Transient Intervention with Oltipraz Protects against Aflatoxin-induced Hepatic Tumorigenesis


ABSTRACT

Oltipraz [5-(2-pyrazinyl)-4-methyl-1,2-dithiole-3-thione] protects against aflatoxin B1-induced hepatocarcinogenesis in rats when fed before and during carcinogen exposure; however, such an exposure-chemoprotection intervention paradigm is not directly relevant to most human populations. To model and assess the possible efficacy of short term interventions targeted at individuals at risk for sustained exposure to aflatoxins, 175-g male F344 rats were treated daily with 25 µg of aflatoxin B1, p.o., for 28 days. One week after the start of aflatoxin B1 exposure, half of the animals were fed a diet supplemented with 0.075% oltipraz for 10 days; these rats were then restored to the unsupplemented AIN-76A diet for the remainder of the experimental period. Livers were analyzed 2 or 3 months after the last aflatoxin B1 dose for burden of glutathione S-transferase P (GST-P)-positive foci, as an index of presumptive preneoplastic alterations in the metabolism and disposition of aflatoxin B1 induced by oltipraz. Glutathione S-transferases catalyze the detoxication of aflatoxin-8,9-oxide and were found to be rapidly induced in the livers of animals after the beginning of the oltipraz intervention. Glutathione S-transferase activity remained significantly (P < 0.05) higher after 9 days than after the end of the oltipraz intervention. In contrast, levels of hepatic aflatoxin-DNA adducts were not significantly reduced until 4 days after the beginning of the intervention but remained significantly (P < 0.05) lower up to 11 days after the end of the intervention. The cumulative reduction in levels of hepatic aflatoxin-DNA adducts (~25%) by the oltipraz intervention underestimated the reduction in the hepatic burden of GST-P-positive foci. The significant protection against presumptive preneoplastic tumors, despite the delay of intervention, suggests that oltipraz may exert substantial activity against the cytotoxic and aut促进了 action of repeated exposures to aflatoxin B1, and supports the utility of intervention trials with oltipraz in individuals chronically consuming aflatoxin B1-contaminated foods, particularly in regions with high incidences of liver cancer.

INTRODUCTION

Oltipraz [5-(2-pyrazinyl)-4-methyl-1,2-dithiole-3-thione] was developed in the late 1970s as an antischistosomal agent, and cure rates of up to 90% have been achieved with single doses of this drug in clinical field trials (1). During the course of studies on the mechanisms of antischistosomal activity of oltipraz, Bueding et al. (2) noted that administration of the drug to rodents led to significant elevations in many tissues of phase II enzymes involved in the detoxication of carcinogens, as well as increased glutathione levels. These biochemical studies led, in turn, to the prediction that oltipraz and related 1,2-dithiole-3-thiones might be excellent candidate compounds for cancer chemoprotection studies. The initial confirmation that 1,2-dithiole-3-thiones may exert chemoprotective effects in vivo came from the demonstration by Ansher et al. (3) that oltipraz or anethole dihydrothioleone protected against the toxicities of carbon tetrachloride and acetaldehyde in mice. Subsequent studies have demonstrated protection by oltipraz against the acute hepatotoxicities of allyl alcohol and acetaminophen in hamsters (4, 5) and AFB13 in rats (6). Wattenberg and Bueding (7) first established cancer chemoprotective properties of oltipraz by inhibiting diethylnitrosamine-, benzo[a]-pyrene-, or uracil mustard-induced neoplasia in the lungs and/or fore-stomachs of mice with this drug. Oltipraz has subsequently been shown to have chemoprotective activity against different classes of carcinogens targeting the breast, bladder, colon, skin, trachea, and liver in rodents (reviewed in Ref. 8). The potency of oltipraz is highlighted by the observations in F344 rats that dietary concentrations of 0.02 and 0.04% oltipraz significantly reduced tumor incidence and multiplicity in azoxymethane-induced intestinal carcinogenesis (9), while 0.075% oltipraz in the diet afforded complete protection against AFB1-induced hepatocarcinogenesis (10). This broad range of anticarcinogenic activity, coupled with its apparently low mammalian toxicity, has prompted the continued development of oltipraz as a potential human chemoprotective agent. Oltipraz is currently undergoing phase I clinical trials in the United States to determine its pharmacokinetic properties and dose-limiting side effects during prolonged administration (11).

In the design of a chemoprotection trial a number of issues must be considered in addition to the choice of the intervention agent, including (a) the dose to be used, (b) the stage of the carcinogenic process to be interrupted, (c) the appropriate outcome measure, and (d) the study population. Mechanistic studies indicate that individuals most likely to benefit from oltipraz are those exposed to carcinogens amenable to detoxication by phase II enzymes. As a consequence, trials examining the efficacy of oltipraz should study populations exposed to high concentrations of airborne pollutants or food-borne mycotoxins such as aflatoxins (12). However, in many cases, exposure to such environmental carcinogens begins in utero or at birth. In most of the experimental models employed to date, oltipraz was administered both before and during carcinogen exposure to ensure maximal protective outcomes. Thus, such exposure-chemoprotection intervention paradigms are not directly relevant to most human populations. In the present study, we have begun to develop paradigms for short term interventions that model phase II clinical trials targeted at individuals at risk for sustained exposure to aflatoxins. In this setting, oltipraz has been fed to rats for 10 days in the middle of a 4-week exposure to AFB1. Such models provide opportunities to assess the efficacy of transient interventions, as well as allowing for the development and validation of intermediate biomarkers as short term measures predictive of outcome.

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The abbreviations used are: AFB1, aflatoxin B1; GST, glutathione S-transferase; GST-P, glutathione S-transferase P.
MATERIALS AND METHODS

Chemicals. Oltipraz was obtained from the Chemoprevention Branch, National Cancer Institute, and was determined to be >97% pure by high performance liquid chromatography (15). AFB1 was obtained from Aldrich Chemical Co. (Milwaukee, WI) and [3H]-AFB1 (24 Ci/mmol) from Moravek Biochemicals (Brea, CA). GST-P antibody was purchased from Medlabs (Dublin, Ireland). All other chemicals and reagents were of the highest quality obtainable commercially.

Animals and Diets. Male F344 rats (175 g; Charles River Breeding Laboratories) were housed under controlled conditions of temperature, humidity, and lighting. Food and water were available ad libitum. Purified diet of the AIN-76A formulation without the recommended addition of 0.02% ethoxyquin (14) was used, and fresh diet was provided to animals at least every other day. Oltipraz at a concentration of 0.075% was mixed into the AIN-76A diet with a V-blender and diet was stored at 4°C. Rats were acclimated to the AIN-76A diet for 1 week before beginning the experiments.

Analysis of Foci and Extent of Fibrosis. After the 1-week acclimation period, all rats received 25 μg of AFB1 in 100 μl dimethylsulfoxide by gavage each morning for 28 consecutive days. Half of the animals were switched to a diet supplemented with 0.075% oltipraz for 10 days after the sixth dose of AFB1 (days 7–16). These animals were then returned to the AIN-76A diet for the remainder of the experiment. This treatment protocol is presented schematically in Fig. 1. Two or 3 months after cessation of AFB1 dosing, the animals were sacrificed, livers were removed and weighed, and multiple 2-mm-thick sections of the right lateral and median lobes were obtained. The liver samples were fixed in cold acetone and stained by a peroxidase-antiperoxidase method for GST-P expression (15). Enzyme-altered focal lesions were scored, with the large majority of focal cells being stained. Numbers and sizes (areas) of the GST-P-positive focal and total surface areas of the liver sections were determined using an image analysis system (16). The volume percent of liver occupied by GST-P-positive foci, the number of foci/cm², and the mean diameter of foci were determined from the observed number and size of the focal sections by the quantitative stereological methods of Pugh et al. (17). Approximately 3 cm² of liver from each rat were analyzed for foci.

The extent of fibrosis and cirrhosis in the livers of all animals was evaluated histologically using a graded scale. The observer was blinded to the treatment protocol. The extent of the fibrotic lesions ranged from proliferation, limited to the immediate perilobular region (grade 1), to collagen deposition across the lobule to the terminal hepatic venule (grade 2). The lobular pattern of cirrhosis with collagen deposition ranged from mild (grade 3) to extensive, with thick septa of collagen (grade 4).

Enzyme Assays and Isolation of DNA. A second cohort of rats were placed on the experimental protocol described in Fig. 1, except that animals were treated with [3H]-AFB1 (134 μCi/μmol). Two rats from each treatment arm were killed on each of the first 27 days, 2 h after dosing with AFB1, and livers were excised, rinsed, minced in 4 volumes of ice-cold 50 mM Tris-HCl buffer, pH 7.0, containing 0.25 M sucrose, and finally homogenized for 20 s using a Polytron. Homogenates were first centrifuged at 10,000 × g for 15 min. The supernatant was then centrifuged at 105,000 × g for 60 min and the resulting fluid was used for GST assays with 1-chloro-2,4-dinitrobenzene as substrate (18). Protein was determined by the method of Bradford (19), using bovine serum albumin as the standard. The 10,000 × g pellet was resuspended in Tris-sucrose buffer containing 0.05% Triton X-100 centrifuged, and washed twice more. The nuclear preparation was subsequently suspended in 10 ml of the original Tris-sucrose buffer, to which 1.5 ml of 10% sodium dodecyl sulfate, 3.5 ml of 1 M NaCl, and then 15 ml of chloroform/isoamyl alcohol (24:1, v/v) were added. The resulting emulsion was shaken vigorously for 20 min at ambient temperature and the phases were separated by centrifugation at 2500 × g for 10 min. The extraction of the aqueous phase was repeated and the nucleic acids were recovered from the aqueous phase by precipitation with 2 volumes of ice-cold ethanol. The DNA was dissolved in water and hydrolysed (20). DNA content was determined by the method of Giles and Meyers (21). Nucleic acid hydrolysates were counted directly to determine the specific activity of aflatoxin-DNA binding.

Statistical Analyses. The exact Wilcoxon rank-sum test was used to compare the volume percent of liver expressing GST-P-positive foci in the two treatment arms (22). A two-way analysis of variance of treatment (i.e., control diet or oltipraz-supplemented diet) and time of necropsy (i.e., 2 or 3 months) was undertaken. The relationship between the extent of hepatic fibrosis/ cirrhosis and GST-P-positive foci was examined by correlation of the grade of fibrosis and the volume percent of GST-P-positive foci for each rat liver. Linear regression methods were used to describe the effects of the transient intervention with oltipraz on GST activity and AFB1-DNA adducts, in which the data were transformed logarithmically since values for both GST activity and adduct levels were skewed to the right. A piecewise linear regression approach (22) was used to incorporate the overall nonlinear pattern of the data, thereby allowing different (but connected) lines from days 7 to 11, 12 to 16, 17 to 21, and 22 to 27. The main-effects model allows for a constant effect of oltipraz over time. To assess whether the effect of oltipraz was different at different times, we tested for the presence of interactions between days. In particular, if Y denotes the biomarker level at day t (1 ≤ t ≤ 27), the piecewise linear regression model for the group not receiving oltipraz is:

\[
\log Y_t = \alpha + \sum_{i=1}^{4} \beta_i (t - 7 + 5(i - 1)) + \epsilon_t
\]

and the corresponding model, including interaction, for the group receiving oltipraz is:

\[
\log Y_{t*} = \alpha + \alpha^* + \sum_{i=1}^{4} (\beta_t + \beta_t^*) (t - 7 + 5(i - 1)) + \epsilon_{t*}
\]

The intercepts α and (α + α*) represent the mean values at day 7 for the

![Fig. 1. Experimental protocol for evaluating the effects of a transient chemoprotective intervention with oltipraz on the development of GST-P-positive foci.](image)

Rats received 25 μg AFB1, p.o., daily for 28 consecutive days. Half of the animals were switched to an AIN-76A diet supplemented with 0.075% oltipraz for 10 days after the sixth dose of AFB1 (treatment arm B). The remaining animals were maintained on the basal AIN-76A diet throughout the experiment (treatment arm A). Arrows, days of AFB1 dosing; X, days of sacrifice for GST-P analyses.

3500
nonintervened and intervened groups, respectively. The cumulative sums of \( \beta \) and \( \beta^* \) values represent the slopes of lines joining at days 12, 17, and 22. The residuals \( e \), and \( e^* \) are independent and follow a gaussian distribution with mean zero and variance \( \sigma^2 \). A parameter of central interest was the ratio of the biomarkers for the intervened to the nonintervened group, which in the context of our piecewise linear model was expressed as the antilogarithm of linear combinations of the regression coefficients. Thus, the ratio of the mean value of \( Y_i^* \) divided by the mean value of \( Y_i \) was estimated as the antilogarithm of:

\[
\alpha^* + \sum_{i=1}^{4} \beta^*[r - 7 - 5(i - 1)] \quad [r \geq 7 + 5(i - 1)]
\]

To test whether such ratios were equal to 1 (i.e., no effect of oltipraz), we used standard procedures of maximum likelihood methods to calculate 95% confidence intervals for linear combinations of estimates of regression coefficients.

**RESULTS**

**Effect of Transient Intervention with Oltipraz on Hepatic GST-P-positive Lesions in AFB, treated Rats.** The effect of a transient dietary intervention with oltipraz in the middle of a sustained exposure to AFB, was evaluated for possible inhibitory effects on the development of presumptive preneoplastic lesions in AFB, treated rats. Presented in Table 1 are the data obtained from microscopic observation of GST-P-positive lesions in livers of rats sacrificed either 2 or 3 months after cessation of dosing with AFB,.

The 10-day intervention with oltipraz in the middle of the AFB, exposure reduced the levels of GST-P-positive foci/cm\(^2\) from 36.4 to 12.4 and from 21.4 to 16.3 at 2 and 3 months, respectively. Foci were observed in the livers of all animals. The number of foci/cm\(^2\) and their transsectional areas tended to be smaller in the oltipraz-treated groups. However, statistical analyses of these two-dimensional data are inappropriate because the data may not represent the actual number of foci in the liver (17). Statistical analysis of the volume of liver occupied by the GST-P-positive foci is appropriate, and oltipraz treatment reduced the focal burden by 54% (\( P = 0.047 \)) and 72% (\( P = 0.004 \)) at 2 and 3 months, respectively. The distribution of the hepatic burden of GST-P-positive foci in individual animals is depicted in Fig. 2. There was marked heterogeneity in the response of rats to the 28-day protocol for AFB, dosing. At both 2 and 3 months there was a >5-fold range in response. Nonetheless, a majority (9 of 13) of the animals receiving the transient oltipraz intervention exhibited hepatic burdens of GST-P-positive foci lower than the lowest level observed in the 12 animals receiving AFB, alone. The aggregate reduction in focal burden by oltipraz was statistically significant (\( P = 0.003 \)). There was no statistical difference within treatment groups between hepatic focal burdens of animals sacrificed at 2 and 3 months, in either the intervention or no-intervention groups.

Hepatic fibrosis and/or cirrhosis were observed in a majority of the rats. The extent of the fibrotic lesions ranged from proliferation, limited to the immediate perportal region, to collagen deposition across the lobule to the terminal hepatic venule. The lobular pattern of cirrhosis ranged from mild to extensive, with thick prominent septae of collagen. In the livers with the most extensive cirrhosis, most but not all cirrhotic nodular parenchymal tissue was GST-P positive. In the oltipraz intervention group, the extent of fibrosis was significantly limited (\( P = 0.013 \)). However, the extent of fibrosis within either treatment arm did not differ between 2 and 3 months (\( P = 0.196 \)).

The degree of fibrosis was positively correlated (\( r = 0.896, P < 0.01 \)) with the hepatic volume percent of GST-P-positive foci. Of interest was the observation that the focal diameter but not the number of foci/cm\(^3\) of liver was highly correlated (\( r = 0.797, P < 0.01 \)) with the extent of fibrosis. Two rats in the oltipraz intervention group examined at 2 months did not respond to the intervention with a reduction in the hepatic burden of GST-P-positive foci. These two rats exhibited livers as severely cirrhotic as the most affected livers of rats receiving only AFB,.

Hepatic fibrosis and cirrhosis are well known consequences of AFB,. Hepatic fibrosis and cirrhosis are related to aflatoxin carcinogenesis (23). Furthermore, the extent of hepatic fibrosis serves as an index of earlier hepatic injury and implies that hepatotoxicity is an important determinant of tumorigenesis.

**Induction of Glutathione S-Transferase Activity and Inhibition of AFB, DNA Adduct Formation.** The ability to induce GST activities is a property common to many chemoprotective agents (24), and the induction of this family of glutathione-conjugating enzymes appears to be an important mechanism for diminishing AFB, carcinogenicity (25, 26). Oltipraz is an effective inducer of hepatic GSTs when fed to naive rats (27); however, it was unclear whether oltipraz-mediated induction of GSTs could occur under conditions of recurrent hepatotoxicity caused by AFB,.

Thus, animals were treated as detailed in Fig. 1 and pairs of animals from each group were sacrificed daily for determination of hepatic GST activity and AFB,-DNA adduct levels. As shown in Figs. 3 and 4, a pharmacodynamic action of oltipraz was readily demonstrable as a consequence of the brief intervention. Depicted in Fig. 3 is the time course for the induction of hepatic GST activity. As has been observed before (28), repetitive treatment with AFB, alone induced hepatic GST activity. The specific activity of GST doubled after 1 week of exposure and remained elevated throughout the AFB, dosing period. The oltipraz intervention superimposed an additional elevation in GST activity in the livers of these animals. Hepatic GST activity was twice that of the animals receiving only AFB, within several days and remained at this elevated level for the remainder of the oltipraz intervention.

The extent of hepatic fibrosis serves as an index of earlier hepatic injury and implies that hepatotoxicity is an important determinant of tumorigenesis.

Table 1  Effect of transient oltipraz intervention on aflatoxin-induced GST-P-positive foci in rat liver

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Months after dosing</th>
<th>No. of rats</th>
<th>No. of foci/cm(^2) of liver</th>
<th>Focal area (mm(^2) \times 100)</th>
<th>Volume (% of liver)</th>
</tr>
</thead>
<tbody>
<tr>
<td>AFB,</td>
<td>2</td>
<td>6</td>
<td>36.4 ± 5.7*</td>
<td>42.8 ± 9.4</td>
<td>14.9 ± 2.5</td>
</tr>
<tr>
<td>AFB, + oltipraz</td>
<td>2</td>
<td>6</td>
<td>12.4 ± 2.1</td>
<td>54.3 ± 37.9</td>
<td>6.8 ± 4.2</td>
</tr>
<tr>
<td>AFB,</td>
<td>3</td>
<td>6</td>
<td>21.4 ± 1.8</td>
<td>51.3 ± 17.3</td>
<td>10.7 ± 3.5</td>
</tr>
<tr>
<td>AFB, + oltipraz</td>
<td>3</td>
<td>7</td>
<td>16.3 ± 1.8</td>
<td>18.8 ± 4.8</td>
<td>3.0 ± 0.8*</td>
</tr>
</tbody>
</table>

* Mean ± SE.

* Differs from AFB, P < 0.05 using Wilcoxon rank-sum test.

Fig. 2. Effects of a transient intervention with oltipraz on the hepatic focal burden of AFB,-induced GST-P-positive foci. Rats were treated as described in Fig. 1. GST-P-positive foci were counted in livers obtained either 2 or 3 months after the last dose of AFB,. X, volume percent of liver occupied by GST-P-positive foci in individual animals receiving AFB, alone (treatment arm A); O, volume percent of liver occupied by GST-P-positive foci in individual animals receiving AFB, plus oltipraz (treatment arm B). Bars, means.
CHEMOPROTECTION BY TRANSIENT OLTIPRAZ INTERVENTION

Fig. 3. Scatter plot of the effects of a transient intervention with oltipraz on the daily activities of hepatic GST in AFB₁-exposed rats. *, GST activities in the first 6 days; X, GST activities in animals receiving AFB₁ alone (treatment arm A); ○, GST activities in treatment arm B while animals are receiving oltipraz; ●, GST activities in treatment arm B after animals have stopped receiving oltipraz. Lines, results of fitting piecewise linear regression models to the data after day 6.

Fig. 4. Scatter plot of the effects of a transient intervention with oltipraz on the daily levels of hepatic AFB₁-DNA adducts in AFB₁-exposed rats. *, AFB₁-DNA adduct levels in the first 6 days; X, AFB₁-DNA adduct levels in animals receiving AFB₁ alone (treatment arm A); ○, AFB₁-DNA adduct levels in treatment arm B while animals are receiving oltipraz; ●, AFB₁-DNA adduct levels in treatment arm B after animals have stopped receiving oltipraz. Lines, results of fitting piecewise linear regression models to the data after day 6.

...tion level about 1 week after cessation of dietary supplementation, indicating that the sustained presence of the drug was required to preserve maximum induction. Shown in Fig. 4 are the effects of the intervention on hepatic DNA adduct levels. The oltipraz intervention engendered a sustained 25% reduction in hepatic levels of DNA adducts, beginning several days after introduction of oltipraz into the diet. Fig. 5 presents the effects of oltipraz on these parameters, expressed as fold changes from the corresponding no-intervention values following piecewise linear regression analysis. This analysis indicates that hepatic GST activities increased 1.3-fold after 1 day of the oltipraz intervention and remained significantly elevated until 9 days after intervention with oltipraz was stopped. The levels of AFB₁-DNA adduct formation did not significantly differ between the oltipraz-intervened rats and their respective controls until 4 days after oltipraz was added to the diet, at which point the intervened rats had 0.84 times the adduct level of the nonintervened rats. The rats fed oltipraz continued to have significantly lower levels of AFB₁-DNA adducts throughout the 11-day postintervention period. The effects of the oltipraz intervention on the dynamics of the two biochemical markers were different. Notably, elevation in GST activity preceded the reduction in AFB₁-DNA adduct levels. However, unlike the situation with GST activity 1 week following termination of the oltipraz intervention, levels of AFB₁-DNA adducts remained depressed throughout the postintervention period. This apparent divergence might reflect the contribution of non-GST mechanisms to the reduction of DNA adducts. In addition, the use of 1-chloro-2,4-dinitrobenzene as a surrogate substrate to measure GST activity underestimates AFB₁-glutathione-conjugating activity (29).

DISCUSSION

Numerous studies in animal models have examined the temporal relationships between administration of chemoprotective agents and carcinogen exposure. In these studies chemoprotectors are typically...
Liu et al. (6) have shown that five daily doses of 250 μg/kg AFB1, administered before, during, and/or after the carcinogen. Such studies provide important insights regarding possible mechanisms of action, i.e., attribution of “blocking” versus “suppressing” effects (30). While informative and experimentally expedient, these protocols largely fail to represent an accurate paradigm for interventions in human populations, where lifelong exposures to carcinogens may occur. In the present study, we have modeled in rats a clinical phase II intervention with oltipraz against AFB1-induced hepatic tumorigenesis. Human exposure to aflatoxins begins in utero and continues at high levels throughout life in many regions of the world (31–33). Thus, significant genetic damage (i.e., initiation) is likely to occur well before middle-age adults could be recruited into intervention trials. Our present findings indicate that even a delayed and transient (10-day) intervention with oltipraz significantly reduces the hepatic burden of presumptive preneoplastic foci in rats repeatedly treated with AFB1 over a 4-week period. While studies with cancer as an endpoint have yet to be conducted, based upon the well established association between expression of enzyme-altered foci and subsequent development of neoplasia our results suggest that even short term interventions in humans may have a positive impact on disease outcomes. In this context, it is also intriguing to recall that an early, short term (1-month) intervention with oltipraz resulted in a statistically significant 43% reduction in the development of lymphomas in male F344 rats in a 2-year bioassay (10).

Oltipraz is an effective chemoprotective agent in nearly a dozen different animal models when administered before and during carcinogen exposure and is generally believed to act as a “blocking” or anti-initiating agent. However, because the animals in the present study receive no protective intervention during the first week of aflatoxin exposure, significant damage to the genome is likely to have occurred in all animals. Maximum levels of carcinogen binding to hepatic DNA are observed following the first few doses of AFB1, and decline thereafter, despite continued exposure to aflatoxin. Levels of AFB1-DNA adducts drop relative to controls within several days of the introduction of oltipraz into the diet and a significant persistent diminution is observed, even many days after cessation of the dietary intervention. However, the reduction in levels of adducts is modest when integrated over days 7 through 27. Clearly, a substantial amount of the total hepatic DNA adduct burden has accumulated during the first week of AFB1 exposure. Subsequent doses of AFB1 contribute proportionally less to the overall adduct burden, in part because of altered metabolic processing of AFB1, as well as diminished numbers of viable hepatocytes in the livers of these animals. Earlier studies by Liu et al. (6) have shown that five daily doses of 250 μg/kg AFB1, p.o., result in >50% loss of thymidine pre-label in rat hepatocytes and that this cytotoxicity can be abrogated by concurrent feeding of oltipraz. The remarkable efficacy of the transient intervention with oltipraz in reducing the hepatic burden of GST-P-positive foci and protecting against fibrosis and cirrhosis highlights the important role that recurrent cytotoxicity likely plays in aflatoxin-induced hepatocarcinogenesis. Inhibition of this autopromoting component of AFB1 action may be a significant aspect of the anticarcinogenic activity of oltipraz. These findings also suggest that overall indices of genetic damage, such as DNA adduct levels, are likely to underestimate the degree of protection afforded by interventions. However, detailed analyses will be required to relate cumulative levels of hepatic DNA adducts [and, more pragmatically, levels of the biomarkers 8,9-dihydro-4,4'-N2-guananyl]-9-hydroxyaflatoxin B1 in urine and AFB1-albumin adducts in serum] to cancer risk in individual animals.

This study also provides insight into a number of practical aspects for the possible use of oltipraz (or similarly acting agents) in human populations. The pharmacodynamic actions of oltipraz, such as induction of GST activity, occur within 1 day after feeding of oltipraz to the animals and persist well beyond the termination of the intervention. Significant elevations in hepatic GST activity were observed up to 9 days after removal of oltipraz from the diet. Thus, intermittent exposures to oltipraz may be sufficient to maintain long term elevations in GSTs and other phase II enzyme activities. While transient high tissue concentrations of oltipraz are required to trigger the enzyme induction response, presumably through the “antioxidant response element” found in the regulatory region of the genes of several phase II enzymes (34), the sustained response reflects dynamics of enzyme expression rather than drug pharmacokinetics. As a consequence, the opportunity to schedule “drug holidays” for individuals on intervention protocols may improve drug tolerance and consequently compliance. It is also encouraging to note that enzyme induction by oltipraz is observed in livers of animals undergoing substantial hepatotoxic stress from repeated exposures to AFB1. It remains plausible that humans at highest risk for AFB1 exposure, particularly those with chronic active hepatitis, may be able to induce carcinogen-detoxification enzymes in response to oltipraz treatment. Although the impact of monofunctional phase II enzyme inducers in human tissues following in vivo exposure has yet to be assessed, Morel et al. (35) recently reported that 1,2-dithiole-3-thiones including oltipraz markedly increased steady state levels of mRNA for α-class GSTs in primary human hepatocyte cultures. Gordon et al. (36) have also noted that 1,2-dithiole-3-thione induces NAD(P)H:quinone reductase activity in peripheral human lymphocytes in vitro.

Exposure of humans to dietary carcinogens including aflatoxins can show remarkable seasonal variability (32, 37). Seasonal variation in food levels of aflatoxins is a reflection of both climatic change and the food harvesting and storage practices of the societies. Contamination of crops is increased in the wet hoi seasons. Thus, it may be possible to design transient interventions focused on those periods of highest aflatoxin ingestion. In this manner, relatively short term interventions may protect against a significant percentage of the total annual exposure to these carcinogenic mycotoxins.

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CHEMOPROTECTION BY TRANSIENT OLTIPRAZ INTERVENTION


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