (lane 6). Hybridization of the same blot to *c-Fos* or *LRF-1* cDNA probes did not detect any messages (data not shown).

DISCUSSION

Many naturally occurring compounds found in vegetables and fruits can act as chemopreventive agents in experimental models of carcinogen-induced carcinogenesis in animals. One of the mechanisms by which these compounds are able to prevent the development or retard the progression of carcinogenesis is presumably *via* preventing carcinogens from reaching or reacting with cellular targets (3). This mechanism has been postulated to involve the induction of phase 2 drug metabolizing enzymes such as GSTs, quinone reductases, epoxide hydrolases, and UDP-glucuronosyltransferases (4). However, the molecular events that lead to phase 2 enzymes gene activation in response to chemopreventive agents remain unclear. Utilizing a human hepatoma Hep G2 cell line stably transfected with a 1.6 kb 5′-flanking region of the rat GSTya subunit gene fused to a reporter CAT gene construct, we investigated the transcription regulation by several chemopreventive agents such as OLT, TAM, GEN, SUL, and various phenyl isothiocyanates.

The reasons these compounds were chosen as candidates were described as follow. OLT is a synthetic dithiolthione, has been shown to increase the level of GST activity, and resulted in marked inhibition of aflatoxin B1-induced hepatic tumorigenesis in rats, and it decreased the depth of tumor invasion in mice

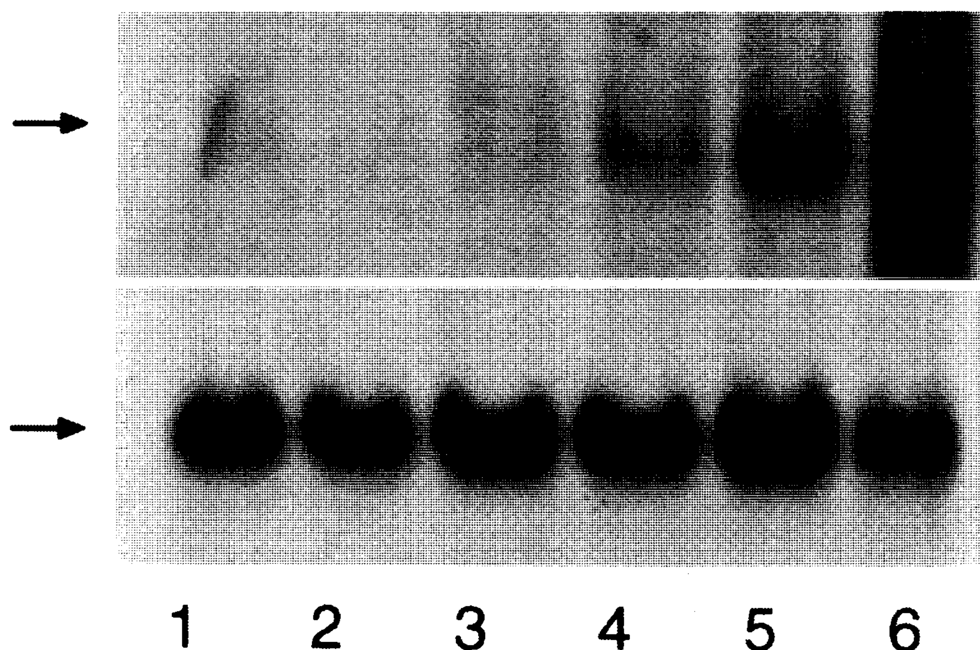

Fig. 4. Kinetics of induction of *c-Jun* mRNA after SUL (20 μM) treatment of the 1.6-CAT HepG2 cell line. Lanes 1 (0 hr), 2 (0.5 hr), 3 (1 hr), 4 (2 hr), 5 (4 hr), and 6 (6 hr). The blot was hybridized to cDNA of *c-Jun* and human β-actin.

(8). TAM, an anti-estrogen receptor antagonist, is controversial over its safety and efficacy in preventing breast cancer (9). TAM has been shown to exhibit multiple cellular effects which includes alteration of secretion of transforming growth factors, restoring the E-cadherin function in human breast cancer (10), as well as induction of many metabolizing enzymes including GST (submitted for publication). GEN, an ingredient of soy bean is a tyrosine kinase inhibitor (11) which blocks or attenuates cascade followed by tyrosine phosphorylation and has been postulated to prevent breast cancer (12). SUL, I3C, and PEITC, consumed with cruciferous vegetables, have been associated with a decreased cancer risk in both animal experiments and clinical human trials (5). By evaluating the transcriptional regulation of gene expression by these compounds in a cell line, insights would be gained as to their potential biological mechanisms in protection against cancer in human.

Using a rGSTya-1.6-CAT transfected cell line model, we have demonstrated that there is a concentration- and time-dependent induction or up-regulation of the CAT reporter gene by these chemopreventive agents. TAM showed an increase of about 3 fold induction of CAT mRNA and protein. This induction supported the *in vivo* animal data that TAM induced *GSTya* mRNA in rat liver in a dose-dependent fashion (submitted for publication). OLT, an antischistosomal agent, had been shown to display a unique ability to inhibit chemically induced hepatic carcinogenesis in rat, by increasing the alpha-class GST heterodimer comprising Yc1 and Yc2 subunits (13). Our results indicated that the Ya subunit was also up-regulated by OLT. Our observations, both *in vivo* and CAT assay, agreed with the recent publication by Buetler et al. (14) that OLT induced GSTya gene expression. I3C had been shown previously to induce rat GST Yc2 subunit gene expression (15), and here we found that it was able to up-regulate the GSTya-CAT. GEN, a constituent of soy bean, shown for the first time that it could induce GSTya-CAT in this cell line model. Future experiments would investigate whether its induction was related to its ability to inhibit tyrosine phosphorylation (12). The isothiocyanates chemopreventive agents, PMITC, PEITC, PBITC, PPITC and SUL, shown here that they all have the ability to up-regulate the expression of rGSTya-CAT.

The effects of varied alkyl carbon chain length of phenyl isothiocyanates on the induction of CAT mRNA (8 hr) and protein (22 hr) at the same concentration were: 8.8/3.5 (PMITC); 7.5/3 (PBITC); 5.2/4.2 (PEITC); and 0.84/5.5 (PPITC), respectively. With increasing carbon chain length, there was a decrease in the fold of induction of CAT mRNA, and a concomitant increase in the fold of induction of CAT protein with the exception of PBITC. Furthermore, PPITC induced CAT protein without inducing CAT mRNA. The exact mechanism for the difference is not known, but one potential mechanism could involve post-transcriptional or post-translational regulation of CAT mRNA/protein.

Previous studies from different laboratories have shown that the 5′-flanking region of rGSTy$_a$ gene can be activated by different antioxidants such as tBHQ at the transcriptional level through the ARE in rat, or the EpRE in mouse (2). Xenobiotics such as aromatic hydrocarbon can transcriptionally regulate the expression of rGSTy$_a$ gene through the XRE/AhRE (7). However, the transcriptional activation of the GRE remains unknown at this time. In an attempt to elucidate the molecular mechanisms that mediate the cellular response to chemopreventive agents, the study on the expression of transcriptional factors was performed. The result showed that only the *c-Jun* mRNA was induced by sulforaphane. Recent publications had found the presence of two adjacent AP-1-like binding sites in the ARE/EpRE (16). Antioxidants (parent compounds and/or their metabolites), such as α-naphthoflavone, 3-methylcholantrene, tBHQ, dioxin, 4-trans-phenyl-3-butene-2-one and phenobarbital, might produce transducing signals responsible for AP-1 activation and induce AP-1 activity (16). This would subsequently led to an induction of *c-Fos* and *c-Jun* gene expression with the accumulation of the respective mRNAs and a *de novo* synthesis of the AP-1 protein components (16). However, recent studies showed that there was no induction of *c-Fos* mRNA by the antioxidant tBHQ (17), which agreed with our finding, but instead, a *c-fos* related gene, *fra-1* and *c-Jun* were induced. We have recently performed gel mobility shift experiments to DNA binding elements of ARE, AP-1 and NF-κB, and found that TAM was able to increase the binding to all three DNA elements (submitted for publication), suggesting that these transcription factors/DNA binding elements may be important in the regulation of gene expression by TAM. Other laboratory had reported the activation of NF-κB and AP-1 by oltipraz in human colon cell line HT29, which may be responsible for the induction of human quinone reductase (18). Future studies on the signal transduction pathways and subsequent activation of the transcription factors by chemopreventive agents, would yield insights as to the mechanisms of induction of phase 2 detoxifying genes (and other genes) which may play a vital role in the protection against cancer in animals and in humans.

In summary, the diverse group of chemopreventive agents tested in this study, all have the ability to up-regulate the gene expression of rGSTya-CAT in a hepatoma cell line model. These results, coupled with *in vivo* animal data, would suggest that a possible common mechanism for the observed chemopreventive effects against chemical-induced carcinogenesis in animals by these compounds. That may involve the activation of transcription factors leading to the enhancement of transcription of genes including phase 2 genes such as GST, and ultimately leading to increase detoxification of the carcinogens. Question remains whether human has the same ability of up-regulation of these genes by the chemopreventive compounds, and future clinical studies would help clarifying this issue.

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REFERENCES

1. B. Mannervik and H. Jensson. Binary combinations of four protein subunits with different catalytic specificities explain the relationship between six basic glutathione S-transferases in rat liver cytosol. *J. Biol. Chem.* **257**:9909–9912 (1982).
2. T. H. Ruchmore, C. B. Pickett, and A. Y. H. Lu. Regulation of Expression of Rat Liver Glutathione S-transferases: Xenobiotic and Antioxidant Induction of the Ya Subunit Gene. In F. C. Kauffman (ed.), *Conjugation-Deconjugation reactions in Drug Metabolism and Toxicity*, Springer-Verlag, Berlin Geidelberg, 1994, pp. 79–107.
3. L. W. Wattenberg. Chemoprevention of cancer. *Cancer Res.* **45**:1–8 (1985).
4. T. Prestera and P. Talalay. Electrophile and antioxidant regulation of enzymes tha detoxify carcinogens. *Proc. Natl. Acad. Sci. USA* **92**:8965–8969 (1995).
5. C. E. Szarka, G. Grana, and P. F. Engstrom. Chemoprevention of cancer. *Curr. Probl. Cancer* 4-78 Jan./Feb. (1994).
6. A.-N. T. Kong, P. Fei, and B. K. Wong. Differential expression of the phenol family of UDP-Glucuronosyltransferases in Hepatoma cell lines. *Pharm. Res.* **12**:309–312 (1995).
7. T. H. Rushmore and C. B. Pickett. Transcriptional regulation of the rat glutathione S-transferase Ya subunit gene. *J. Biol. Chem.* **265**:14648–14653 (1990).
8. R. A. C. Moon, G. j Kelloff, C. J. Detrisac, V. E. Steele, and C. F. Thomas. Chemoprevention of OH-BBN-induced bladder cancer in mice by oltipraz alone and in combination with 4-HPR and DFMO. *Anticancer Res.* **14(1A)**:5–11 (1994).
9. M. A. Anzano, S. W. Byers, J. M. Smith, C. W. Peer, L. T. Mullen, C. C. Brown, A. B. Roberts, and M. B. Sporn. Prevention of breast cancer in the rat with 9-cis retinoic acid as a single agent and in combination with tamoxifen. *Cancer Res.* **54**:4614–4617 (1994).
10. M. E. Bracke, C. Charlier, E. A. Bruyneel, C. Labit, M. M. Mareel, and V. Castronovo. Tamoxifen restores the E-Cadherin function in human cancer MCF-7/6 cells and suppresses their invasive phenotype. *Cancer Res.* **54**:4607–4609 (1994).
11. T. Akiyama, J. Ishida, S. Nakagawa, H. Ogawara, S. Watanable, N. Itoh, M. Shibuya, and Y. Fulami. Genistein, a specific inhibitor of tyrosine-specific protein kinases. *J. Biol. Chem.* **262**:5592–5595 (1987).
12. T. Takano, K. Takada, H. Tada, S. Mishiyama, and N. Amino. Genistein but not staurosporine can inhibit the mitogenic signal evoked by lithium on rat thyroid cells. *J. Endocrinol.* **143**:221–226 (1994).
13. T. Primiano, P. A. Egner, T. R. Sutter, G. J. Kelloff, B. D. Roebuck, and T. W. Kensler. Intermittent dosing with oltipraz: Relationship between chemoprevention of aflatoxin-induced tumorigenesis and induction of glatathione S-transferases. *Cancer Res.* **55**:4319–4324 (1995).
14. T. M. Buetler, E. P. Gallagher, C. Wang, D. L. Stahl, J. D. Hayes, and D. L. Eaton. Induction of phase I and Phase II drug-metabolizing enzyme mRNA, protein, and activity by BHA, ethoxyquine, and oltipraz. *Toxicol. Appl. Pharmacol.* **135**:45–57 (1995).
15. D. M. Stresser, D. M. Williams, L. I. Mclellan, T. M. Harris, and G. S. Bailey. Indole-3-carbinol induces a rat liver glutathione transferase subunit (Yc2) with high activity toward aflatoxin B1 exo-epoxide. *Drug Met. Disp.* **22**:392–399 (1994).
16. R. S. Friling, S. Bergelson, and V. Daniel. Two adjacent AP-1 like binding sites from the electrophile-responsive element of the murine glutathione S- transferase Ya subunit gene. *Proc. Natl. Acad. Sci. USA* **89**:668–672 (1992).
17. K. Yoshioka, T. Deng, M. Cavigelli, and M. Karin. Antitumor promotion by phenolic antioxidants: Inhibition of AP-1 activity through induction of Fra expression. *Proc. Natl. Acad. Sci. USA* **92**:4972–4976, 1995.
18. K.-S. Yao and P. J. O'Dwyer. Involvement of NF-κB in the induction of NAD(P)H:quinone oxidoreductase (DT:diaphorase) by hypoxia, oltipraz and mitomycin C. *Biochem. Pharmacol.* **49**:275–282, 1995.