CHEMOPROTECTION BY ORGANOSULFUR INDUCERS OF PHASE 2 ENZYMES: DITHIOLETHIONES AND DITHIINS

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SUMMARY

One of the major mechanisms of protection against carcinogenesis, mutagenesis, and other forms of toxicity mediated by carcinogens is the induction of enzymes involved in their metabolism, particularly phase 2 enzymes such as glutathione *S*-transferases, UDP-glucuronosyl transferases, and quinone reductases. Animal studies indicate that induction of phase 2 enzymes is a sufficient condition for obtaining chemoprevention and can be achieved by administering any of a diverse array of naturally-occurring and synthetic chemopreventive agents. Alliaceous and cruciferous plants are rich in organosulfur compounds with inducer activity. Indeed, monitoring of enzyme induction has led to the recognition or isolation of novel, potent chemopreventive agents such as 1,2-dithiole-3-thiones, dithiins and the isothiocyanate sulforaphane. For example, oltipraz, a substituted 1,2-dithiole-3-thione originally developed as an antischistosomal agent, possesses chemopreventive activity against different classes of carcinogens targeting multiple organs. Mechanistic studies in rodent models for chemoprevention of aflatoxin B_1 (AFB_1)-induced hepatocarcinogenesis by oltipraz indicates that increased expression of phase 2 genes is of central importance, although inhibition of phase 1 activation of aflatoxin B_1 can also contribute to protection. Exposure of rodents to 1,2-dithiole-3-thiones triggers nuclear accumulation of the transcription factor Nrf2 and its enhanced binding to the Antioxidant Response Element, leading to transcriptional activation of a score of genes involved in carcinogen detoxification and attenuation of oxidative stress. *Nrf2*-deficient mice fail to induce many of these genes in response to oltipraz and the impact of this genotype on the chemopreventive efficacy of dithiolethiones is currently under investigation. To test the hypothesis that enzyme induction is a useful strategy for chemoprevention in humans, three key elements are necessary: a candidate agent, an at-risk population and modulatable intermediate endpoints. Towards this end, a placebo-controlled, double blind clinical trial of oltipraz was conducted in residents of Qidong, P.R. China who are exposed to dietary aflatoxins and who are at high risk for the development of liver cancer. Oltipraz significantly enhanced excretion of a phase 2 product, aflatoxin-mercapturic acid, a derivative of the aflatoxin-glutathione conjugate, in the urine of study participants administered 125 mg oltipraz by mouth daily. Adminis-

tration of 500 mg oltipraz once a week led to a significant reduction in the excretion of the primary oxidative metabolite of AFB_1, aflatoxin M_1, when measured shortly after drug administration. While this study highlighted the general feasibility of inducing phase 2 enzymes in humans, a longer term intervention is addressing whether protective alterations in aflatoxin metabolism can be sustained for extended periods of time in this high-risk population. Food-based approaches to chemoprotection, targeted both to the general population and high-risk individuals, offer many practical advantages compared to the use of pharmaceutical agents. Thus, identification and utilization of naturally-occurring organosulfur chemoprotectors including dithiins should be a high priority.

KEY WORDS

oltipraz, 1,2-dithiole-3-thiones, dithiins, chemoprotection, *Allium*, Cruciferae

1. ROLE OF ENZYME INDUCTION IN CANCER CHEMOPROTECTION

The multiple stages of carcinogenesis offer many potential strategies for protection. However, in the majority of tests conducted in animal models, protection has been achieved by administering the chemopreventive agent prior to and/or concurrently with the exposure to the carcinogen. Given this temporal relationship between administration of anticarcinogen and carcinogen, it seems likely that these agents act, at least in part, to alter the metabolism and disposition of carcinogens, thereby altering events critical to the initial interactions of chemical carcinogens with biomolecules.

Many chemicals require metabolic activation to electrophilic intermediates to exert carcinogenic activity /1/. If not detoxified, these intermediates can react with and thereby functionally modify nucleophilic moieties on critical biomolecules. Nucleophilic groups in DNA are among those targeted by electrophiles; the interaction of carcinogen metabolites with DNA can cause point mutations and other genetic lesions, which can result in activation of protooncogenes and inactivation or loss of tumor suppressor genes. The importance of metabolic activation in carcinogenesis is highlighted by the fact that

target organ specificities and even species susceptibilities can be determined through the presence or absence of metabolic pathways. Commonly, the processing of chemicals to proximate carcinogens involves an initial two-electron oxidation. This phase 1 reaction can be catalyzed by a number of enzymes, particularly those comprising the cytochrome P450 superfamily. A second metabolic step involves the transfer or conjugation of an endogenous, water-soluble substrate to the functional group introduced during phase 1 biotransformation, thereby facilitating elimination of the carcinogen. These phase 2 reactions, which include sulfation, acetylation, glucuronidation, and conjugation with glutathione, typically lead to carcinogen detoxification. Thus, the amount of ultimate carcinogen available for interaction with its target represents, in part, a balance between competing activating and detoxifying reactions. While this balance is under genetic control, it is readily modulated by a variety of factors including nutritional status, age, hormones, and exposure to drugs or other xenobiotics /2/. In this setting, chemopreventive agents can profoundly modulate the constitutive metabolic balance between activation and inactivation of carcinogens through their actions on both phase 1 and 2 enzymes.

As summarized in Table 1, there is now expansive experimental evidence supporting the concept that induction of phase 2 enzymes is an effective means to enhance resistance to environmental carcinogens. Most important, screening of pharmaceutical agents and natural products has led to the recognition or isolation of novel classes of chemoprotective agents and/or more potent analogs of known protective compounds. The prediction that the substituted 1,2-dithiole-3-thione, oltipraz (5-(2-pyrazinyl)-4-methyl-1,2-dithiole-3-thione), would be an effective chemoprotective agent was predicated upon its activity as an inducer of phase 2 enzymes in multiple tissues following administration to mice /3/.

2. OCCURRENCE OF DITHIOLETHIONES AND DITHIINS IN CRUCIFERAE AND *ALLIUM* PLANTS AND IDENTIFICATION AS INDUCERS

In 1958, Jirousek and Stárka reported, without experimental details, the isolation of two 1,2-dithiole-3-thiones from cabbage and Brussels sprouts /4/. On the basis of UV spectrum, polarographic and

TABLE 1

Evidence for a major role for induction of phase 2 enzymes in chemoprotection

Natural sensitivity or resistance to carcinogens such as AFB_1 correlates with expression of phase 2 enzymes (*e.g.*, GSTs)
Overexpression of GSTs protects cells against carcinogen DNA adduct formation and cytotoxicty (*e.g.*, benzo[a]pyrene, AFB_1 and PhIP)
Targeted deletion of GST genes or genes for transcription factors controlling their expression enhance sensitivity to DNA adduct formation and carcinogenicity (*e.g.*, GST-P and Nrf2)
Deficiencies in expression of phase 2 enzymes may be important determinants of susceptibility to cancer in humans (*e.g.*, polymorphisms in GSTs and QR)
Monitoring of phase 2 enzyme induction has led to the recognition or isolation of novel chemoprotective agents (*e.g.*, dithiolethiones, dithiins, sulforaphane, terpenoids, dimethyl fumarate, phytoalexins)

chromatographic behavior, and several simple chemical and color tests, these workers tentatively identified one of the dithiolethiones as the unsubstituted 3*H*-1,2-dithiole-3-thione (D3T). The second dithiolethione was suggested to have an oxygenated aromatic ring at position 5. Further work on the structure of these two substances has not appeared in the literature. Several years ago, Marks and coworkers /5/, using current separation and detection techniques, reinvestigated the question of whether the unsubstituted dithiolethione occurs naturally in cabbage. These workers were able to show that 3*H*-1,2-dithiole-3-thione was not present in their cabbage sample at concentrations greater than 1 ppb. However, in view of the many factors that might influence the levels of dithiolethiones in plants (*e.g.*, there is >1000-fold variation in the level of the chemoprotective organosulfur compound sulforaphane as a function of age and cultivar of broccoli), the question of whether dithiolethiones occur naturally in plants of the Brassica family must be regarded as unresolved.

Nonetheless, the work of Marks and coworkers /5/ makes it less likely that structurally simple dithiolethiones are major components of these plant species.

Another possibility that seems not to have been considered is that chemoprotective agents related to the dithiolethiones may arise *in vivo* by biotransformation of naturally-occurring precursors. For example, 3*H*-1,2-dithiole has been detected in cooked white asparagus /6/, in garlic /7-9/, and in the Amazonian garlic bush /10/. Oxidation of this compound to produce 1,2-dithiole-3-thione, a known chemoprotective agent /11/, might be expected to be facile. Similarly, the methylated derivatives of 3*H*-1,2-dithiole found in garlic oil /9/ might be expected to give rise to methylated 1,2-dithiole-3-thiones *in vivo*.

Members of the *Allium* genus, including onion, garlic, leek, chive, shallot, and scallion, are very rich in organosulfur compounds. Although the chemoprotective properties of alliaceous plants seem well established, it is not clear which component or components are responsible for the observed activity /12/. Since the most obvious characteristic of these species is their aroma and flavor, it is logical to assume that the compounds responsible for these features are also responsible for their chemoprotective properties. A survey of *Allium* components revealed a potentially important class of organosulfur compounds, the dithiins. These are cyclic enethiol ethers possessing the grouping C=C-S- in a ring. Moreover, like the dithiolethiones, some of these compounds possess a disulfide bond, also located in the ring. The structures of dithiins A and B as well as of two important dithiolethiones, oltipraz and D3T, are shown in Figure 1. Although relative minor components (1-3%) of some *Allium* plants such as garlic, the chemical similarities of dithiins to dithiolethiones warranted their evaluation as enzyme inducers and chemoprotectors.

Several synthetic, substituted 1,2-dithiole-3-thiones exhibit antioxidant, chemotherapeutic, and radioprotective properties /13-16/. One of these agents, oltipraz, has shown significant antischistosomal activity in experimental animals and in humans. Cure rates of up to 90% have been achieved with a single dose of oltipraz in field trials /17/. During the course of studies on the mechanisms of antischistosomal activity of 1,2-dithiole-3-thiones, Bueding *et al.* /18/ initially noted that administration of oltipraz to mice infected with *Schistosoma mansoni* caused a reduction in the glutathione stores of

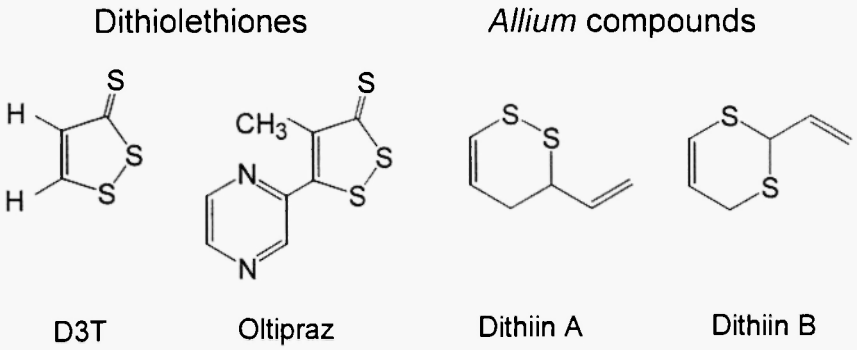

Fig. 1: Structures of 1,2-dithiole-3-thiones and dithiins.

the parasites, but increased levels of glutathione in many tissues of the host. Subsequent studies demonstrated that oltipraz and many related dithiolethiones were potent inducers of enzymes concerned with the maintenance of reduced gluathione pools as well as enzymes important to the detoxification of electrophiles and free radicals. Genes now recognized to be induced by dithiolethiones *in vivo* include alpha, mu and pi isoforms of glutathione *S*-transferases (GSTs), UDP-glucuronosyl transferases, NAD(P)H:quinone reductase (QR), epoxide hydrolase, aflatoxin aldehyde reductase, γ-glutamylcysteine synthase, manganese superoxide dismutase, catalase, heavy and light chains of ferritin, and leukotriene B_4 dehydrogenase /18-21/. The activities of oltipraz and D3T as well as dithiins A and B as inducers of two prototypical phase 2 enzymes, GST and QR, in the liver of rats are shown in Table 2.

3. PROTECTION AGAINST EXPERIMENTAL CARCINOGENESIS BY DITHIOLETHIONES AND DITHIINS

The biochemical manifestations of oltipraz in schistosome-infected mice prompted Bueding to predict that this drug might have cancer chemoprotective properties. The initial confirmation that 1,2-dithiole-3-thiones such as oltipraz may exert chemoprotective effects *in vivo* came from the demonstration that oltipraz protected against the hepatotoxicity of carbon tetrachloride and acetaminophen in mice /22/.

TABLE 2

Induction of the phase 2 enzymes GST and QR, and protection against AFB_1 adduct formation in rat liver, by dithiolethiones and dithiins

	QR activity (nmol/min/mg protein)		**GST activity** (nmol/min/mg protein)		**AFB_1-DNA adducts** (pmol/mg DNA)	
Control	202 ± 12[a]		326 ± 10		27.4 ± 6.1	
Dithiin A	313 ± 42	(1.6)[b]	390 ± 17	(1.2)	10.5 ± 1.0[c]	(62%)[d]
Dithiin B	483 ± 32	(2.4)	345 ± 18	(1.1)	16.6 ± 1.6	(31%)
Oltipraz	693 ± 50[c]	(3.4)	609 ± 25[c]	(1.9)	6.9 ± 2.1[c]	(75%)
D3T	1337 ± 76[c]	(6.6)	655 ± 41[c]	(2.0)	1.6 ± 0.5[c]	(94%)

Organosulfur compounds (0.2 mmole/kg-body weight) were gavaged on days 6, 4, 2 and 7 h prior to autopsy. AFB_1 (25 μg) was administered by gavage 2 h prior to autopsy.

[a] Mean ± SE (n=5)

[b] Values in parentheses are ratios of treated/control enzyme activities.

[c] $p < 0.01$, differs from control.

[d] Percent reduction of adduct burden as compared to control.

Subsequent studies have demonstrated protection by oltipraz against the acute hepatotoxicities of allyl alcohol and acetaminophen in the hamster /23/ and AFB_1 in the rat /24/. Toxin-induced elevations in liver function tests were blunted in all cases, clearly indicating protection. Pretreatment with oltipraz also substantially reduced the mortality produced by either acute or chronic exposure to AFB_1 /24/.

To directly test the cancer chemoprotective efficacy of oltipraz, Wattenberg and Bueding /25/ examined the capacity of oltipraz to inhibit carcinogen-induced neoplasia in mice. Oltipraz was administered either 24 or 48 hours before treatment with each of three chemically diverse carcinogens: diethylnitrosamine, uracil mustard, and benzo[a]pyrene. This sequence of oltipraz and carcinogen administration was repeated once a week for 4 to 5 weeks. Oltipraz reduced by nearly 70% the number of both pulmonary adenomas and tumors of the forestomach induced by benzo[a]pyrene. Pulmonary

adenoma formation induced by uracil mustard or diethylnitrosamine was also significantly reduced by oltipraz pretreatment, but to a lesser degree. As reviewed elsewhere /26,27/, oltipraz has now shown chemoprotective activity against different classes of carcinogens targeting the trachea, lung, stomach, small intestine, colon, pancreas, liver, urinary bladder, mammary gland, hematopoietic cells, and skin. The most dramatic actions occur in the colon and liver, where dietary administration results in significant reductions in both tumor incidence and multiplicity.

Dietary concentrations of 200 and 400 ppm oltipraz significantly reduced tumor incidence and multiplicity in azoxymethane-induced intestinal carcinogenesis /28/, while 750 ppm oltipraz afforded complete protection against AFB_1-induced hepatocarcinogenesis in F344 rats /29/. Moreover, dietary concentrations as low as 100 ppm engendered >90% reduction in the hepatic burden of presumptive preneoplastic lesions in the aflatoxin model /30/. As depicted in Figure 2, administration by gavage (before and during exposure of rats

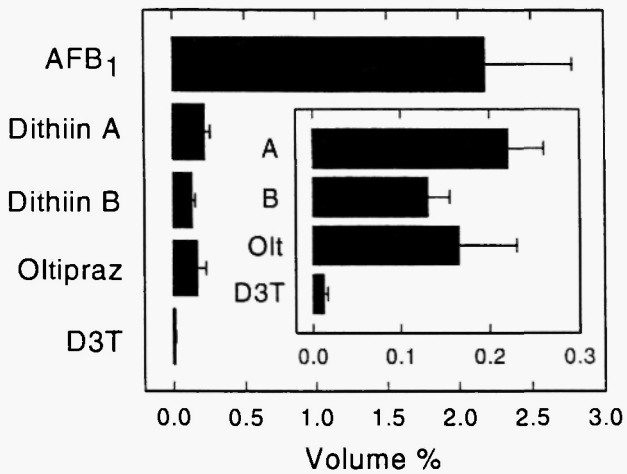

Fig. 2: Inhibition of hepatic tumorigenesis by dithiolethiones and dithiins. Male F344 rats were gavaged 8 times with 0.2 mmol of the organosulfur compounds/kg-body weight, *q.o.d.* Beginning after the third dose, rats were gavaged with 25 μg of AFB_1 for 10 consecutive days. All animals were killed 5 weeks after the last dose of AFB_1 and livers were analyzed by light microscopy to identify and quantify the presumptive preneoplastic foci expressing the placental form of GST (GST-P) /10/.

to AFB_1) of 0.2 mmol/kg-body weight of oltipraz, 3*H*-1,2-dithiole-3-thione, dithiin A and dithiin B significantly reduced the hepatic burden of GST-P positive lesions. In accord with other bioassay results /10/, 3*H*-1,2-dithiole-3-thione was the most effective organosulfur compound examined. Nonetheless, both dithiins as well as oltipraz reduced presumptive prenoplastic lesion burden by >90%.

4. MECHANISMS OF PHASE 2 ENZYME INDUCTION BY ORGANOSULFUR COMPOUNDS

Initial molecular studies in rats and subsequent studies in humans indicated that increases in mRNA and protein levels of several phase 2 genes in response to dithiolethiones and other chemoprotective agents were mediated through the transcriptional activation of these genes /31,32/. Two families of phase 2 enzyme inducers exist. Prochaska and Talalay /33/ have coined the terms *bifunctional* and *monofunctional* inducers to describe these families. Bifunctional inducers (*e.g.*, polycyclic hydrocarbons, dioxins, azo dyes, flavones) can all be characterized as large planar polycyclic aromatics, and they elevate phase 2 as well as selected phase 1 enzymatic activities such as aryl hydrocarbon hydroxylase. These compounds are potent ligands for the aryl hydrocarbon (*Ah*) receptor, and the direct participation of the *Ah* receptor in the activation of aryl hydrocarbon hydroxylase gene transcription has been demonstrated /34/. Moreover, since phase 2 enzyme inducibility by bifunctional inducers segregates in mice that possess functional *Ah* receptors, it is presumed that these enzymes are under the direct control of the *Ah* receptor. Monofunctional inducers (phenols, lactones, isothiocyanates, dithiocarbamates, dithiins, and 1,2-dithiole-3-thiones) elevate phase 2 enzymatic activities without significantly elevating the aforementioned phase 1 activities and do not possess an obvious defining structural characteristic. Several, but not all, phase 2 enzyme inducers have also been shown to inhibit the activities of some cytochrome P450 enzymes /35,36/, but the contribution of this component to their chemoprotective actions may be limited /37/.

There is no evidence at this time to suggest that monofunctional inducers function through a receptor-mediated pathway. However, Talalay *et al.* /38/ have identified a chemical signal present in some monofunctional inducers: the presence or acquisition of an electro-

philic center. Many monofunctional inducers are Michael reaction acceptors (*e.g.*, olefins or acetylenes conjugated to an electron withdrawing group such as a carbonyl function) and potency is generally paralleled by their efficiency as Michael reaction acceptors. These generalizations can account for the inducer activity of many types of chemopreventive agents and have led to the identification of other novel classes of inducers, including acrylates, fumarates, maleates, vinyl ketones and vinyl sulfones. Other classes of monofunctional inducers, notably peroxides, vicinal dimercaptans, heavy metals, arsenicals and the 1,2-dithiole-3-thiones, exhibit a common capacity for reaction with sulfhydryls by either oxidoreduction or alkylation /39/.

Several regulatory elements controlling the expression and inducibility of the Ya subunit of rodent GSTs by bifunctional and monofunctional inducers have been characterized /40,41/. A 41 bp element in the 5′-flanking region of the rat GST Ya gene, termed the "Antioxidant Response Element" (ARE), has been identified using a series of 5′ deletion mutants fused to the chloramphenicol acetyl transferase gene and then transfected into hepatoma cells. To date, AREs have been detected in the promoters of nearly a score of genes. All share a common RTGACnnnGC motif /42/. Prestera *et al.* /39/ observed that members of eight distinct chemical classes of monofunctional inducers stimulate expression of a reporter gene, growth hormone, through the ARE when an ARE-growth hormone construct is transfected into murine hepatoma cells. Comparisons of potency for induction of reporter gene expression and QR activity in the same cells indicated a striking concordance over a 4-log range for the two endpoints. Further, when 25 dithiolethiones and related analogs were evaluated for their activities as inducers of QR and as activators of the transfected ARE construct in this model system, a strong correlation was seen in the potencies of 21 active 1,2-dithiole-3-thiones to elicit the two responses /43/. Moreover, no dithiolethiones were inactive in only one system. Collectively, these results suggest that the ARE mediates most, if not all, of the phase 2 enzyme inducer activity of these compounds.

The transcription factors that bind to the ARE consensus sequence have not been fully identified and are likely to vary between cell types and species. Nrf1 and Nrf2, members of the basic-leucine zipper NF-E2 family of transcription factors that regulate expression of globin

genes during erythroid development /44,45/, are known to bind and activate the ARE. Overexpression of either Nrf1 or Nrf2 in human hepatoma cells enhances the basal and inducible transcriptional activity of an ARE reporter gene /46/. Because other basic-leucine zipper transcription factors typically form heterodimers, Nrf1/Nrf2 may also dimerize with other factors in order to activate the ARE. The tissue specific expression profiles of a number of transcription factors suggest that an Nrf2/small Maf heterodimer best mirrors the pattern for induction of phase 2 genes *in vivo*. Using recombinant Nrf2 and mafK proteins in an electromobility shift assay with the promoter sequence of the murine GST Ya gene, Yamamoto and colleagues /47/ demonstrated binding of the heterodimer complex to this promoter. Oligonucleotides containing the ARE effectively competed for the binding of this heterodimeric complex to the GST Ya promoter. This issue has also been directly examined by exploring the effects of disruption of the *nrf2* gene *in vivo* on induction of phase 2 enzymes /47/. 3*H*-1,2-Dithiole-3-thione vigorously induced GST, QR and other phase 2 activities and mRNA expression in livers of wild type mice, but not in the homozygous *nrf2*-mutant mice /48/. Collectively, these results suggest that Nrf2/small Maf heterodimers may be one of the key regulators of phase 2 gene expression. However, the details of how the chemical signals produced by enzyme inducers interact with this molecular signaling pathway remain to be elucidated.

5. CLINICAL STUDIES WITH DITHIOLETHIONES

Phase I clinical trials are designed to characterize the pharmacokinetics and tolerability of the chemopreventive agent /49/. Dose and schedule of administration are based on achieving plasma drug levels that are very likely to be safe and likely to show efficacy based upon preclinical studies in *in vivo* and *in vitro* models. Single dose Phase I studies with oltipraz indicated that administration of 500 mg orally would produce a peak plasma concentration of about 20 μM while 125 mg produced a peak of only 2 μM /50/. Dose escalation studies with repeated administration suggested that 125 mg oltipraz was close to the maximum tolerated dose following administration daily for 6 months /51/. Although the steady-state concentrations of oltipraz are rather low, reflecting the rapid clearance of the drug from the body, the peak concentrations following administration of 125-500 mg

oltipraz/day are comparable to those required to induce phase 2 enzyme expression in rodent and human cell culture models. Gupta *et al.* /50/ reported a doubling in the specific activity of GST in peripheral lymphocytes obtained from Phase I study participants 10 h after administration of 125 mg oltipraz. Elevations in levels of glutathione were also observed. In a dose-finding study with 125, 250, 500 or 1,000 mg/m^2 oltipraz as a single oral dose; increases in GST activities were seen in peripheral mononuclear cells and colon mucosa biopsies at the lower, but not higher, doses /52/. Four to five fold increases in mRNA transcripts for γ-glutamylcysteine synthetase and quinone reductase were seen in colon mucosa at 250 mg/m^2. Higher doses were not more effective. mRNA content increased after dosing to reach a peak on day 2 and declined to baseline levels over the subsequent week. Collectively, these results demonstrate that oltipraz triggers the expression of phase 2 enzymes in humans.

To more directly test the hypothesis that oltipraz can modulate the metabolism of carcinogens in humans, we conducted a Phase IIa intervention trial with oltipraz. The primary goals of Phase IIa studies, in addition to establishing the general feasibility of conducting biomarker measurements, are to characterize the dose-response of biomarker modulation, the tolerance or loss of effect of biomarker modulation over time, and drug toxicity with chronic administration /49/. Study participants for this Phase IIa trial were recruited from residents of Daxin Township, Qidong, P.R.China, where dietary exposures to aflatoxins and risk for hepatocellular carcinoma are high /53/. This trial with oltipraz was a randomized, placebo-controlled, double-blind study. Two hundred forty adults in good general health without any history of major chronic illnesses and with detectable serum aflatoxin-albumin adduct levels at baseline were randomized into one of three intervention arms: (A) placebo; (B) 125 mg oltipraz administered daily; or (C) 500 mg oltipraz administered weekly. The methods, participant characteristics, compliance and adverse events as well as initial results on modulation of biomarkers from this trial have been reported /54-56/.

Urine samples were collected at 2-week intervals throughout the active 8-week intervention period as well as during the 8-week follow-up period. To date, aflatoxin metabolites have been assayed in urine samples from one cross-section in time: after the first month on the active intervention /56/. Sequential immunoaffinity and liquid chro-

matography coupled to mass spectrometry and fluorescence detection were used to identify and quantify the phase 1 metabolite, AFM_1, and the phase 2 metabolite, aflatoxin-mercapturic acid, in these urine samples. One month of weekly administration of 500 mg oltipraz led to a significant decrease (51%) in median levels of AFM_1 excreted in urine compared to placebo, but had no effects on levels of aflatoxin-mercapturic acid. By contrast, daily intervention with 125 mg of oltipraz led to a significant, 2.6-fold increase in the median levels of aflatoxin-mercapturic acid excretion, but had no pronounced effect on excreted AFM_1 levels. Thus, sustained low dose oltipraz increased phase 2 conjugation of aflatoxin, yielding higher levels of mercapturic acid, but did not appreciably affect formation of AFM_1. Intermittent, high dose oltipraz inhibited the phase 1 activation of aflatoxin, as reflected by lowered excretion of aflatoxin M_1. Potential effects of induction of phase 2 enzymes, *i.e.*, GSTs, in this arm appear to be masked by the inhibition of aflatoxin-8,9-epoxide formation. Indeed, Langouet *et al.* /35/ have reported 2 to 4-fold increases in the protein levels of alpha and mu classes of GSTs in primary human hepatocytes treated with 50 μM oltipraz, but found this inductive effect was not associated with an increased formation of aflatoxin-glutathione conjugates because it was overridden by the inhibitory effect of oltipraz on AFB_1 activation. As previously noted in experimental models, it would appear that both mechanisms are likely to contribute to reduced genotoxicity and other chemopreventive actions of this drug.

In addition to the cross-sectional measurements of effects of oltipraz on urinary aflatoxin metabolites, longitudinal analyses of effects of slopes of aflatoxin-albumin adducts have been conducted /55/. There were no consistent changes in albumin-adduct levels in the placebo arm, nor in the 125 mg oltipraz daily arm over the 16-week observation period. However, individuals receiving 500 mg oltipraz once a week for 8 weeks showed a triphasic response to oltipraz. No effect was observe during the first month of the intervention, whereas a significant diminution in adduct levels was observed during the second month of active intervention and during the first month of follow-up. A partial rebound in adduct levels toward baseline values was observed during the second month of follow-up. Linear regression models up to week 13 confirmed a significant weekly decline in biomarker levels in this group. Because modulation of aflatoxin-albumin adducts and diminution of AFM_1 levels were both observed

in the 500 mg weekly arm, comparisons were made of the albumin adduct slopes with levels of AFM_1. Individuals ranked in the lowest third of AFM_1 levels showed the greatest decline in aflatoxin-albumin adduct levels. This moderate correlation suggests that inhibition of cytochrome P450 activity could contribute to the observed decline in albumin adducts.

The larger question of whether modulation of carcinogen metabolism, either by enzyme inhibition or enzyme induction, can substantively reduce the risk of cancer in individuals at high risk for exposure to environmental carcinogens remains open. A Phase IIb intervention trial with oltipraz in Qidong began in 1999 and is evaluating the efficacy of 250 mg or 500 mg oltipraz given weekly to modulate levels of aflatoxin biomarkers over a one-year period in comparison to a placebo group. Biomarkers exploring the multiple mechanisms of action of oltipraz will be used. The Phase IIb study should serve as a foundation for selecting a safe and effective dose for a Phase III trial. Phase III trials are used to actually establish the efficacy of the drug in chemoprevention, and unless the biomarker is a strong predictor of cancer prevention, rely on reduced incidence of disease as the endpoint.

6. CONCLUSIONS

1,2-Dithiole-3-thiones are five-membered cyclic sulfur-containing compounds with antioxidant, chemotherapeutic, radioprotective and cancer chemoprotective properties. 1,2-Dithiole-3-thiones are unusual among classes of cancer chemoprotective compounds in that they were first identified on the basis of their biochemical properties, which include induction of carcinogen detoxification enzymes and anti-oxidative enzymes, and subsequently evaluated to confirm their anti-carcinogenic activities. Although dithiolethiones have been identified in plants, the most actively studied members of this chemical class are synthetic agents. One such compound, oltipraz, has been extensively evaluated in clinical trials and shown to modify the disposition of the dietary carcinogen, AFB_1, in exposed individuals, in part through induction of phase 2 enzymes. As a pharmaceutical, oltipraz will most likely be targeted towards individuals at high risk for occupational or environmental exposures to genotoxic carcinogens. Conversely, the apparently limited presence of 1,2-dithiole-3-thiones in natural

products suggests that they are not likely to contribute substantively to nutritionally-based interventions in the general population. Nonetheless, the abundance of naturally-occurring inducers of phase 2 and antioxidative enzymes in plants (*e.g.*, isothiocyanates, flavones, indoles, cinnamates, coumarins, terpenes and dithiins) suggests that consumption of cruciferous and alliaceous vegetables rich in organosulfur compounds should enhance the resistance of humans to carcinogenesis.

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