Mechanism of Protection against Aflatoxin Tumorigenicity in Rats Fed 5-(2-Pyrazinyl)-4-methyl-1,2-dithiol-3-thione (Oltipraz) and Related 1,2-Dithiol-3-thiones and 1,2-Dithiol-3-ones


Abstract

1,2-Dithiol-3-thiones, reported constituents of cruciferous vegetables, are five-membered cyclic sulfur-containing compounds with antioxidant, chemotherapeutic, and chemoprotective activities. The effects of dietary administration of a substituted 1,2-dithiol-3-thione, oltipraz [5-(2-pyrazinyl)-4-methyl-1,2-dithiol-3-thione], a potent antischistosomal agent, on aflatoxin B1 (AFB1) metabolism, DNA adduct formation, and hepatic tumorigenesis were examined in male F344 rats. Rats were fed graded doses of oltipraz (0.01–0.1%) for 4 wk. During the second and third wk of oltipraz feeding rats were gavaged with 250 μg of AFB1/kg five times a wk. Rats were finally restored to control diet 1 wk after cessation of AFB1 dosing. At 4 months focal areas of hepatocellular alteration were identified and quantitated by staining sections of liver for γ-glutamyl transpeptidase activity. Treatment with oltipraz at all doses reduced by >90% the volume of liver occupied by γ-glutamyl transpeptidase-positive foci. Levels of AFB1 bound to hepatic DNA were reduced between 40 and 80% in animals fed increasing doses of dietary oltipraz (0.01–0.1%) for 1 wk prior to a single exposure to AFB1. Feeding of the higher levels of oltipraz led to marked increases in the specific activity of glutathione S-transferases, presumably serving to facilitate the detoxication of the ultimate electrophilic form of AFB1, the 8,9-oxide. At low dietary concentrations of oltipraz (0.01%), the only inductive effects seen were on the activities of selected cytochrome P-450 monooxygenases. Therefore, the protection afforded by oltipraz may be due to both the enhancement of electrophile detoxication pathways as well as modified oxidative metabolism of AFB1. In vitro metabolism studies with hepatic postmitochondrial supernatant, low-dose oltipraz pretreatment facilitated the oxidative production of aflatoxin P1 and Q1, but not M1, from AFB1. High-dose (0.1%) oltipraz pretreatment enhanced the primary metabolism of AFB1 to aflatoxins P1, M1, and Q1 as well as the formation of chloroform-insoluble metabolites. Feeding studies with a series of 1,2-dithiol-3-thione and 1,2-dithiol-3-one derivatives of oltipraz demonstrated that the inductive activity for cytochrome P-450-dependent monooxygenases and electrophile detoxication enzymes, such as glutathione S-transferases, could be readily separated by minor modifications of the 1,2-dithiol-3-thione structure. The unsubstituted 1,2-dithiol-3-thione nucleus strongly induced electrophile detoxication enzymes, but not the monooxygenases, and was the most effective inhibitor of the binding of AFB1 to hepatic DNA in vivo. The high potency, low toxicity, and selectivity of enzyme inductive effects may render some of the 1,2-dithiol-3-thiones as excellent compounds for chemoprotection in humans.

Introduction

Several substituted 1,2-dithiol-3-thiones are used medicinally as antischistosomal agents, choleretics, and to stimulate sialic acid secretion (2–5). Other 1,2-dithiol-3-thiones are used as antioxidant additives in rubber, metals, and commercial oils and greases (2). During the course of studies on the mechanisms of antischistosomal activity of one 1,2-dithiol-3-thione, oltipraz,3 it was noted by Bueding et al. (3) that this agent provoked elevations in cellular thiol levels in host tissues in mice infected with the parasite. Subsequent studies demonstrated that oltipraz and related 1,2-dithiol-3-thiones were potent inducers of enzymes concerned with the maintenance of reduced glutathione pools as well as enzymes important to electrophile detoxication (6). Notably, elevated NAD(P)H:quinone reductase, epoxide hydrolase, and glutathione S-transferase activities have been observed in tissues of rats and mice treated with oltipraz. The murine hepatoma cell line Hepa 1c1c7 also responds to exposure with oltipraz by elevation of NAD(P)H:quinone reductase (7). In contrast to the marked inductions of these Phase II enzymes,4 cytochrome P-450 levels and Phase I enzyme activities are only modestly elevated by oltipraz (8).

The elevation of electrophile detoxication systems has been recognized as characteristic of the action of chemoprotective agents as exemplified by the antioxidants BHA, BHT, and ethoxyquin (8–12). Like these compounds, oltipraz has recently been shown to protect against the development of pulmonary and forestomach cancers induced by benzo(a)pyrene in female ICR/Ha mice (15). Oltipraz and a related 1,2-dithiol-3-thione, anethole dihydrothiole, also reduce the hepatic toxicity of acetaldehyde and carbon tetrachloride in mice (14). 1,2-Dithiol-3-thiones have been reported to be present in cruciferous vegetables, such as cabbage and Brussels sprouts (15). Epidemiological evidence suggests that consumption of these vegetables is associated with a reduction in the incidence of cancer in humans (16–18). In an experimental setting, the inclusion of lyophilized cabbage or cauliflower in rat diet reduces AFB1-induced carcino genesis (19). Feeding of lyophilized cabbage to mice mimics the effects of oltipraz in that induction of NAD(P)H:quinone reductase and glutathione S-transferase activities as well as elevation of glutathione levels is observed (6). Other studies have demonstrated an increased activity of cytochrome P-450-dependent monooxygenases in individuals consuming a cruciferous-supplemented diet (20). Yet, whether these and the chemoprotective effects caused by feeding of cruciferous vegetables can be attributed to their content of 1,2-dithiol-3-thiones remains uncertain since vegetables contain many other chemoprotective compounds, including indoles, phenols, aromatic

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3 The abbreviations used are: oltipraz, 5-(2-pyrazinyl)-4-methyl-1,2-dithiol-3-thione; aflatoxin B1 (AFB1), aflatoxin B2 (other aflatoxins are similarly abbreviated); CDNB, 1-chloro-2,4-dinitrobenzene; DCNB, 3,4-dichloronitrobenzene; GGT, γ-glutamyl transpeptidase.

4 The enzymes involved in the metabolism of xenobiotics have been classified into two broad categories. Phase I enzymes (which include the cytochromes P-450) functionally detoxify compounds by oxidation, reduction, or hydrolysis, whereas Phase II enzymes carry out the conjugations of functionalized compounds with endogenous ligands (e.g., glucuronic acid, glutathione, and sulfate).
isothiocyanates, flavonoids, and protease inhibitors (18).

The carcinogenicity of the potent hepatocarcinogen, AFB₁, can be inhibited by concurrent feeding of either BHA, BHT, or ethoxyquin (21, 22). Consistent with these findings we have reported that these antioxidants, as well as oltipraz, substantially inhibit the formation of aflatoxin-DNA adducts in target tissues in the rat (8). A major determinant for these protective actions appears to be elevation of glutathione S-transferase activities, presumably facilitating the detoxication and elimination of the ultimate electrophilic form of aflatoxin, the 8,9-oxide (12). In the present studies we demonstrate dramatic protective effects of dietary oltipraz on the formation of GGT-positive foci in the livers of aflatoxin-exposed rats. Mechanistic studies indicate that this protection afforded by oltipraz is due to both the enhancement of electrophile detoxication pathways as well as a reduction in aflatoxin activation. However, studies with analogues of oltipraz demonstrate that pronounced protection can occur solely through the induction of Phase II enzymes without concomitant effects on the Phase I monooxygenases.

### MATERIALS AND METHODS

**Chemicals.** Oltipraz (RP 35,972) and two of its analogues [5-(2-pyrazinyl)-1,2-dithiol-3-thione (RP 33,851) and 5-(2-pyrazinyl)-4-methyl-1,2-dithiol-3-thione (RP 36,652)] were kindly provided by Rhône-Poulenc, Paris, France while anethole dithiolthione, 4-phenyl-1,2-dithiol-3-thione (129L), 5-phenyl-1,2-dithiol-3-thione (116L), 1,2-dithiol-3-thione, and 1,2-dithiol-3-one were the generous gifts of Latema, Suresnes, France. The structures of these compounds are presented in Fig. 1. All 1,2-dithiol-3-thiones and 1,2-dithiol-3-ones were subjected to high performance liquid chromatography using a 5-μm CIS ultra-sphere column. The compounds were eluted with 65% methanol/H₂O at a flow rate of 1 ml/min at ambient temperature. The retention times were 4.3, 10.1, 17.8, 20.6, 7.8, 10.3, 5.9, and 3.5 min, for compounds 1–8, respectively, while the void volume eluted at 1.8 min. Using an HP3390A integrator the purity of all compounds was greater than 97% as adjudged by integrated areas at 254 nm. The UV/visible absorption characteristics of the compounds were determined with a scanning Beckman DU-7 spectrophotometer and were identical to published values (23). NADH, NADPH, aniline, aminopyrine, glutathione reductase, UDP-glucuronic acid, and p-nitrophenyl-β-D-glucuronide were obtained from Sigma Chemical Co. (St. Louis, MO), p-nitrophenol and CDNB from Eastman Organic Chemicals (Rochester, NY), and DCNB and AFB₁ from Aldrich Chemical Co. (Milwaukee, WI). [³²⁵]AFB₁ (50 mCi/mmol) and [³²⁵]HAFB₁ (24 Ci/mmol) were purchased from Moravek Biochemicals (City of Hope, CA) and [³²⁵]Cystereine 7,8-oxide from Amersham (Arlington Heights, IL). All other chemicals were of the highest quality obtainable commercially.

**Animals and Diets.** Male F344 rats, 75–100 g (Charles River Breeding Laboratories, Wilmington, MA), were housed singly in wire-bottomed cages under controlled conditions of temperature, humidity, and lighting. Food and distilled water were available ad libitum. Purified powdered diets of the AIN-76A formulation lacking the recommended addition of 0.02% ethoxyquin (24) were used, and fresh diet was provided to the animals at least every other day. Oltipraz was incorporated in the AIN-76A diet at the indicated concentrations and stored at 4°C. Rats were acclimated to the control diet for 1 wk before switching to the experimental diet. All rats were weighed weekly. The 0.1% oltipraz diet produced a 5% reduction in body weight over a 4-wk feeding period; lower concentrations of oltipraz had no effect on animal growth rates. For the structure-activity studies, 1,2-dithiol-3-thiones and 1,2-dithiol-3-ones were incorporated into the diet at a final concentration of 0.075% to compare the efficacy of these analogues to the maximally tolerated dose of oltipraz. No effects on growth rates were observed over the 1-wk feeding period.

**Analysis of Foci.** After 1 wk on the experimental diets, rats received by gavage 250 μg of AFB₁/kg of body weight 5 days a wk for the next 2 wk (days 8–12 and 15–19). One week following cessation of dosing all animals were restored to the control AIN-76A diet. This treatment protocol is presented schematically in Fig. 2. Twelve wk after restoration of rats to control diets, they were sacrificed, livers removed and weighed, and 4 liver samples were taken by the use of a 15-mm diameter cork borer. Liver samples were then frozen in liquid nitrogen and processed for GGT histochemistry as described previously (25). All GGT-positive lesions scored were well defined, with the majority of cells being stained. Numbers and sizes (areas) of the GGT-positive focal transections and total areas of the liver sections (2–4 cryostat sections from each liver) were determined using an image analysis system (26). The mean number and mean size of the foci were determined from the observed number and size of the GGT-positive focal transections by the quantitative stereological methods of Pugh et al. (27).

**Enzyme Assays and Isolation of DNA.** Rats maintained on experimental diets were stunned and killed by cervical dislocation. Small portions of liver were removed for glutathione determinations (28) and the remaining tissue homogenized in 4 vol (w/v) of a 50 mm Tris-HCl buffer (pH 7.0) containing 0.25 M sucrose with a Polytron homogenizer. Homogenates were centrifuged at 105,000 × g for 60 min, and the resulting supernatant fluid was used for glutathione S-transferase (CDNB and DCNB as substrates) (29), glutathione reductase (30), glucose 6-phosphate dehydrogenase (31), 6-phosphogluconate dehydrogenase (32), glutathione peroxidase (33), and NAD(P)H:quinone reductase (34) assays. In the isolated microsomes, the levels of cytochrome P-450 were measured by the method of Omura and Sato (35).
CHEMOPROTECTION BY 1,2-DITHIOL-3 THIONES

Assays of NADPH cytochrome P-450 reductase (measured as cytochrome c reductase) were conducted according to Masters et al. (36) and activities of microsomal mixed-function oxidases were measured using either aniline or aminopyrine as substrates (37). Epoxide hydrase activity was assayed using [3H]stere 

The same quantity of postmitochondrial supernatant healed in vitro metabolism studies were conducted essentially as described by Roebuck and Wogan (43). Briefly, to a final volume of 5 ml was added an incubation buffer consisting of 50 mM Tris-HCl (pH 7.4), 2.7 mM KCl, 1.0 mM MgCl2, 2.4 mM glucose 6-phosphate, 0.1 mM NADP, and 0.6 unit of yeast glucose 6-phosphate dehydrogenase and postmitochondrial supernatant equivalent to 0.2 g of liver. The same quantity of postmitochondrial supernatant heated in boiling water for 10 min served as a control. After a 5 min preincubation to generate NADPH, 70 g AFB1 in 50 ml methanol was added to each flask. The final concentration of AFB1 was 0.045 mM and included 0.1 0Ci ring-labeled [3H]AFB1. Duplicate flasks were incubated for 15 min in air at 37°C in a Dubnoff metabolic shaking incubator. Incubations were stopped by addition of 5 ml ice-cold methanol. The incubation medium containing methanol was successively extracted 5 times with 25-ml aliquots of chloroform and the combined extracts evaporated to dryness and reconstituted in 50 ml ethanol. The chloroform-soluble aflatoxins were analyzed by reverse phase chromatography using a 25-cm Zorbax 5-μm octadecyl silane column. Chromatography was performed at ambient temperature using 20 mm triethylammonium formate, pH 3.0 (Regis Chemical Co., Morton Grove, IL) containing 25% ethanol as mobile phase at a flow rate of 1.2 ml/min. Aflatoxins Q4, M1, P1, and B2 eluted at 17, 22, 27, and 37 min, respectively. The effluent was monitored at 362 nm and 0.5-min fractions were collected. The radioactivity in each fraction was determined by a Packard Model 300 liquid scintillation counter. All samples were spiked with authentic standards. The chloroform-insoluble phase was quantitatively recovered and aliquots were taken for determination of radioactivity.

RESULTS

Effect of Oltipraz on GGT-positive Lesions in AFB1-treated Rats. The effects of graded doses of dietary oltipraz ranging from 0.01–0.1% were evaluated for possible inhibitory effects on the development of presumptive preneoplastic lesions in aflatoxin-treated rats. Presented in Table 1 are the data obtained from quantitative 2- and 3-dimensional analyses of GGT-positive lesions in livers of rats treated with 2 dosing cycles of AFB1 and sacrificed at 4 mo. As has been reported previously with this dosing protocol (12, 44), we observed in 2 separate experiments between 1.4 and 1.6 foci/cm2 of liver in rats treated with aflatoxin and maintained on the control diet. Foci were seen in all animals in these groups. In the first experiment, rats placed on a 0.1% oltipraz diet during the dosing period showed a mean number of only 0.1 focus/cm2 of liver and a >95% reduction in the percentage volumes of liver occupied by GGT-positive foci. Foci were observed in the livers of only 2 of 10 rats in this group. In a second experiment, the dose-response characteristics of this inhibition were examined. Doses between 0.075 and 0.01% oltipraz in the diet produced significant reductions (68 to 98%) in the number of GGT-positive foci/cm2 of liver. Despite surveying more tissue in the groups fed the higher levels of oltipraz, foci were not found in 40, 20, and 30% of the rats fed 0.075, 0.04, and 0.02% oltipraz, respectively. Animals in the other treatment groups had at least 1 focus. All doses tested decreased the percentage volume of the livers occupied by GGT-positive foci by at least 90%. Because the number of observed foci were too few in any of the oltipraz-treated groups, it was not possible to calculate or to compare statistically the 3-dimensional values of mean focal diameter and mean focal volume by the techniques of quantitative stereology.

Depicted in Fig. 3C is the relationship between induction of hepatic glutathione S-transferase activity following dietary administration of oltipraz is shown in Fig. 3A. The highest concentration of oltipraz tested, 0.1% in the diet, induced glutathione S-transferase activities 3.2- (DCNB) after 1 wk of feeding. Concentrations as low as 0.02% in the diet yielded modest (50%) but statistically significant increases in enzyme activity, whereas 0.01% oltipraz was without effect on hepatic glutathione S-transferase activities. Shown in Fig. 3B are the effects of graded doses of oltipraz on overall aflatoxin-DNA adduct formation 2 h following intubation with 250 μg AFB1/kg. Two-h postdosing represents the time of maximal aflatoxin-DNA adduct levels in the liver (8). Oltipraz at 0.1% in the diet reduced the levels of aflatoxin bound to hepatic DNA by 78%, while the low dose of 0.01%, which was without effect on basal glutathione S-transferase activity, engendered a 40% reduction in aflatoxin-DNA adduct levels. Depicted in Fig. 3C is the relationship between induction of glutathione S-transferase activity by oltipraz and the degree of inhibition of aflatoxin-DNA binding. An excellent linear correlation (r = 0.97) exists between these two events. However, the fact that significant protection against adduct formation occurs in the absence of glutathione S-transferase induction implies that modification of other processes may also be involved in the protective mechanisms of oltipraz.

Table 1  Effects of oltipraz on aflatoxin-induced γ-glutamyl transpeptidase-positive foci in rat liver

<table>
<thead>
<tr>
<th>Dose of oltipraz (in %)</th>
<th>Area (cm²) of liver examined/rat</th>
<th>No. of foci/cm²</th>
<th>Foci vol (% of total liver)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Experiment 1</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control 0.1</td>
<td>4.90 ± 0.30*</td>
<td>1.60 ± 0.28</td>
<td>0.1097 ± 0.0257</td>
</tr>
<tr>
<td>0.01</td>
<td>5.18 ± 0.27</td>
<td>0.11 ± 0.09*</td>
<td>0.0035 ± 0.0032</td>
</tr>
<tr>
<td>Experiment 2</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control 0.1</td>
<td>7.68 ± 0.25</td>
<td>1.41 ± 0.29</td>
<td>0.4357 ± 0.1927</td>
</tr>
<tr>
<td>0.01</td>
<td>7.26 ± 0.25</td>
<td>0.44 ± 0.11*</td>
<td>0.0311 ± 0.0084*</td>
</tr>
<tr>
<td>0.02</td>
<td>16.08 ± 0.47</td>
<td>0.21 ± 0.10*</td>
<td>0.0089 ± 0.0055*</td>
</tr>
<tr>
<td>0.04</td>
<td>15.49 ± 0.25</td>
<td>0.17 ± 0.06*</td>
<td>0.0084 ± 0.0032*</td>
</tr>
<tr>
<td>0.075</td>
<td>18.35 ± 0.52</td>
<td>0.03 ± 0.01*</td>
<td>0.0022 ± 0.0017*</td>
</tr>
</tbody>
</table>

* Mean ± SE.  
* Differs from control, P < 0.01.
ometrically following OKI, extraction and separation by high-performance liquid chromatography.

The presence of a NADPH-generating system. Metabolites were quantified radiometrically following CDNB and DCNB determined by least-squares regression analysis. Points, means of duplicate determinations done on each of 4 animals; bars, SE.

Table 2 Induction of hepatic microsomal enzymes following feeding of oltipraz

<table>
<thead>
<tr>
<th>% oltipraz in diet for 7 days</th>
<th>Cytochrome P-450 (nmol/mg)</th>
<th>Cytochrome P-450 reductase (nmol cytochrome c consumed/min/mg)</th>
<th>Aniline hydroxylase (nmol p-aminophenol formed/min/mg)</th>
<th>Aminopyrine N-demethylase (nmol HCHO formed/min/mg)</th>
<th>UDP:glucuronyl transferase (nmol p-nitrophenol conjugated/min/mg)</th>
<th>Epoxide hydrase (nmol styrene diol formed/min/mg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>0.65 ± 0.07b</td>
<td>282.6 ± 22.2</td>
<td>1.3 ± 0.1</td>
<td>7.9 ± 0.4</td>
<td>3.5 ± 0.1</td>
<td>3.0 ± 0.2</td>
</tr>
<tr>
<td>0.01</td>
<td>0.69 ± 0.05</td>
<td>277.5 ± 11.1</td>
<td>1.8 ± 0.1b</td>
<td>9.8 ± 0.4b</td>
<td>4.2 ± 0.3</td>
<td>3.0 ± 0.2</td>
</tr>
<tr>
<td>0.10</td>
<td>0.89 ± 0.07b</td>
<td>366.1 ± 35.3b</td>
<td>2.0 ± 0.2b</td>
<td>12.9 ± 0.6b</td>
<td>13.6 ± 1.0b</td>
<td>5.4 ± 0.4b</td>
</tr>
</tbody>
</table>

* Mean ± SE (n = 5).
# Differs from control, at P < 0.01.

A similar selectivity in inductive effects by the low dose of oltipraz was observed when AFB, was used as a substrate for Phase I metabolism. As detailed in Table 3, significant increases in the rate of formation of AFQ, and AFP, were observed when AFB, was incubated with fortified postmitochondrial supernatant prepared from livers of rats fed a diet containing 0.01% oltipraz for 1 wk. By contrast, no enhancement in the rate of AFB, metabolism was noted, nor was there any effect on the amount of chloroform-insoluble derivatives of aflatoxin. The chloroform-insoluble species presumably reflect conjugation metabo- 

tables of AFB, Since no cofactors were added to the incubation mixture to support glucuronide or sulfate conjugation, it is likely, but not directly determined, that the predominant conjugate in the chloroform-insoluble fraction would be the glutathione conjugate of aflatoxin. Feeding of 0.1% oltipraz resulted in accelerated formation of AFQ, AFP, and AFM, as well as chloroform-insoluble metabolites, suggesting that both Phases I and II metabolism of AFB, is uniformly induced by the high dose of oltipraz.

Structure-Activity Studies with 1,2-Dithiol-3-thiones. The effects of dietary administration of a series of 1,2-dithiol-3-thione and 1,2-dithiol-3-one analogues of oltipraz on hepatic AFB,-DNA adduct formation as well as on the specific activities of several Phases I and II enzymes were also determined. As shown in Fig. 4, the feeding of 7 analogues of oltipraz at 0.075% in the diet each resulted in substantial reduction of aflatoxin binding to hepatic DNA 2 h after p.o. administration of the carcinogen compared to animals on the control AIN-76A diet. The unsubstituted 1,2-dithiol-3-thione (compound 1) produced a greater than 90% reduction in AFB,-DNA adduct level and was the most effective compound examined. Clearly, only the dithioloethene ring structure is required for protective activity. Addition of an oxygen atom to position 4 to form 1,2-dithiol-3-one analogues of oltipraz markedly reduced the protective actions of 1,2-dithiol-3-one analogues of oltipraz.
CHEMOPROTECTION BY 1,2-DITHIOL-3-THIONES

When compared to the unsupplemented controls, 0.075% produced a significant (P < 0.05) reduction in aflatoxin-DNA binding. Rats were fed diets supplemented with 0.075% of the indicated compound for 1 wk and then treated with 250 μg [3H]AFB1/kg intragastrically 2 h before sacrifice. Control binding levels were 500 pmol AFB1 bound/mg DNA. Bars, means ± SE of individual determinations made on 4 animals. Each compound produced a significant (P < 0.05) reduction in aflatoxin-DNA binding when compared to the unsupplemented controls.

Table 4 Effect of feeding 1,2-dithiol-3-thione analogues on hepatic enzyme induction

<table>
<thead>
<tr>
<th>Enzyme activity as ratio of treated/control values for compound</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
<th>7</th>
<th>8</th>
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<tr>
<td>Glutathione</td>
<td>3.0*</td>
<td>2.6*</td>
<td>1.8*</td>
<td>1.5*</td>
<td>1.3</td>
<td>1.4</td>
<td>1.9*</td>
<td>2.5*</td>
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<tr>
<td>Glutathione peroxidase</td>
<td>0.4*</td>
<td>0.9</td>
<td>0.8</td>
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<td>0.9</td>
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<tr>
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<td>0.8</td>
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<td>1.4</td>
<td>0.8</td>
<td>1.1</td>
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<tr>
<td>Glucose 6-phosphate dehydrogenase</td>
<td>2.6*</td>
<td>2.0*</td>
<td>1.7*</td>
<td>1.6*</td>
<td>1.6*</td>
<td>1.6*</td>
<td>1.4</td>
<td>2.8*</td>
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<tr>
<td>6-Phosphogluconate dehydrogenase</td>
<td>1.3</td>
<td>1.1</td>
<td>1.2</td>
<td>1.0</td>
<td>1.1</td>
<td>1.4</td>
<td>1.2</td>
<td>1.3</td>
</tr>
<tr>
<td>NAD(P)H:quinone reductase</td>
<td>7.1*</td>
<td>2.3*</td>
<td>2.2*</td>
<td>2.2*</td>
<td>5.6*</td>
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<td>UDP glucuronyl transferase</td>
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<td>2.1*</td>
<td>1.6*</td>
<td>1.8*</td>
<td>2.7*</td>
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<td>Glutathione S-transferase (DCNB)</td>
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<td>2.0*</td>
<td>2.9*</td>
<td>3.2*</td>
<td>1.8*</td>
<td>1.1</td>
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<tr>
<td>Glutathione S-transferase (CDNB)</td>
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<td>1.8*</td>
<td>1.6*</td>
<td>1.6*</td>
<td>2.6*</td>
<td>2.8*</td>
<td>1.4*</td>
<td>1.3</td>
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<tr>
<td>Epoxyde hydrolase</td>
<td>2.6*</td>
<td>1.6</td>
<td>1.4</td>
<td>1.5</td>
<td>2.1*</td>
<td>1.9*</td>
<td>1.7*</td>
<td>1.4</td>
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<td>Cytochrome P-450</td>
<td>1.0</td>
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<td>1.4</td>
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<tr>
<td>Cytochrome P-450 reductase</td>
<td>0.9</td>
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<td>1.2</td>
<td>1.0</td>
<td>1.3</td>
<td>1.4</td>
<td>1.1</td>
<td>1.2</td>
</tr>
<tr>
<td>Aminopyrine N-demethylase</td>
<td>1.0</td>
<td>1.4</td>
<td>1.4</td>
<td>1.6*</td>
<td>1.6*</td>
<td>1.8*</td>
<td>1.7*</td>
<td>1.4</td>
</tr>
<tr>
<td>Aniline hydroxylase</td>
<td>1.0</td>
<td>1.3*</td>
<td>1.4*</td>
<td>1.3*</td>
<td>1.7*</td>
<td>1.6*</td>
<td>1.4*</td>
<td>1.4*</td>
</tr>
</tbody>
</table>

* Differs from control at P < 0.01.
* Differs from control at P < 0.05.

DISCUSSION

1,2-Dithiol-3-thiones are a novel class of chemoprotective compounds that are demonstrating a broad spectrum of anti-carcinogenic actions against structurally diverse carcinogens at multiple sites and may represent a useful class of compounds for the inhibition of human neoplasia. Wattenberg and Bueding (13) have recently reported the inhibitory effects of oltipraz on carcinogenesis induced by benzo(a)pyrene, diethyltin, and uracil mustard in the lung and forestomach of mice. Our present findings that dietary concentrations of oltipraz as low as 0.01% powerfully inhibit the formation of presumptive pre-neoplastic lesions in the livers of rats exposed to AFB1, adds further credence to the possible use of 1,2-dithiol-3-thiones in protection against human cancer. Lifetime anticarcinogenic studies are presently underway to ascertain the cancer chemoprotective properties of oltipraz in F344 rats treated with AFB1.
in a feeding protocol identical to that used in the present studies.

A major limitation to the proposed use of chemoprotective agents in humans is the inherent toxicity of the anticarcinogen. In this regard, limited toxicological studies suggest that 1,2-dithiol-3-thiones are attractive candidates for such a use inasmuch as they exhibit low mammalian toxicity. For example, the p.o. 50% lethal dose of oltipraz in mice and rats is about 10–20 g/kg (47). Additionally, this 1,2-dithiol-3-thione is not mutagenic in <i>Samonaella</i> histidine reversion assays either in the presence or absence of the S9 metabolic activating system (46).

However, unlike the situation with the commercial food antioxidants (48), the possible presence of carcinogenic or tumor promoting properties of oltipraz have not been examined directly by bioassay. Two 1,2-dithiol-3-thiones have been administered to large numbers of people: anethole dithioliathione as a choleretic agent and to counteract the inhibition of salivation caused by antidepressant drugs and oltipraz for the treatment of schistosomiasis (2, 4, 5). Anethole dithioliathione (Sulfarlem) has been used outside of the United States for many years without apparent adverse effects. Oltipraz, however, has recently been withdrawn from clinical trial as an antischistosomal agent due to phototoxic responses in tropical areas (49). Since the structural determinants for the antischistosomal activity of oltipraz are quite rigorous (3) (as compared to the chemoprotective and enzyme inductive effects described in this study), it is possible that this toxicological effect of oltipraz may be readily dissociated from the chemoprotective actions of other 1,2-dithiol-3-thiones.

Many chemoprotectors appear to inhibit the neoplastic process by altering the metabolic fate of carcinogens by modulating the activities of either or both Phases I and II drug-metabolizing enzymes. Phenobarbital and β-naphthoflavone are potent inhibitors of AFB<sub>1</sub> carcinogenesis in rats (50, 51), and both of these agents induce cytochrome P-450 isozymes that accelerate Phase I metabolism of AFB<sub>1</sub> to hydroxylated products which are considerably less genotoxic than either AFB<sub>1</sub> or its 8,9-oxide (52, 53). Presumably these inductions serve to alter the balance between the metabolic activation and detoxication of aflatoxin. Dietary antioxidants, such as BHA, BHT, and ethoxyquin also inhibit AFB<sub>1</sub>, carcinogenesis when fed simultaneously with the carcinogen (21, 22). These protective effects arise largely from enhanced carcinogen inactivation through the selective induction of Phase II detoxication pathways which facilitate the clearance of activated metabolites through conjugation reactions, particularly with glutathione (8, 12). The inhibitory effects of oltipraz against aflatoxin actions appear to involve components of both of these mechanisms. At low doses of oltipraz, considerable protection occurs against carcinogen-DNA binding and GGT-positive foci formation in the absence of any effects on the activities of electrophile detoxication enzymes. Under these conditions selective inductions of P-450 activities are observed to occur and may serve to shunt the oxidative metabolism of aflatoxin away from the 8,9-oxide. At the higher dietary levels, oltipraz markedly increases the activities of glutathione S-transferases to facilitate the conjugation of glutathione to the aflatoxin 8,9-oxide and its ultimate elimination. Enhanced biliary elimination of the glutathione conjugate, 8,9-dihydro-8-(S-glutathionyl)-9-hydroxyaflatoxin B<sub>1</sub>, has been observed in rats fed 0.1% but not 0.01% oltipraz (1).

The utility of compounds that induce Phase I enzyme activities as general chemoprotectors seems doubtful (54). Many situations have been described in which the different isoforms of cytochrome P-450 can simultaneously serve to metabolically inactivate a procarcinogen while also participating in its metabolic activation. Thus, protection engendered by a Phase I inducer in one setting may be a co-carcinogenic action in another, depending upon the metabolic balance between activation and detoxication. By contrast, Phase II enzymes such as glutathione S-transferase predominantly participate in the detoxication of xenobiotics, although there exist some notable exceptions to this premise (55). In any case, since the actions of a Phase II enzyme on a carcinogen are generally unidirectional as opposed to Phase I reactions which may activate and/or inactivate the same substrate, the use of Phase II inducers as chemoprotective agents, especially in situations where the occupational or environmental carcinogen(s) are known (and hence, the metabolic outcome predictable), is plausible. The value of 1,2-dithiol-3-thiones as potential chemoprotective agents in humans is based on the observations that they are potent chemoprotectors in rodents, reasonably nontoxic, and that their actions on Phase I versus Phase II enzymes can be easily dissociated by appropriate structural modifications. Further examination and development of this class of chemoprotectors appears warranted.

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