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Induced Expression of Drug Metabolizing Enzymes by Preventive Agents: Role of the Antioxidant Response Element

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Abstract

Identifying agents that block tumor initiation is a goal of cancer prevention. The ability of a chemically varied group of agents to induce various drug metabolizing genes in livers of rats was examined. Sprague-Dawley rats were treated for seven days with various agents in the diet or by gavage. The agents examined, which might be expected to respond via specific nuclear receptors (CAR, AhR) as well as antioxidant response elements (AREs), included Phase I/II inducers [5,6 benzoflavone (BF, 5000 mg/kg diet), diallyl sulfide (DAS, 500 mg/kg BW/day), ethoxyquin (EXO, 300 mg/kg BW/day) and phenobarbital (PB, 500 mg/kg diet)] or pure Phase II inducers [1,2 dithiol-3-thione (DTT, 500 mg/kg diet), and cyclopentadithioldthione (CPDTT, 175 mg/kg BW/day)]. Liver RNA expression was analyzed employing oligonucleotide microarrays. The agents yielded unique expression profiles. In genes with known AREs, the induction ratios (Levels Treated/Levels Controls) were: Quinone Oxidoreductase (BF, 8:1; DTT, 3.2:1; CPDTT, 3:1; DAS, 1.8:1; Exo, 1.7:1), Glutatione Transferase Pi (DTT, 36:1; CPDTT 34:1; EXO, 8:1; DAS, 5:1; BF, 2.5:1), and aldehyde keto reductase 7A3 (AFAR) (DTT and CPDTT, 14:1; DAS 6:1; EXO 4:1; PB, 1.5:1). When the search included a wider variety of Phase II drug metabolizing enzymes, no clear pattern was observed. Agent induced gene expression and preventive activity in published carcinogen induced tumor models showed limited correlation; questioning whether measuring the induction of one or two genes (e.g., quinone reductase) is a surrogate for overall Phase II inducing (antioxidant) and potential anti-tumor activity.

Keywords

Chemopreventive Agents; Phase I/II Inducers; Antioxidant Response Element

1. Introduction

Five decades ago Miller and coworkers observed that treatment with certain xenobiotics could block the carcinogenic effects of known hepatocarcinogens [1,2]. These investigators were examining the ability of xenobiotics (polycyclic hydrocarbons, phenobarbital and

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Sudan dyes) to block the hepatocarcinogenic activity of aminoazo dyes and acetylaminofluorene. These efforts stimulated further work by Wattenberg, Sporn, and coworkers [3,4] relative to the use of xenobiotics to prevent the development of cancer. Initially, preventive agents were separated into: a) blocking agents that would inhibit the initiation of tumors by electrophiles (active chemical carcinogens, oxidants), and b) agents that inhibit the promotion and progression of neoplastic lesions once they have been initiated [3]. The latter group would include SERMS, NSAIDs, etc. that have proven to be effective in large scale clinical prevention trials [5,6]. However, it was the anti-initiators that were the primary focus of the field of chemoprevention during the 1950s – 1970s [1-3,7].

Many of the early xenobiotics that were identified as being anti-initiators proved to be mixed inducers [2,3,7] which increased levels of both Phase I (primarily cytochrome P450s) and Phase II (primarily conjugating enzymes) drug metabolizing enzymes. Although the mixed inducers were highly effective anti-initiators in various carcinogen induced models, questions were raised whether the induction of Phase I enzymes might actually serve to activate certain procarcinogens [8]. Therefore, there was a systematic effort to identify agents that might preferentially or solely induce the Phase II enzymes [9,10]. This examination was initially performed by looking for agents that would induce one or more of the Phase II enzymes; particularly quinone oxidoreductase [10]. Based on this screening assay, they identified a wide variety of xenobiotics and naturally occurring agents that induced quinone oxidoreductase; many were chemical antioxidants [11,12].

More recently, Pickett and coworkers [13] identified specific DNA sequences in the promoter region of genes coding for certain Phase II enzymes (e.g., quinone reductase, GST Yc2, GST Pi) which appear to control the transcriptional response to many agents; e.g., tert-butylhydroxyanisole. Since many of the active agents were antioxidants, the resulting DNA sequences were initially called antioxidant response elements (AREs). A wide number of genes with AREs have been identified among the Phase II enzymes, including quinone reductase, GST Pi, AFAR, GST Yc2, etc. [13]. Subsequently, it has been shown that transcription at these AREs is mediated by a transcription complex that includes the transcription factor Nrf-2. This complex involves the binding of Nrf-2 to a cytoskeleton protein KEAP which modulates the degradation of Nrf-2 [14,15]. This binding between Nrf-2 and KEAP is altered by multiple sulfhydryl groups on the KEAP protein whose oxidation/reduction status can be changed by antioxidants; including most of the agents identified by Talalay and coworkers [11,12].

In the present studies, an Affymetrix microarray was employed to examine induction of a wide variety of Phase I/II drug metabolizing genes by a structurally varied group of agents (Fig. 1). Certain of these agents were known mixed inducers (BF, PB, DAS and EXO) while the structurally related 1,2 dithiol 3-thione (DTT) and cyclopentadithiolthione (CPDTT) are primarily Phase II inducers [16-18]. Questions examined included: 1) Is there a coordinate induction of genes with known AREs? Coordinate induction was defined as a strong inducer of one gene with a known ARE would similarly be a strong inducer of other genes with AREs; 2) Is there coordinate induction of genes coding for Phase II enzymes, including genes not necessarily known to have AREs?; 3) Is there a coordinate response of various cytochromes P450?; and 4) What is the relationship between induction of the various Phase II drug metabolizing enzymes and efficacy in certain carcinogen induced tumor models?

2. Materials and Methods

Female Sprague-Dawley rats were obtained from Harlan Sprague-Dawley, Inc. (Indianapolis, IN) at 4 weeks of age. The animals were kept in a room lighted 12 hours each day and maintained at 22°C. Teklad diet (Harlan Teklad, Madison, WI) and tap water were

provided ad libitum. Beginning at 7 weeks of age, the rats were treated with the various agents for 7 days (5/group). The dose levels of the agents administered were: 5,6-benzoflavone (BF), 5000 mg/kg diet; phenobarbital (PB), 500 mg/kg diet; 1,2-dithiol-3-thione (DTT), 500 mg/kg diet; diallyl sulfide (DAS), 500 mg/kg BW/day, i.g.; ethoxyquin (EXO), 300 mg/kg BW/day, i.g.; and cyclopentadithiolthione (CPDTT), 175 mg/kg BW/day, i.g. Compounds were added to the feed by mixing into mash diet using a Patterson-Kelly blender with intensifier bar. DAS, CPDTT and EXO were mixed with corn oil for administration i.g. to the rats (0.5ml/treatment). 5,6 BF, DAS, EXO and PB were obtained from Sigma Chemical Co. (St. Louis, MO). DTT and CPDTT were obtained from the chemical repository of the NCI Chemoprevention Agent Development Research Group.

2.2 RNA Isolation and Amplification

RNA was isolated and processed similarly to that described previously (19). Total liver RNA from untreated rats and rats treated with DAS, DTT, EXO, PB, CPDTT, and BF was isolated by Trizol (Invitrogen, Carlsbad, CA) and purified using the RNeasy Mini Kit and RNase-free DNase Set (QIAGEN, Valencia, CA) according to the manufacturers protocols. In vitro transcription-based RNA amplification was then performed on the samples. cDNA for each sample was synthesized using a Superscript cDNA Synthesis Kit (Invitrogen) and a T7-(dT) 24 primer: 5′-GGCCAGTGAATTGTAATACGACT-CACTATAGGGAGGCGG-(dT)24-3′. The cDNA was cleaned using Phase-lock gel (Fisher Cat ID E0032005101) phenol/chloroform extraction. Then, the biotin-labeled cRNA was transcribed in vitro from cDNA using a BioArray High Yield RNA Transcript Labeling Kit (ENZO Biochem, New York, NY), and purified again using the RNeasy Mini Kit.

2.3 Affymetrix Gene Chip Probe Array and Gene Cluster

The labeled cRNA was applied to the Affymetrix RAE 230A Gene Chips (Affymetrix, Santa Clara, CA) according to the manufacturer's recommendations. Every gene or EST is represented by a probe set consisting of approximately 16 probe pairs (oligonucleotides) of 25-mer oligonucleotides. One sequence of a probe pair represents the complementary strand of the target sequence while the other has a one base pair mismatch at the central base pair position. This mismatch sequence serves as an internal control for specificity of hybridization. Array normalization and gene expression estimates were obtained using Affymetrix Microarray Suite 5.0 software (MAS5). The array mean intensities were scaled to 1500. These estimates formed the basis for statistical testing. Differential expression was determined using the t-test with $p < 0.05$. Genes meeting the criteria were called positive for differential expression. Hierarchical clustering was performed by selecting Phase 1 and Phase II genes from the array data, including CYP450s, NADPH quinone oxidoreductases, epoxide hydrolases, GSTs, aldoketoreductases, and UDPGs. For the selected genes, expression indexes were transformed across samples to an N (0,1) distribution using a standard statistical Z-transform. These values were input to the Gene Cluster program of Eisen [20] and genes were clustered using average linkage and correlation dissimilarity.

3. Results

3.1 Gene expressions of Phase I and Phase II genes in livers after agent treatment

Sixty genes coding for Phase I and Phase II drug metabolizing enzymes were identified from the array with 30 CYP genes and 30 Phase II enzymes (Fig. 2). Among these genes, roughly one-half showed significant induction ($P< 0.02$, relative induction >1.5) by at least two of the agents. DAS, DTT, CPDTT and EXO induced a wide variety of Phase II and some Phase I genes, whereas the classic mixed inducers phenobarbital and 5,6 benzoflavone preferentially highly induced various cytochrome P450s. DAS and DTT altered the greatest number of genes (Table 1). When confined to the sixteen Phase II drug metabolizing

enzymes (Non CYP) and only genes which were induced at least 2×, it was found that there were 13 and 12 genes induced by DAS and DTT, respectively (Table 1). In fact, the agents yielded four clusters of genes that they strongly induced (Fig. 2). Thus, PB, EXO, and DAS highly induced a variety of genes (Cluster A) which included CYPs (2B1, 2C) and UDPG 1A1. DTT and CPDTT highly induced a variety of Phase II related genes (Cluster B) including aldoketoreductase 7A3, GST Pi, and GST theta, while BF (Cluster C) highly induced a variety of CYPs (1A1, 1A2, 1B1) and NADPH dehydrogenase quinone 1 (quinone reductase). Finally, there was a cluster of genes (D) that was induced by DAS, and DTT that included GST Mu1, GST Yc2, epoxide and hydrolase 1 (microsomal).

We subsequently examined induction of two sets of specific genes. First, genes which were known from the literature to have AREs and, second, a number of cytochrome P450s associated with known DNA response elements (CYP1A1, 1B1, 2B1 and 3AI) (Fig. 4).

3.2 Specific effects on genes with known AREs (Fig. 3, Table 1)

Glutathione S Transferase Pl (Fig. 3A, Table 1)—Of the known genes with AREs, GST PI was the most highly induced of any of the Phase II enzymes that were examined. Thus, DTT and CPDTT induced this gene almost 35×, while EXO and DAS induced 8× and 5×, respectively. Finally, BF induced this agent roughly 3×, while PB did not significantly induce this gene.

Glutathione S Transferase Yc2 (GST 5-5) (Fig. 3B, Table 1)—Each of the agents, except PB, significantly induced the RNA coding for GST Yc2 ($P<0.025$). However, the agents yielded different relative levels of induction. Thus, DAS and DTT induced expression of this gene 4-5×, while CPDTT, BNF, PB CPDTT, and EXO induced 2-2.5×.

Quinone Reductase: (NADPH dehydrogenase, quinone reductase 1) (Fig. 3C, Table 1)—All agents except PB significantly induced quinone reductase ($P<0.025$). Induction ratios were: BF 8:1, DTT and CPDTT 3:1, DAS 1.8:1, EXO 1.7:1, and PB 1.2:1.

Aldoketoreductase 7A3 (Aflatoxicol Reductase) (AFAR) (Fig. 3D, Table 1)—This enzyme preferentially reduces aflatoxicol which is a hydration product of aflatoxin 7,8 oxide. DTT and CPDTT induced this gene almost 15×, while DAS and EXO induced 8× and 5×, respectively. Finally, BF and PB failed to significantly induce this gene.

3.3 Effects on Non CYP Drug Metabolizing Genes (Table 1)

The glutathione and glucuronyl transferases are perhaps the best characterized group of Phase II enzymes. In Table 1, we have given results for five glutathione transferases, two glucuronyltansferases, aldoketoreductase 7A3, aldehyde dehydrogenase 1A1, quinone oxidoreductase, and epoxide hydrolase (microsomal). The results for the four genes with known AREs mentioned above are presented in Fig. 3 and Table 1. As can be seen, there is no consistent pattern in induction insofar as no single agent preferentially induces most or all the various non CYP drug metabolizing genes.

4. Discussion

The objectives of this study were to evaluate structurally varied chemopreventive agents (Fig. 1) to determine whether they yielded a coordinate response in genes coding primarily for non CYP drug metabolizing genes in general, and genes with known AREs elements in particular. Coordinate induction is defined here as “if an agent induced one gene to half the maximal levels, it would similarly induce other genes to roughly half the maximal levels”. Specific questions included: 1) can induction of a single gene or limited number of genes be

used to assess the total drug induction of genes coding for drug metabolizing enzymes for a given agent or mixture?; 2) will the use of one or two genes heavily skew the results?; and 3) is there any clear relationship between the gene induction levels achieved in this study and known activities of these agents in carcinogen induced models of cancer in rodents?

The agents are structurally varied with the exception of DTT and its cyclopenta derivative (CPDTT), and all except PB have been employed in recent preclinical literature as chemopreventive agents. However, phenobarbital treatment blocks the carcinogenic activity of azo dyes and aflatoxin B1 (1). Furthermore, epidemiologic data showed that phenobarbital actually inhibited development of bladder tumors in epileptics who were taking this agent and who smoked [21]; presumably by blocking tumor initiation.

The induction of Phase I drug metabolizing enzymes (mostly cytochrome P450s) has been attributed to specific nuclear receptors and specific response elements [22]. Thus, CYPs 1A1 and 1B1 are mediated via the Ah (dioxin) receptor, the nuclear transporter protein Arnt, and the corresponding XREs (xenobiotic response elements) in the DNA. Induction of CYPs 2B is mediated by CAR (Constitutive Androstenedione Receptor) and corresponding DNA elements [23,24]. The CAR receptor has much less ligand specificity (Fig. 1) than most receptors, and may not directly bind many potential inducers.

Recently, a mechanism that can mediate induction of genes coding for Phase II drug metabolizing enzymes has been described [13-15]. The system was initially described by its DNA response elements and designated ARE because it responded to antioxidants such as BHA and BHT. The AREs have now been shown to be activated by a complex including the transcription factor NFE2-p45 related factor 2 (Nrf2) and various co-factors [14,15].

Two comments should be made. First, induction of the genes coding for the various Phase I/II enzymes was by RNA analysis and did not measure protein levels, or enzymatic activities. This was because the arrays allow one to simultaneously examine a wide range of genes. In addition, studies have shown a relatively good correspondence between increases in gene expression, protein levels, and enzymatic activities when dealing with many of the Phase I and Phase II enzyme genes (16-17, 25). Second, the specific doses employed were not equal doses or equimolar; rather we used doses previously shown to be effective chemopreventive agents in vivo. We have previously examined induction by both AhR and CAR induces and found a sigmoid response curve with gene induction reaching some plateau. Thus, it seems unlikely that we have achieved some supra-optimal dose for any of these agents in which induced gene levels decline. This does not preclude the possibility that higher doses of certain of the agents might have achieved higher levels of gene induction. However, the doses employed had proved to be effective preventive doses in previously published studies.

In Fig. 3 A-D, four genes with known ARE responses were examined. Two of the four are not specifically Phase II enzymes (quinone oxidoreductase, aldoketoreductase 3A7). However, the enzymes coded by these genes can both reduce quinones, ketones, or aldehydes to entities which can be more readily conjugated and excreted. In addition, quinone reductase has often been used to screen for antioxidants/Phase II inducers/chemopreventive anti-initiators (10,11). QOR was highly induced by BF (8×), moderately induced by the two dithiolthiones DTT and CPDTT (3×), and weakly induced by DAS and EXO (Fig. 3A). In contrast, levels of aldoketoreductase were induced roughly 15× by DTT and CPDTT, 8× and 5× by DAS and EXO, respectively, and was unaffected by BNF. We also examined two glutathione transferases with known AREs; GST Pi, and GST Yc2. RNA levels of GST Pi were profoundly induced by DTT and CPDTT. GST Yc2, the form of GST that preferentially conjugates the AFB1-7,8-oxide, was induced 4-5× by DTT and DAS and roughly 2× by BNF, CPDTT, and EXO.

Since all of these genes have been shown to have AREs in their promoter region, it is perhaps surprising that there does not appear to be strong coordinate activation between the various genes and inducers. Thus, DTT and CPDTT gave by far the highest inductions of GST Pi and AFAR. DAS either directly or through one of its metabolites proved to be a relatively strong inducer of GST Yc2, and AFAR was a much weaker (albeit statistically significant), inducer of GST Pi and QOR. Almost 15 years ago we showed that DAS was both a PB type inducer (CYP2B) [17,25] and simultaneously induced quinone reductase. Ethoxyquin was a CYP 2B inducer and a moderate inducer of GST Yc2, AFAR, and GST Pi. These results confirm previous results by Eaton and coworkers [16]. A portion of this lack of coordinate response presumably reflects the fact that multiple receptors can affect expression of many of the drug metabolizing genes. Thus, XREs that can respond to the Ah receptor are clearly associated with quinone oxidoreductase, GST Ya and UDPG 1A6, as well as the various CYP 1A genes. Similarly, the CAR receptor can affect DNA elements on various genes inducing GST alpha type 2, epoxide hydrolase, GST Mu, UDPG phenobarbital-induced as well as CYP 2B1.

Finally, it was observed that BF highly induced QOR, but was a relatively weak inducer of the other genes. The high induction of QOR can be explained, in part, by the fact that this gene (in addition to its ARE) has an XRE through which it can respond to this agent. The more surprising aspect is that one would expect BF to be metabolized to quinones that would signal via the ARE pathway [10,11]. BF has been used as a positive control for cell culture studies examining a wide variety of ARE related genes, and its activity has been attributed to the production of quinones which signal via the AREs. In contrast, quinones may be readily reduced and subsequently conjugated in vivo in the liver.

A wider range of genes coding for Phase II or Phase I related enzymes were examined (Table 1). Most genes were modulated by multiple agents. DAS and DTT caused the most striking increases in the greatest number of genes. DTT was the strongest inducer of a number of genes including the ARE genes listed above, whereas DAS was the strongest inducer of many other non-CYP related drug metabolizing genes; including GST Mu2, ALDH 1A1, liver UDPG (PB inducible), and epoxide hydrolase. Finally 5,6-BF was the strongest inducer of UDPG 1A6 and ALDH 1A1, as well as quinone oxidoreductase. These results show no clear coordinate response when looking at a wide variety of Phase II enzymes. Although there were differences in induction patterns between the various inducers, four main gene induction clusters (A,B,C,D, Fig. 2) were identified that related to induction by DAS/EXO/PB (Cluster A: CYP2B, 3A, UDPG2A1), DTT (Cluster B: e.g., aldoketoreductase 7A3; GST Pi, GST Yc2), BF (Cluster C: CYP 1A1, CYP 1B1, and quinone reductase), and by both DTT and DAS epoxide hydrolase (Cluster D: microsomal epoxide hydrolase and GST mu 1). These cluster signatures may prove useful in the characterization of unknown compounds or in examining complex mixtures. However, examining only one or two non CYP genes is unlikely to give insights into the overall induction of Phase II drug metabolizing genes.

The last groups of genes examined were various cytochrome P450s genes. We examined expression of CYP 1A1, 1B1, 2B1 and 3A (Table 1 and Fig. 4) and found there is strong specificity regarding induction of these genes. Thus, CYP 1A1 and 1B1 are both profoundly induced by 5,6- BF. CYP 2B1 was highly induced by three (PB, DAS and EXO). CYP3A was also induced by these three agents; although induction levels were much lower.

Potentially the most important question is what (if anything) does induction mean with respect to the ability of these various agents to inhibit carcinogenesis. In Table 2, we have included and compared induction data with that showing the efficacy of these agents in prevention of carcinogenesis in multiple models (based primarily on data in the literature).

Thus, all the agents at or below doses employed in the present studies can profoundly inhibit AFB1 induced carcinogenesis in rat liver [26-30]. In that model, inhibition of liver tumors is presumably due in major part to induction of GST Yc2 and somewhat less so to the induction of AKR 7A3 (AFAR) [31]. GST Yc2 preferentially conjugates aflatoxin 7, 8 epoxide while AFAR reduces the diol metabolite aflatoxicol. However, metabolism to less toxic metabolites by CYP induction may also play some part regarding the efficacy of the mixed inducers (BF, DAS, EXO) [31], given that BF is highly effective but only a moderate inducer of these Phase II genes. Thus, we know that both GST Yc2 and AKR 7A3 have a role in aflatoxin induced carcinogenesis. However, when the most important enzymes affecting carcinogenesis are not known extrapolation is problematic; e.g., what decreases DMBA induced carcinogenesis in the rat mammary gland, or when the carcinogenic agent is not even known (as is often the case in human cancers). In Table 2, the anticarcinogenic effects of these various agents based on data in the literature (as well as on our unpublished results with the DMBA tumorigenesis model) are compared with the induction of a limited number of genes. The DMBA induced model of mammary carcinogenesis has been employed by a wide variety of investigators to look for agents that may block chemically induced carcinogenesis [32]. We found that BF (5000 mg/kg diet), 1,2 DTT (500 mg/kg diet), DAS (500 mg/kg BW/day) and ethoxyquin (3000 mg/kg diet) reduced DMBA induced mammary tumor multiplicity by 95, 40, <20, and 80%, respectively (Grubbs, C.J., Lubet, R.A., data not shown). The profound efficacy of the Ah receptor agonist is in line with prior studies showing that indole-3-carbinol (another mixed Phase I/II inducer) was similarly effective in reducing mammary tumorigenesis [33]. In fact, the results confirm efficacy studies by Malejka-Gilanti and coworkers [34]. This high efficacy may be mediated in part by the metabolism of DMBA to less carcinogenic products via specific CYP proteins. Similarly, we have shown earlier that BF and indole-3- carbinol were significantly more effective than DTT in inhibiting smoke induced P32 adducts in rats [35,36]. Thus, BF which is not one of the more striking inducers of the various non CYP related drug metabolizing genes is highly effective in various models.

The results presented imply that merely measuring expression of a single gene or protein in the liver or cell culture is unlikely to clearly parallel the chemopreventive efficacy of that agent against a wide range of potential carcinogens in multiple organs. This relatively facile determination is even more problematic when one considers that GST Pi and aldoketoreductase family 7 member A3 (which are highly induced by DTT in liver) are minimally induced in colon cancer (Lubet, R. A. data not shown). Thus, the extrapolation of potential preventive activity of unknown agents or dietary constituents by measuring gene or protein changes in only one or two specific genes does not seem reasonable as surrogates for overall gene expression of drug metabolizing enzymes or for measuring some generalized chemopreventive efficacy.

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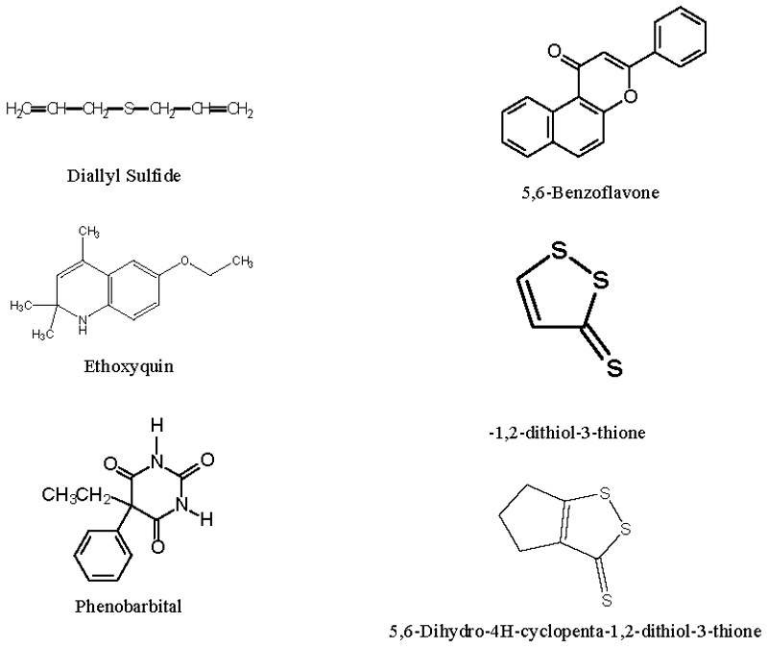


Figure 1.
Chemical Structures of the Various Agents

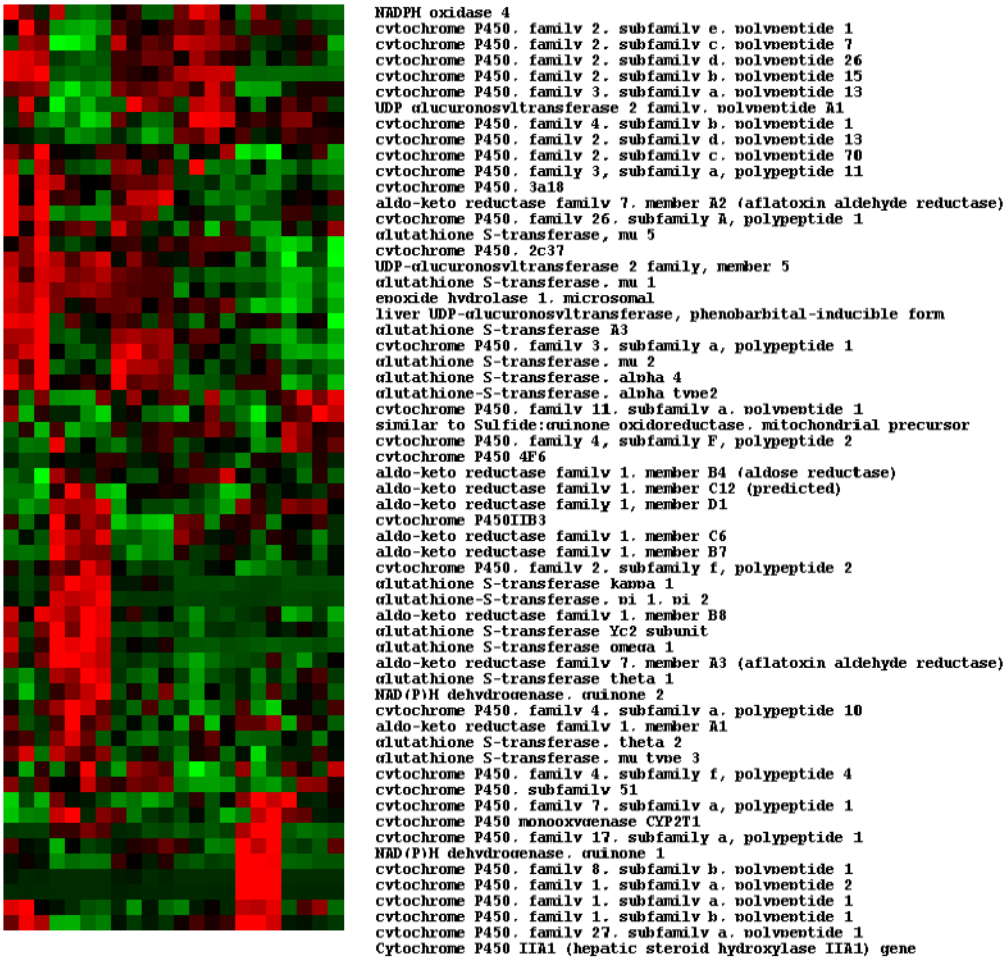


Figure 2.

Gene Cluster Analysis of Both Phase I and Phase II Enzymes. Group A: genes preferentially induced by PB, DAS and EXO; Group B: genes induced by DTT; Group C: genes induced by BF; Group D: genes induced by DAS and DTT.

Fig 3A

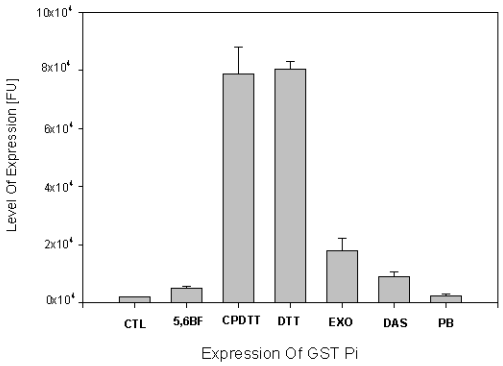


Fig 3B

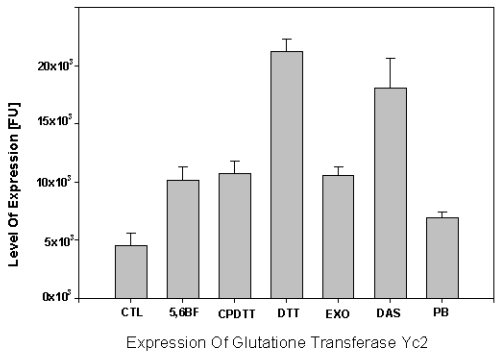


Fig 3C

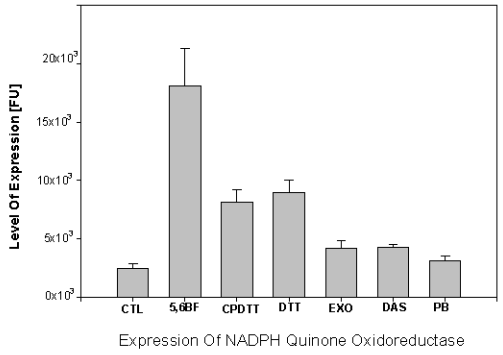


Fig 3D

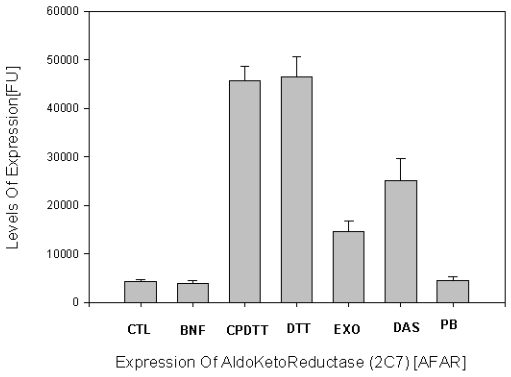


Figure 3.

Gene Expression In Livers Of Control Or Treated Groups. Examination of Genes Known to have ARE. (3A) GST Pi; (3B) GST Yc2; (3C) Quinone Oxidoreductase; (3D) Aldoketoreductase 3A7. Individual means and standard deviations are based on FUs as described in the Materials and Methods Section. N= 4 rats/group. Statistical analysis of treatment groups vs controls is shown in Table 1.

Figure 4 A

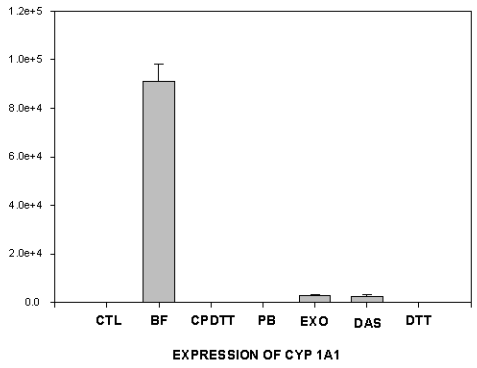
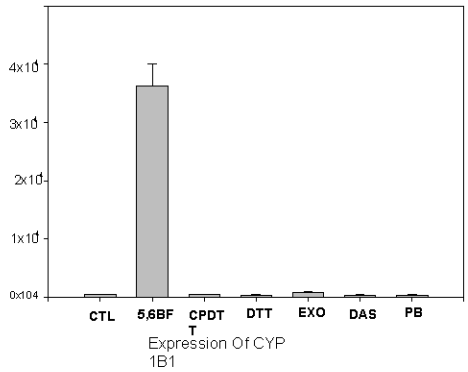


Figure 4 B



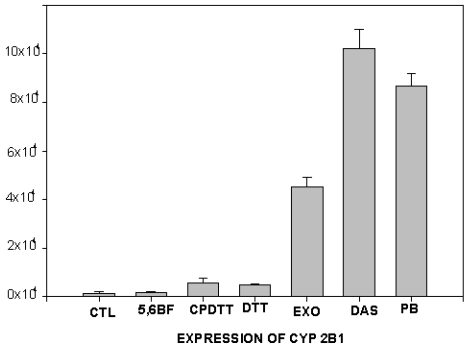


Figure 4 C

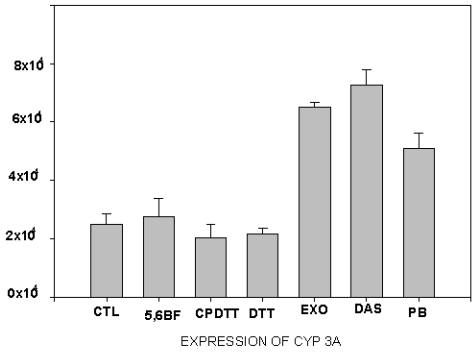


Figure 4 D

Figure 4.

Gene expression in Livers of Control or Treated Groups, Examination of Cytochrome P450 Genes. (4A) Cyp 1A1; (4B) CYP 1B1; (4C) CYP 2B1; (4D) CYP. Individual means and standard deviations are based on FUs as described in the Materials and Methods Section. N= 4 rats/group. Statistical analysis of treatment groups vs controls is shown in Table 1.

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Table 1

Modulation effects of different agents on Phase II enzyme genes expression in liver

	Non CYP Drug Metabolizing Genes		Fold Changes (>1.4)					
Accession #	**Gene Title**	**Symbol**	**DAS**	**DTT**	**EXO**	**PB**	**BF**	**CPDTT**
NM-02240	Aldehyde dehydrogenase 1A1		**6.5** **	3.0 **	2.3 *	1.7 *		2.8 *
NM_013215	aldo-keto reductase family 7, member A3 **(ARE)**	Akr7a3	5.6 *	**14.2** **	3.3 *			13.8 **
NM_012844	epoxide hydrolase 1, microsomal **(PRE)**	Ephx1	**3.2** **	2.8 *	2.4 *	1.6		2.4
AI234527	glutathione S-transferase, alpha 4	Gsta4	**1.7** *		1.7 *			
NM_020540	glutathione S-transferase, M4	Gstm4	**6.8** *			3.1	1.8	
M28241	glutathione S-transferase, mu 1	Gstm1	**3.3** **	3.2 *	2.7 *	1.5 *		2.9 *
AI169331	glutathione S-transferase, mu 2	Gstm2	**2.4** *		2.4 *	1.5 *	1.5 *	
NM_053293	glutathione S-transferase, theta 1	Gstt1	1.6	2.4 **				2.1 **
AA945082	glutathione S-transferase, Yc2 subunit **(GST5-5) (ARE, PRE)**	Yc2	3.9 **	**4.7** **	2.4 *		2.2 *	2.8 *
NM_017013	glutathione-S-transferase, alpha type2 **(ARE)**	Gsta2	**3.3** **	2.0 *	2.7 *		2.2 *	1.8 *
X02904	glutathione-S-transferase, pi 1 **(ARE)**	Gstp1	4.4	**36.7** **	8.6 **		2.5 *	34.5 **
J02679	**NAD(P)H dehydrogenase, quinone 1 (ARE,XRE)**	Nqo1	1.6 *	3.5 **	1.6 *		**7.0** **	2.2 *
NM_053524	NADPH oxidase 4	Nox4	4.2 *	1.8	1.9	**4.6** *	2.5	1.5
J02612	UDP glycosyltransferase 1 family, polypeptide A1 **(XRE, PRE)**	Ugt1a1	2.2 *		2.0 *	1.5 *	**2.8** **	
M31109	UDP glycosyltransferase 2 family, member 3	Ugt2b3		**2.6** **				2.2 **
NM_031980	UDP-glucuronosyltransferase 2 family, member 4	Ugt2b4	**1.6** *	1.5 *				
	CYP Mediated Drug Metabolizing Genes							
D38381	cytochrome P450, 3a18	Cyp3a18	**3.1** *	1.8	2.1 *			
X00469	cytochrome P450, family 1, subfamily a, polypeptide 1 [XRE]	Cyp1a1	33.4		36.6 *		**1227** **	
K02422	cytochrome P450, family 1, subfamily a, polypeptide 2 [XRE]	Cyp1a2	1.5		1.7		**3.4** **	
NM_012940	cytochrome P450, family 1, subfamily b, polypeptide 1 [XRE]	Cyp1b1			1.8		**94.7** **	
AI454613	cytochrome P450, family 2, subfamily b, polypeptide 15 [PRE]	Cyp2b15	**75.9** **	3.5 *	33.3 **	64.4 **		
NM_013105	cytochrome P450, family 3, subfamily a, polypeptide 1 [PRE]	Cyp3a1	**2.9** **		2.6 **	2.0 *		

Accession #	Non CYP Drug Metabolizing Genes Gene Title	Symbol	Fold Changes (>1.4) DAS	DTT	EXO	PB	BF	CPDTT
U09742	cytochrome P450, family 3, subfamily a, polypeptide 11 [PRE]	Cyp3a11	**5.4** **	2.2	5.3 **	3.5		

* $p < 0.05$

** $p < 0.01$

Abbreviations: XRE, Xenobiotic Response Element (Dioxin Inducible) [AhR Mediated]; ARE, Antioxidant Response Element; PRE, Phenobarbital Response Element (Phenobarbital Inducible) [CAR Mediated]. The induction ratio for a specific gene that is underlined and in bold was the agent that most highly induced that specific gene.

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Table 2

Effects of various agents on expression of drug metabolizing genes and their chemopreventive efficacy in various models

AGENT	AFB1 LIVER CANCER[a]	DMBA MAMMARY CANCER[b]	SMOKE ADDUCTS[c]	GST Pi[d]	QUINONE REDUCTASE[e]	CYP 1A1[f]	CYP 2B1[g]
5,6-Benzoflavone	++++	++++	+++	+	+++	++++	NE
Diallyl Sulfide	++++	++	ND	++	+	NE	++++
Ethoxyquin	++++	+++	ND	++	+	NE	+++
Phenobarbital	++	NE	ND	NE	NE	NE	++++
Dithiolthione	++++	++	+	++++	++	NE	NE

ND; Not Determined; NE, No Significant Effect

[a] AFB1 Induced liver cancer based on previously published data in the literature at or below the doses used in this study. 5,6-benzoflavone (26), diallyl sulfide (27), ethoxyquin (28): ++++, >80% inhibition; ++, 25-50% inhibition.

[b] DMBA mammary cancer data from Lubet, Grubbs Unpublished ++++, >85% reduction; +++, 60-85% reduction; ++, 40-60% reduction; NE, <25% reduction.

[c] Smoke induced adducts in lung (36,37): +++, >75% reduction; + 25-50% reduction.

[d] GST Pi (Table 1): ++++, >25× Induction; ++, 5-10× Induction, + >2× Induction, NE, <2× Induction.

[e] Quinone reductase (Table 1): +++, >6× Induction; ++, 3-5× Induction; +, 1.5 - 3× Induction; NE, <1.5× Induction

[f] CYP 1A1 (Table 1): ++++, >100× Induction; NE, <4× Induction

[g] CYP 2B1 (Table 1): ++++, >50 Induction; +++, 30-50 × induction; NE, <4× Induction