Detoxication Enzymes and Chemoprevention (44169)

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Abstract. **Detoxication enzymes protect cells from a wide variety of xenobiotics and endogenous toxins. Current data suggest that the balance between the Phase I carcinogen-activating enzymes and the Phase II detoxifying enzymes is critical to determining an individual's risk for cancer. Human deficiencies in Phase II enzyme activity, specifically glutathione-*S*-transferase (GST), have been identified and associated with increased risk for colon cancer. The increased frequency of the GST M1 null genotype among individuals with primarily smoking-related cancers has been documented. Induction of Phase II enzymes by naturally occurring or synthetic agents represents a promising strategy for cancer prevention. Both the required characteristics of potential chemopreventive agents and the role of the antioxidant response element in the monofunctional induction of Phase II enzymes have been discussed. The synthetic dithiolthione oltipraz induces a battery of Phase II enzymes and inhibits chemically induced tumors in a variety of target organs. Its ability to induce Phase II enzymes in human colon tissue and blood lymphocytes has been reported. Other promising inducers with chemopreventive activity include the isothiocyanates and polyphenols. These data collectively support the future development of Phase II enzyme inducers as clinical chemopreventive agents.** [P.S.E.B.M. 1997, Vol 216]

Experimentation continues to document the pivotal role of detoxication enzymes in cellular protection. Efficient inactivation of both xenobiotics and endogenous toxins results in the preservation of cellular integrity and inhibition of the cytotoxic events, which lead to several diseases, including cancer. The contribution of several families of enzymes (monooxygenases, dehydrogenases, reductases, peroxidases, oxidases, hydrolases, and conjugation catalyzing transferases [1]) results in protection against hazardous agents, which are very diverse in their chemical, physical, and bioactive properties. The ability of these enzymes to provide cellular defense continues to be challenged by modern advances in the food (i.e., preservatives, artificial substitutes, and dietary supplements) and chemical (i.e., synthetic compounds, environmental pollutants) industries.

Detoxication enzymes have been categorized into two groups based upon their functional properties (Table I). Phase I enzymes, including cytochrome P450s, metabolically activate xenobiotics to generate products which are highly reactive electrophiles (i.e., epoxides and reactive oxygen species). In contrast, Phase II detoxication enzymes both compete with the Phase I activating enzymes to inhibit the formation of electrophiles and catalyze the conversion of the electrophiles to inactive conjugates, making them more water soluble and more readily excreted from the cell. It is the cellular balance between the Phase I carcinogen activating enzymes and the Phase II detoxifying enzymes that contributes to one's risk of developing chemically induced cancer.

Detoxication Enzyme Deficiencies

Strong support for the relationship between deficient cellular protection and cancer susceptibility has been provided by the glutathione-*S*-transferase (GST) multigene family of detoxication enzymes. These enzymes (α, μ, π and θ) catalyze the conjugation of a variety of structurally diverse compounds (both endogenous and exogenous) with the nonprotein thiol glutathione (for review see Ref. 3). Endogenous substrates include organic hydroperoxides and oxidative products of arachidonic acid and cholesterol metabolism. The enzymatic reaction catalyzed by the GSTs inhibits reactive electrophiles from reaching cellular targets and results in the production of a thioether-linked glutathionyl conjugate, which is, in most instances, less cytotoxic.

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Table I. List of Enzymes That Are Often Included as Drug-Metabolizing Enzymes

Phase I
P450s, flavin-containing monooxygenases (FMOs), hydroxylases, lipoxygenases, cyclooxygenases, peroxidases, oxidases, monoamine oxidases (MAOs), dioxygenases, and reductases
Phase II
UDP glucuronosyl-, glutathione-, and sulfotransferases
Transaminases, acetyltransferases, methyltransferases, and acyltransferases
Quinone reductases, aldoketoreductases, and carboxylesterases
NAD- and NADP-dependent alcohol, and steroid dehydrogenases
Glycosylases, glucuronidases, various hydrolases, and esterases

Note. Although some colleagues might not consider most reductases, dehydrogenases, epoxide hydrolases, and esterases to be representative of Phase I or Phase II metabolism, for the purposes of the hypothesis set forth in this review we shall lump all drug-metabolizing enzymes into only the two categories. (Reprinted, by permission of the publisher, from Nebert D.W. Drug-metabolizing enzymes in ligand-modulated transcription. Biochem Pharmacol **47**:25–37. Copyright 1994 by Elsevier Science Inc. [2].)

Elevation of GST activity both in cell lines resistant to chemotherapeutic agents (4, 5) and in tissues protected from carcinogenic exposure by chemopreventive agents (6) implies the critical role of these enzymes in cellular protection.

Data from this laboratory continue to suggest a relationship between decreased GST expression and increased risk for cancer. Blood lymphocytes from individuals with a family or personal history of colon cancer, or a personal history of colon polyps, were found to have significantly lower levels of GST activity than those of healthy controls (7). GST activity was lowest in individuals with a history of colon polyps. Although the data suggested an inverse correlation between the level of GST activity in blood lymphocytes and the number of risk factors present, this trend did not reach statistical significance. An analysis of the GST activity of paired blood lymphocyte and colon mucosa samples revealed a strong correlation between the activity of these two tissues ($r = 0.87$). This finding suggested that the GST activity of blood lymphocytes may be used to identify individuals who are at increased risk of colorectal cancer and who may benefit from chemopreventive regimens that induce cellular protection.

Examination of matched pairs of liver tissue from 32 patients with hepatocellular carcinoma indicated that the total GST activity of tumor tissue (162.9 ± 32 nmoles/min/mg, mean ± SEM) was decreased significantly from that of adjacent normal tissue (348.3 ± 30 nmoles/min/mg) (8). Western blot analyses revealed that the μ class of GST isozymes was lost during tumor formation. GST μ was detected in the normal tissue from 87.5% of the subjects but only in 28.6% of the corresponding tumor tissues. Additional experimentation suggested that GST activity within the human liver is compromised by hepatitis B viral infection and further decreased during hepatocellular tumorigenesis.

Genetic polymorphisms in the GST μ and θ class isozymes have been identified. The μ class isozyme (GST M1) is absent in 40%–60% of the general population due to a gene deletion (9). This polymorphic expression, when combined with the ability of M1 to inactivate highly reactive environmental epoxides such as the aflatoxin B_1-8,9-*endo*-epoxide (10) and benzo[*a*]pyrene-4,5-oxide (11), has prompted a detailed investigation of the role of the null genotype in determining personal susceptibility to various cancers. Several studies have suggested an association between the GST M1 null genotype and increased risk for a variety of cancers, including lung (9), bladder (12, 13), larynx (12), skin (14, 15), gastric adenocarcinomas (16), high-grade astrocytomas (17), and pituitary adenomas (18). The inability of 40% of the tested population to conjugate halomethanes such as soil fumigants and pesticides has been associated with the absence of the θ class gene (GST T1) (19). To date, a relationship between the T1 null genotype and increased cancer risk is currently under investigation.

Subsequent experimentation in a mouse model of induced colitis has provided additional evidence for the contribution of detoxication enzyme deficiencies to cancer risk (20). Chronic colitis was induced in Swiss Webster mice by administering dextran sulfate sodium (DSS) in the drinking water for four cycles (each cycle consisted of 7 days of DSS and 14 days of untreated water). GST activity was reduced significantly within the colon as early as Day 2 of treatment and reached 52% of control by the end of Cycle 4. Similar depletion of both γ-glutamylcysteine synthetase (γGCS) activity (the rate-limiting enzyme in glutathione synthesis) and glutathione levels was observed (56% and 29% of control, respectively) within the colon of mice with DSS-induced colitis. These data, when combined with the observation that these animals subsequently develop dysplasia and invasive carcinomas, suggest that detoxication enzyme depletion is an early and important event in the progression of ulcerative colitis to colon cancer.

Although mutations in other detoxication enzymes have been reported, their contribution to cancer risk remains to be elucidated. For example, several laboratories have reported the presence of a homozygous C → T transition at position 609 of the NAD(P)H:quinone oxidoreductase gene (NQO1) (21–24). NQO1 catalyzes the two-electron reduction of quinones and their derivatives, thus preventing their participation in redox cycling. This mutation, which occurs in the population at a frequency of 6%–17%, results in the loss of NQO1 protein and activity. This mutation was originally detected in human colon carcinoma cell lines (25). Renal carcinoma cell lines deficient in NQO activity have also been identified (23). In the case of epoxide hydrolase, mutant alleles of this detoxication enzyme have been found to be overrepresented in individuals with primary hepatocellular carcinoma (26).

It should be noted that the detrimental effects of Phase

II enzyme deficiencies may be exacerbated by the corresponding presence of polymorphisms in cytochrome P450 (CYP). For example, polymorphisms in CYP1A1, CYP2D6, and CYP2E1 have been identified, and their importance in cancer susceptibility remains an area of intense investigation (for review see Ref. 27). A significantly increased risk for lung cancer has been associated with the presence of either the *Msp* I (28) or Ile-Val (28, 29) genotype of CYP1A1 in patients possessing a homozygous deletion of GST M1. An association between the high inducibility of CYP1A1 transcription by TCDD (2,3,7,8-tetrachlorodibenzo-*p*-dioxin) and the GST M1 null genotype has been reported recently by Vaury and colleagues (30).

Induction of Phase II Detoxication Enzymes

The ability of chemical agents to block the carcinogenic process in animals was reported as early as the 1920s, when Berenblum (31) demonstrated that the epidermal neoplasia induced by tar painting could be inhibited by topically applying mustard gas. Because many of the initially identified chemoprotectors were themselves carcinogenic, it was not until the early 1970s, when similar results were obtained with dietary constituents, that chemoprevention appeared to be a plausible approach to cancer prevention. Wattenberg (32) demonstrated that dietary supplementation with the antioxidants butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT), and ethoxyquin could inhibit the formation of chemically induced forestomach and mammary tumors.

Induction of detoxication enzymes by either naturally occurring substances or synthetic agents continues to be a promising chemopreventive strategy. The overall theory behind this approach is that the elevation of detoxication enzyme activity in individuals deficient in protection should provide additional defense against carcinogens by inhibiting them from reaching their cellular targets. The threshold of detoxication enzyme expression required to confer maximum cellular protection remains to be determined.

Molecular Mechanisms of Induction

The mechanism of action of candidate chemopreventive agents provides the basis for both the selection of compounds for in-depth evaluation and the establishment of biochemical screening assays with which to monitor drug response. Detoxication enzyme inducers have been divided into two classes based upon their effects on Phase I and Phase II enzyme activity. Bifunctional inducers increase the activity of both classes of enzymes, while monofunctional inducers increase the activity of Phase II detoxifying enzymes without affecting that of the Phase I group of activating enzymes. Prochaska and Talalay (33) determined that bifunctional and monofunctional induction of Phase I and Phase II detoxication enzymes occurs through distinct mechanisms. Bifunctional induction by β-naphthoflavone (β-NF), polycyclic aromatics, TCDD, and azo dyes was accompanied by increases in the aryl hydrocarbon hydroxylase (AHH) activity of Hepa 1c1c7 cells. In contrast, monofunctional induction by *tert*-butylhydroquinone (*t*-BHQ), 3,5-butylcatechol, bisethylxanthogen, 1,2-dithiol-3-thione, oltipraz, and benzylisothiocyanate occurred without significant induction of AHH activity. The dependence of only bifunctional induction on an intact *Ah* system was confirmed using cell lines deficient in either *Ah* receptor function or AHH enzyme activity (33). Subsequent studies have defined the genetic element responsible for the *Ah*-dependent inducible activity of a wide variety of primarily Phase I genes as the xenobiotic-responsive element (XRE). This element was originally discovered in the cytochrome *P-450c* gene (34) and later determined to be a binding site for the *Ah* receptor (35).

Antioxidant/Electrophile-Responsive Element

Two fundamental discoveries have significantly enhanced our current understanding of the mechanism responsible for monofunctional induction of Phase II enzymes. First, the finding that Phase II enzyme inductive ability is correlated with Michael acceptor strength provided an explanation for the apparent lack of structural similarity among monofunctional inducers (36). Interestingly, this previously disregarded chemical property is also related to GST substrate suitability (36, 37). Second, the genetic element putatively responsible for mediating monofunctional induction by Michael acceptors has been identified and characterized as the antioxidant-responsive element (ARE) (38, 39), or electrophile-responsive element (EpRE) (40, 41) (Table II). The work involved with the characterization of this element has been necessarily complex, meriting, in our opinion, considerable detail. Characterization of the transcription factors that can bind the ARE and that are functionally responsible for its basal and inducible activities is an area under investigation in several laboratories.

Regulatory regions responsible for β-NF inducible activity of a CAT reporter construct were discovered originally in the GST Ya (α class) subunit genes of the rat (38) and the mouse (40). Further studies by each group determined the specific nucleotide sequences responsible for induction. In each case, β-NF–mediated induction of the Phase II GST Ya gene was dependent on an intact *Ah* receptor and AHH activity (39, 41). Comparison of the EpRE and the ARE reveals a marginal difference of two of 41 bases of the originally proposed regulatory regions. Rigorous experimentation then determined the ARE core consensus from the rat Ya gene using deletion and point mutational analyses (46) as 5′-gTGACNNNGC (Table II). Point mutation of any of the TGAC nucleotides reduced basal activity and abolished inducibility, while mutation of the 3′-GC nucleotides abolished inducibility but had no effect on basal expression of the CAT reporter constructs (46). This work indicated that one of the two unique nucleotides is responsible for a substantive functional difference between the rat and the murine regulatory regions. The murine GST Ya

Table II. Comparison of the ARE/EpRE Sequences Found in Phase II Genes

Designation	Gene[a]	Sequence[b]	Location
		ARE Consensus - g**TGAC**NNN**GC**	
ARE	Rat GST Ya (38, 39)	GAGCTTGGAATGGCATTGCTTAATGG**TGAC**ATT**GC**	−722 to −682
EpRE	Mouse GST Ya (40, 41)	TAGCTTGGAA**TGAC**ATT**GC**TAATGG**TGAC**AAA**GC**	−754 to −713
ARE	Rat quinone reductase (42)	TCTAGAGTCACAG**TGAC**TTG**GC**AAAATCTGA	−434 to −404
ARE	Human γ-GCS (43)	CCTGACAGGTCATT**GC**TCT**GTCA**	−875 to −853
		TRE Consensus - TGA G/C T C/A A	
ARE/TRE[c]	Human quinone reductase (44)	AAATCGCAGTCACAG**TGAC**TCA**GC**AGAATCTG	−476 to −445
ARE/TRE	Human heme oxygenase 1 (45)	g**TGAC**TCA**GC**A....	−179 to −169;
		A**TGAC**ACA**GC**A	−127 to −117

[a] The following elements have been reported in the literature. Other genes may reveal consensus core ARE sequences but were not specifically reported as such.
[b] Denoted for coding strand, 5′ to 3′ orientation. Bold type indicates agreement with ARE consensus, and a perfect TGACNNNGC is also depicted in larger font; underlined characters are full TRE consensus sequences; N indicates that any base may be located at that position.
[c] Only the TREs located within the consensus AREs have been listed.

gene EpRE comprises two ARE consensus "core" sequences (40), while a single base difference (A → G) leaves one core sequence in the rat GST Ya gene ARE (Table II).

Interestingly, the ARE/EpRE consensus has a sequence of high similarity to the consensus sequence of the AP-1–binding site, or phorbol 12-tetradecanoate 13-acetate (TPA)–responsive element (TRE). The two sequences are not mutually exclusive (Table II). A number of studies have demonstrated the potential for *fos* family and *jun* family protein heterodimers to bind ARE/EpRE sequences which contain near- (47, 48) or fully- (49) consensus TREs. In the latter study, however, CAT reporter constructs were designed to isolate the effects of the distinct ARE component (a 3′-GC box) from the distinct TRE component (the interior TCA) without disturbing the mutual 5′-ggTGAC sequence.[2] Results indicated that the antioxidant-inducible activity mediated by the ARE was independent of its capacity to function as a TRE. The GC dinucleotide, previously shown to be required for ARE mediated inducibility (46), enhanced both the basal and TPA inducible rates of transcription of the ARE/TRE (49). Interestingly, deletion of the GC box caused reduction of TPA-induced native ARE-mediated transcription. Prior studies by Nguyen and Pickett (50) had indicated that a heterodimeric protein from *t*-BHQ induced HepG2 cell nuclear extracts bound to the ARE consensus, but not to consensus TRE sequences, though the potential for Jun or *jun* family proteins to participate was mentioned. Additionally, DNAse I protection assays indicated an interaction between this protein and the first three G nucleotides of the coding strand. Later gel shift assays were consistent with the existence of non-Fos1, Fos2, Jun1, or Jun2 nuclear proteins capable of preferentially binding the ARE versus the TRE (49).

Prestera and Talalay (51) systematically demonstrated that, while certain experiments supported the possible role of AP-1 transcription factors in ARE/EpRE-mediated transcriptional activation by Phase II enzyme inducers (48, 52), current experimental evidence is inconsistent with AP-1 binding (51) and actually indicates that distinct novel protein(s) bind to activate the native ARE (53). Indeed, treatment with the monofunctional agent *t*-BHQ can induce Jun-Fra heterodimers, which inhibit Jun-Fos heterodimeric binding to consensus AP-1 sites (54). This provides evidence for a potential mechanism by which a monofunctional (Phase II) inducer may cause inhibition of AP-1–mediated tumor promotion.

Model of Bifunctional and Monofunctional Phase I and Phase II Enzyme Induction

The overall ability of the ARE to mediate Phase II enzyme induction was tested (55) using a reporter construct containing the promoter and 41-bp regulatory element (EpRE) of the mGSTA gene. The concentrations of a variety of inducers required to double basal transcription rates were measured by growth hormone production in HepG2 cells. A high correlation (r = 0.89, using ranked data) was observed between the active compound's ability to stimulate NQO activity and induce growth hormone (reporter) production from the EpRE-regulated construct. A smaller fragment lacking a putative ETS-binding site was found to be less inducible (55). Thus, the model originally proposed by Prochaska, De Long, and Talalay (56) has been systematically tested and characterized (33, 36, 51, 55). Many of the specific genetic elements involved in the regulation of bi- and monofunctional induction of such enzymes as rGSTA (38, 39, 46), mGSTA (40, 47, 48), NQO (42, 44), γ-GCS (43), and heme oxygenase (45) have been defined at the molecular level. This model, presented in Figure 1, can be summarized as:

- Activation of Phase I enzymes by bifunctional inducers

[2] In a prior study (46), point mutational analysis determined that the TGAC consensus was essential for basal and inducible transcription, and mutation of the immediately preceding GpG dinucleotide did not affect either type of activity of the reporter construct. However, these authors incorporated the dinucleotide as part of the consensus sequence. As the ARE-designated constructs used in this currently discussed study all contained a ggTGAC sequence, the GpG is included as a "mutual" element. The TRE construct is based on a native human collagenase TRE, which does not contain this precedent GpG.

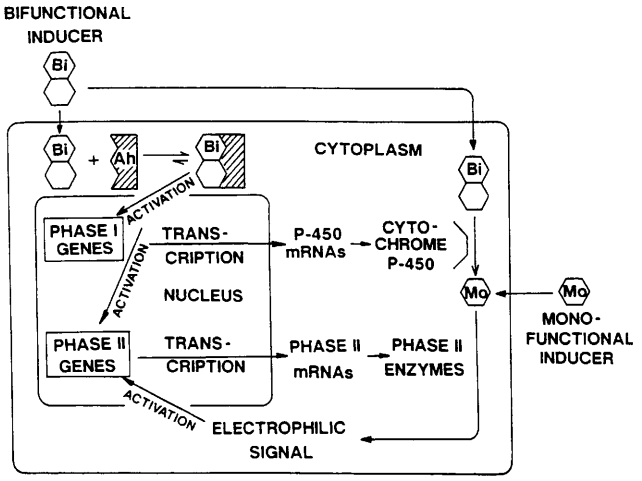

Figure 1. Metabolic cascade model for the relation between the mechanism of action of monofunctional (Mo) and bifunctional (Bi) inducers of Phase I and Phase II enzymes (slightly modified from data of Prochaksa *et al.* [56]). Monofunctional inducers enter the cell and generate the electrophilic signal that stimulates the induction of Phase II enzymes only. Bifunctional inducers require participation of the *Ah* receptor in two distinct mechanisms of induction. Bifunctional inducers enter the cell and bind to the *Ah* receptor, and the resultant complex activates gene transcription for both Phase I and Phase II enzymes. The resulting enhanced AHH activity converts metabolizable bifunctional inducers into compounds analogous in electrophilic properties to monofunctional inducers, which signal Phase II gene transcription. (This figure and legend were adapted with permission from Prochaksa and Talalay [33].)

occurs through an AHH dependent mechanism, putatively through direct XRE-mediated transcription initiated by an inducer-AH receptor complex. This mechanism would also stimulate the transcription of Phase II enzyme genes that are regulated by XREs.

- Monofunctional induction of Phase II enzymes is an AHH-independent process, putatively mediated by a transcription factor that recognizes the ARE/EpRE motif.
- Bifunctional inducers can be metabolized by an AHH-dependent mechanism to monofunctional inducers that then induce ARE- or EpRE-mediated transcription of Phase II enzymes.

It should be noted that other genetic elements have been identified and characterized as having putative roles in the regulation of specific Phase II genes. However, their precise contribution to this model requires additional clarification.

Phase II Inducers as Chemopreventive Agents

A Phase II enzyme inducer requires several characteristics in order to be considered a chemopreventive agent. First, it is imperative that all agents be nontoxic. Unlike chemotherapy trials, which focus on the treatment of individuals with established disease, chemoprevention regimens are designed to target individuals at increased risk of developing cancer who are healthy and asymptomatic. Second, established treatment regimens should include a schedule and route of administration compatible with daily life. Although many classic inducers of Phase II detoxication enzymes have been identified, few are appropriate for long-term usage as clinical chemopreventive agents. Third, the selected agent should be monofunctional, inducing Phase II enzymes without altering the expression of the Phase I activating enzymes. Corresponding induction of the Phase I enzymes could result in the metabolic activation of carcinogens. Fourth, an optimal agent would induce an entire battery of Phase II detoxication enzymes and thus afford protection against an array of structurally diverse compounds. Coordinate induction of the Phase II enzymes has been demonstrated and is thought to be facilitated by the presence of common regulatory elements (i.e., ARE) in the 5′ region of the affected genes. Lastly, an ideal chemopreventive agent would act as an anti-initiator, inhibiting early preneoplastic events in the carcinogenic process. Our inability to determine the exact time of initiation event in carcinogen-exposed individuals dictates the need for chronic drug administration, perhaps for the remainder of our lives. The optimal time to begin chemopreventive treatment with an anti-initiator remains controversial. The compounds listed below are examples of agents that show great promise based upon their monofunctional ability to induce Phase II enzymes and their known chemopreventive activity.

Oltipraz. The Phase II enzyme inducer that best fulfills the above criteria as a chemopreventive agent is oltipraz (5-[2-pyrazinyl]-4-methyl-2,3-dithiol-3-thione). This synthetic dithiolthione was originally marketed by Rhône Poulenc (Vitry-sur-Seine, France) as an antischistosomal agent. Its observed ability to increase the detoxication potential of the host while depleting glutathione within the schistosome to lethal levels provided the first evidence that oltipraz may be effective in increasing cellular protection (57). Characterization of the chemopreventive activity of oltipraz using animal models of chemically induced carcinogenesis demonstrated this compound's unique ability to provide numerous target organs with protection from structurally diverse carcinogens (for review see Ref. 58). Its activity as a radioprotector (59, 60) and an antiviral agent (61, 62) has also been reported.

The effectiveness of oltipraz in inhibiting the carcinogenic process has been attributed to its ability to induce an entire battery of Phase II detoxication enzymes (63–65). This activity as a monofunctional inducer (33, 66) has been localized to its unsubstituted 1,2-dithiol-3-thione nucleus (6). Induction of GST activity is consistently observed following oltipraz treatment and has prompted this laboratory and others to focus on this enzyme as a biomarker of its chemopreventive effect. Both the degree to which GST activity is elevated in the murine liver following a single oltipraz exposure (5-fold) and the extended duration of the response (approximately 2 weeks) (67) suggest that chronic elevation of GST activity may require only intermittent dosing with oltipraz. Nuclear run-on assays revealed that increases in enzymatic activity were attributed to coordinate increases in the rate of transcription of GST α, μ, and π. Involvement of the EpRE enhancer element in the induction

of Phase II detoxication enzymes by oltipraz has been demonstrated (68).

Inhibition of carcinogen-induced DNA adduct formation in the presence of oltipraz has provided compelling evidence for its chemopreventive activity. Dietary administration of oltipraz to aflatoxin B_1 (AFB_1)–treated mice produced a 76% reduction in hepatic DNA adducts as compared with controls receiving unsupplemented diet (69). An inverse correlation ($r = 0.95$) was established between the level of AFB_1-DNA adducts and the induction of hepatic GST. Similar results have been reported recently by Smith *et al.* (70), who observed a dose-dependent decrease in the formation of dibenz[*a,l*]pyrene adducts following exposure of MCF-7 human breast cancer cells to oltipraz. Pretreatment of the cells for 20 hr with 30 μM oltipraz produced a 95% reduction in DNA adduction. Data are emerging from both groups on the translation of these findings to human populations exposed to known carcinogens.

We have designed three chemoprevention trials at Fox Chase Cancer Center to examine the ability of oltipraz to induce detoxication enzyme expression in human target tissue. First, a dose-finding study has been performed in 24 individuals at increased risk for colorectal cancer (single oral dose of 125, 250, 500, or 1000 mg/m^2) (71). No adverse symptoms were reported in patients randomized to the lowest dosages. Three subjects experienced grade I adverse symptoms, specifically diarrhea and vomiting, after receiving the highest dosages (500–1000 mg/m^2). In the three cases, the symptoms resolved following treatment. Biochemical analyses of colon mucosa taken at timed intervals posttreatment demonstrated the elevation of GST activity at the lower dosages. Transcript levels for γ-GCS and NQO_2 peaked within the colon on Day 2–4 post-treatment, reaching 5.7-fold and 4.1-fold, respectively, at 250 mg/m^2; the optimal dosage for induction. A strong correlation was observed between the transcript levels of these enzymes in colon mucosa and peripheral mononuclear cells both before and after drug treatment. We had previously observed a similar correlation between the GST activities of these two tissues at baseline (7). These findings indicated that peripheral mononuclear cells may be used in future chemoprevention trials to assess the detoxication potential of colon mucosa and monitor its responsiveness to Phase II inducers. Second, we have completed a similar Phase I evaluation of chronic oltipraz (125 and 250 mg/m^2 twice weekly for 12 weeks) in patients at increased risk for colorectal cancer. The regimen was well tolerated, and these data are currently being analyzed. Third, a Phase II trial of oltipraz in chronic smokers with lung dysplasia (200 mg twice weekly for 6 months) is in progress. The endpoints of this current trial include both histological and enzymatic measures. It should be noted that the effect of oltipraz on the detoxication enzyme activity of blood lymphocytes from normal volunteers, specifically GST, has been examined by Gupta *et al.* (72).

Isothiocyanates. Naturally occurring isothiocyanates, such as benzyl isothiocyanate and sulforaphane, act as monofunctional inducers, elevating the activity of such enzymes as NQO and UDP glucuronosyltransferase (73). Their chemopreventive properties have been summarized recently by Hecht (74).

Sulforaphane (1-isothiocyanate-4-[methylsulfinyl]butane) has been isolated from broccoli and identified as a potent monofunctional inducer of Phase II detoxication enzymes, specifically GST and NQO (75). Its ability to inhibit CYP2E1 has been demonstrated (76). Although the available data on the chemopreventive activity of Sulforaphane is limited, administration of Sulforaphane to carcinogen-treated rats significantly decreased the incidence and multiplicity of mammary tumors (77).

Polyphenols. Polyphenols currently under evaluation as potential chemopreventive agents include ellagic acid and tea polyphenols (for review see Ref. 78). The mechanism of action of these antioxidants appears to be multifaceted and includes the selective induction of Phase II enzymes. *In vivo* treatment with the plant polyphenol ellagic acid produced elevations in GST, NQO, and UDP glucuronosyltransferase activities (79). Induction of both NQO (80) and GST Ya (81) by ellagic acid is mediated by the ARE of each gene. Inhibition of cytochrome CYP2E1 (79, 82) and 1A1 (80) by ellagic acid has been reported. Increases in glutathione peroxidase, glutathione reductase, catalase, NQO, and GST were observed in various tissues following the administration of green tea polyphenols to SKH-1 hairless mice (83). Treatment with (−)-epigallocatechin-3-gallate, the major and putative active component of green tea, inhibited both cytochrome CYP1A and 2B1 (84).

Conclusion

The induction of Phase II enzymes by monofunctional inducers represents a promising chemopreventive strategy. In the past decade, our understanding of the mechanisms involved in regulating detoxication enzymes has reached the point where inducer activity can be predicted based on chemical structure. Precise details of these mechanisms continue to be elucidated. While several compounds are currently under consideration for their chemoprotective potential, oltipraz appears to have the most promise as an easily administered, long lasting, potent, and, therefore, effective modality for chemoprevention.

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