Chronic Daily Low Dose of 4-Methyl-5-(2-pyrazinyl)-1,2-dithiole-3-thione (Oltipraz) in Patients with Previously Resected Colon Polyps and First-Degree Female Relatives of Breast Cancer Patients[1]

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ABSTRACT

The chemoprevention agent oltipraz, one of the most active chemopreventive compounds in preclinical studies, has been shown to induce glutathione-*S*-transferase (GST) activity in animals. Oltipraz was evaluated in a Phase I trial at daily oral doses of 20 mg (L1), 50 mg (L2), and 100 mg (L3) and twice weekly doses of 125 mg (L4) taken for 6 months with 6 patients entered at L1 and L2 and 7 patients entered at L3 and L4 (26 subjects: 19 females and 7 males). The subject population included patients with previously resected colon polyps and first-degree female relatives of breast cancer patients. Patients with resected colon polyps underwent rectal biopsy for GST and glutathione (GSH) analyses. Of the 26 subjects, the following completed 6 months of therapy: 4 of 6 patients (L1), 4 of 6 patients (L2), 5 of 7 patients (L3), and 4 of 7 patients (L4). Toxicities were mild to severe and included: gastrointestinal symptoms, photosensitivity/heat intolerance, and neurological symptoms. Monthly plasma samples were obtained 2–3 h after oltipraz ingestion with minimally detectable plasma concentrations at L1. There was a significant difference in mean oltipraz concentration across the four doses, with no significant differences in mean oltipraz concentration over time. Rectal tissue and lymphocyte GSH and GST were variable, with no significant difference in mean levels across doses. At the 100-mg/day dose (L3), 1 patient experienced significant increase in rectal tissue GSH and GST activity, whereas 3 additional patients (L1 and L4) had >50% increase in tissue GSH. Lymphocyte GSH level was significantly related to plasma oltipraz concentration. There were no significant correlations between plasma oltipraz concentration and lymphocyte GST level nor any significant correlation between plasma concentration and percentage of change in tissue GSH or GST. Further investigation of dose/schedule and biological end points is ongoing.

INTRODUCTION

Oltipraz[3] is one of the most promising chemopreventive agents in development, based on preclinical models (1–13). There are approximately 12 animal models demonstrating that oltipraz is an effective chemopreventive agent when given before and during carcinogen exposure. For example, oltipraz is protective against aflatoxin B_1-induced hepatic tumorigenesis in rats with an associated decrease in DNA adducts and γ-glutamyl transpeptidase-positive preneoplastic foci. It is postulated that the effect may be secondary to enhancement of electrophilic detoxification pathways and modified oxidative metabolism of aflatoxin B_1. Reduction in the number of pulmonary adenomas and forestomach tumors in mice treated with nitrosamine and uracil mustard has been noted after oltipraz ingestion (3–7). In addition, it has been shown to inhibit the number of azoxymethane-induced colon and small intestinal adenocarcinomas (9).

Interest in oltipraz also originates in part from human epidemiological studies and animal investigations showing that consumption of cruciferous vegetables (*i.e.*, cabbage, Brussels sprouts, and broccoli) is associated with a decrease in the development of cancers such as human colon cancer and aflatoxin-induced hepatic tumors (14–22). Elimination of carcinogens may be responsible for these protective effects secondary to induction of phase II enzymes associated with drug and carcinogen conjugation (23). The dithiolethione group is one of several chemoprotectants found in the *Brassica* family of cruciferous vegetables (24, 25). These compounds have demon-

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[3] The abbreviations used are: oltipraz, 4-methyl-5-[2-pyrazinyl]-1,2-dithiole-3-thione; GST, glutathione-*S*-transferase; GSH, glutathione; G6PD, glucose-6-phosphate dehydrogenase.

strated protection against the induction of tumors in rodent liver, colon, lung, forestomach, and mammary gland tissue by polycyclic aromatic hydrocarbons (23). They induce detoxification enzymes [NAD(P)H:quinone reductase and GST] and increase GSH levels intracellularly (2, 26–28).

Animal studies have shown that oltipraz, as a synthetic substituted 1,2-dithiolethione, is an enhancer of G6PD, epoxide hydrase, glutathione reductase, GST, quinone reductase, and UDP:glucuronyl transferase (2, 29). Oltipraz has been shown to induce GSH and GST levels in rat lymphocytes (30, 31). Limited human studies have suggested the correlation between oltipraz concentration and GSH and GST elevations in lymphocytes and an increase in GSH in colon mucosal biopsies after oltipraz exposure (32, 33).

Toxicities secondary to oltipraz have been evaluated in high-dose, short-duration trials for patients with schistosomiasis and in volunteers entering chemoprevention trials using single low doses of oltipraz or chronic low dose administration (32–37). The most common reversible mild-to-moderate toxicities include gastrointestinal discomfort (diarrhea, nausea, flatulence, cramping, and bloating), thermal and photosensitivity, and headache. Pharmacokinetic evaluations have demonstrated significant interpatient and intrapatient variability (13, 32, 33, 38–40). Despite the variability, serum oltipraz concentrations achieved at low-dose levels (*e.g.*, 125 and 250 mg/day) are similar to rodents fed a diet of oltipraz at which biologically relevant effects have been found (5, 10, 26, 41). Chronic daily doses of oltipraz $>$125 mg are prohibitive because of excessive toxicity (13, 42).

At least 13 metabolites of oltipraz extracted from the urine of mice and human patients with schistosomiasis have been identified (43–45). More recent work evaluated the plasma and urine pharmacokinetics of the desulfurated metabolite (M3; Ref. 46). There was significant and variable conversion of oltipraz to M3. The biological significance of oltipraz metabolites is unknown. Elimination of oltipraz metabolites is chiefly via the kidneys with $<$1% of unchanged oltipraz excreted in the urine. The metabolism of oltipraz appears to be similar in humans and monkeys. Oral oltipraz absorption, however, differs depending upon the animal species and the dose administered (44). In humans, there is a suggestion that bioavailability of oltipraz is increased when administered with food rather than while fasting (38, 47).

The objectives of this study were to define the toxicities, the pharmacokinetics, and biochemical correlations of oltipraz when administered chronically at less than the maximum tolerated dose in patients with previously resected colon polyps and first-degree female relatives of breast cancer patients. Biochemical end points included the investigation of induction of GSH and GST in lymphocytes and rectal tissue over time during chronic oltipraz ingestion.

MATERIALS AND METHODS

Participants. From July 1993 to October 1995, 26 subjects (19 women and 7 men) were entered into the study. Participants at increased risk for breast or colon cancer were eligible, based on being a first-degree female relative of a breast cancer patient or having had a polypectomy with a pathological diagnosis of adenomatous polyp within the previous 2 years. All patients were required to have an Eastern Cooperative Oncology Group performance status of 0–1; no history of serious or uncontrolled cardiac, hypertensive, pulmonary, hepatic, or renal disease; no history of malignancy (except cervical carcinoma *in situ* or nonmelanomatous skin carcinoma); and no G6PD deficiency. Subjects could not have received previous chemotherapy, radiation therapy, or immunotherapy. Mineral oil use was prohibited because it decreases absorption of oltipraz. All participants were required to have adequate contraception if they were of reproductive age. Women of childbearing potential were required to have a negative pregnancy test. All participants provided informed consent in accordance with federal and institutional guidelines.

Treatment. Subjects were to take oltipraz in once-daily oral doses, after a meal, dispensed in calendar packs. The oltipraz was provided by Rhône-Poulenc (Paris, France) to the National Cancer Institute and distributed by Ogden BioServices (Rockville, MD) in 20-, 50-, 100-, and 125-mg capsules. Compliance was assessed by medication calendars filled out by the participants and actual capsule counts done by the study nurse. Frequent telephone calls were conducted by the study coordinators to evaluate toxicities and to monitor compliance.

The starting dose of oltipraz was 20 mg once daily for 6 months. Subsequent daily doses were at the 50- and 100-mg dose levels. The fourth dose level was planned at 125 mg given twice weekly for 6 months. This particular 125-mg regimen was chosen because we had already determined that the maximum chronic daily dose of oltipraz was 125 mg daily (13, 32) and because preliminary data suggest that biological modulation (*e.g.*, induction of GST isozymes) may occur on intermittent dose schedules (31, 33). Participants were to be entered into the specified dose levels in groups of 6. Oltipraz was discontinued for participants who developed persistent symptoms that were related to the study drug (National Cancer Institute Common Toxicity Criteria) and that were unacceptable to the patient. Individuals were permitted to continue daily oltipraz if symptoms were mild (*e.g.*, flatus and mild bloating) and did not interfere with daily activities.

Prior to therapy, all patients had a baseline history, physical exam, performance status, complete blood count, blood urea nitrogen, creatinine, aspartate aminotransferase, bilirubin, alkaline phosphatase, albumin, sodium, potassium, chloride, phosphorous, calcium, TSH, T_4, G6PD, urinalysis, electrocardiogram, and two separate baseline blood samples for lymphocyte separation to determine baseline GSH levels and to measure GST activity. Participants with a prior history of colon polyps had a baseline flexible proctosigmoidoscopy with rectal biopsy to determine GSH levels and to measure GST activity. Biopsies were obtained using a flexible pinch with an inner diameter of 0.8 mm at 7–15 cm from the anal verge. Three to four small biopsy samples were estimated to provide 24–40 mg of tissue. The samples were placed immediately into liquid nitrogen and stored at −80°C. A repeat history and physical examination were performed after 2 weeks of dosing. Thereafter, history, physical exam, performance status, complete blood count, and chemistries were obtained monthly. Plasma oltipraz samples were collected 2–3 h after dosing at 2 weeks, 4 weeks, and then monthly. They were protected from light, centrifuged, and de-

canted into test tubes with 0.1 ml of thiol diglycerol and frozen at −70°C. Blood samples for GSH and GST analyses were collected in heparinized tubes; lymphocytes were isolated within 2 h and stored at −70°C until further analysis. Two baseline samples were obtained, one each at two different pretreatment visits. The samples were obtained biweekly for the first month and monthly thereafter. In addition, at 2 weeks and again at ~6 months, oltipraz levels and lymphocyte separation for GSH and GST activity were obtained just prior to oltipraz ingestion and then at 2 and 4 h after dosing. Oltipraz levels were also obtained at 30 min and 1 h after oltipraz ingestion. Proctosigmoidoscopy with rectal biopsy for the polypectomy patients was repeated at ~6 months just prior to discontinuation of oltipraz to measure GST levels and GST activity. All patients completed a diary documenting pill intake, time of ingestion, toxicities or symptoms, and ingestion of servings of cruciferous vegetables. Observation was to continue for 6 months after treatment cessation.

Oltipraz Plasma Concentration. To quantitate oltipraz in plasma samples, a modification of the extraction method published by Bennett *et al.* (48) was used. One hundred μl of plasma were spiked with 50 μl of internal standard (4 μg/ml ethyl oltipraz; Rhône Poulenc) and extracted twice with 3 ml of heptane. After centrifugation, the supernatant was evaporated to dryness under nitrogen. The samples were reconstituted with 40% methanol and analyzed by reversed phase high-performance liquid chromatography using a μNovapak C_{18} column (10 μm, 3.9 × 300 mm; Waters Associates, Milford, MA). The mobile phase consisted of 60% methanol:40% 50 mM ammonium acetate. The amount of oltipraz in the samples was quantitated by monitoring absorbance at 448 nm. Adequate measures were taken during sample extraction procedures to prevent exposure to light. Extraction efficiency was determined to be >90%, and all samples were analyzed in duplicates. Standards from the National Institute of Standards and Technology were also analyzed. There was <5% deviation between the actual concentrations *versus* those determined by our methods, which were acceptable by National Institute of Standards and Technology.

GSH and GST Activity. For the quantitation of GSH, lymphocytes were isolated by adding 30 ml of blood to an equal volume of RPMI 1640 and layered on Ficoll-Paque as described (49). Final pellets were suspended in 0.1 M potassium phosphate buffer (pH 6.65). The rectal biopsies were thawed, immersed in 1 ml of 10 mM Tris-HCl (pH 7.8), and homogenized. GSH was measured using the glutathione disulfide reductase methods of Tietze (50) and Griffith (51). After two repeated 10-s sonications of the lymphocytes or rectal tissue in phosphate buffer, separated by 30 s, sulfasalicyclic acid (1.7%; Sigma Chemical Co., St. Louis, MO) was added. The samples were placed on ice for 10 min and centrifuged at 500 × *g* for 5 min at 4°C. The supernatant was incubated with NADPH, 5,5′-dithiobis(2-nitrobenzoic acid), and glutathione reductase (Sigma Chemical Co.) in sodium phosphate/EDTA buffer (pH 7.5). Glutathione reductase was added immediately prior to reading at 412 nm. Results were compared with standard curves constructed using increasing concentrations of GSH in place of lymphocyte extract in the reaction mixture. Protein estimations were done according to the method of Bradford (52).

Total GST in the lymphocytes and tissue was determined using a kinetic spectrophotometric assay (53). Frozen lymphocyte pellets or homogenized tissue was rapidly thawed, resuspended, and sonicated twice at 15-s intervals. Supernatant was obtained after centrifugation at 10,000 × *g* at 4°C for 30 min. Reaction mixtures contained 500 μM GSH, cytosolic extract (300 μg total protein), and 3.2 mM 1-chloro-2,4-dinitrobenzene (Eastman Organic Chemicals, Rochester, NY) in 0.01 M potassium phosphate buffer (pH 6.65). The reaction was performed in a cuvette, and the increase in absorbance was monitored at 0.25-min intervals for 2.5 min. GST activity was determined as the linear rate of formation of substrate. Protein estimation was performed according to the method of Bradford (52).

Statistical Analyses. Plasma oltipraz levels, lymphocyte GSH levels, and lymphocyte GST activity were analyzed by a general mixed model ANOVA (54), using PROC MIXED in SAS (55). Mean GSH and GST differences among dose levels were tested for statistical significance. At each dose level, differences in mean levels over time were tested. Pearson correlation coefficients were calculated to describe the association between plasma oltipraz levels and GSH/GST levels.

RESULTS

Twenty-six participants were entered into the study (6 each at the 20- and 50-mg doses and 7 each at the 100- and 125-mg doses). Fourteen were first-degree female relatives of breast cancer patients, and 12 (9 females and 3 males) were persons with a history of resected adenomatous polyps of the colon. Mean age was 48 years (range, 28–75 years), and all had an Eastern Cooperative Oncology Group performance status of 0.

Seventeen of the 26 (65%) participants completed 6 months of the trial, including 4 individuals for each of three dose levels (20, 50, and 125 mg) and 5 at the 100-mg dose. Of the remaining 9 individuals, 8 withdrew because of toxicity (range of time in the study, 2 days to 19 weeks) and 1 discontinued oltipraz because of suspected mild alcohol-induced gastritis.

All persons were evaluable for toxicity. Eight of the participants had no toxicity (3 each at the 20- and 100-mg doses and 2 at the 50-mg dose). Of the remaining 3 individuals receiving 20 mg, 1 had mild (grade 1) toxicities and 2 had severe (grade 3) toxicities. At the 50-mg dose, 2 experienced mild and 2 noted moderate toxicity. Of the remaining 4 participants at the 100-mg dose level, 1 had mild and 3 experienced moderate toxicities. Five participants developed mild toxicities, and 2 had moderate toxicities at the 125-mg dose.

Detailed episodes of toxicity are summarized in Table 1. There were a total of 80 episodes. The common toxicities were mild nausea, bloating, cramps, increased flatus, and a change in stool consistency, accounting for 30% of the episodes. In some cases, symptoms improved after eating. The severity of toxicities was variable, ranging from very mild with no effect on daily activities to severe effects resulting in limitation of functional capabilities. The second most frequently reported toxicity was photosensitivity and/or thermal sensitivity noted in 6 individuals. This sensation was characterized by erythema (sunburn), warmth, tingling, and/or pruritus on exposure to sun, even with the use of sunscreen, or when hands were immersed in warm water. Neurological toxicities included paresthesias in 4 partic-

Table 1 Number of patients experiencing toxic symptoms

Toxicity[a]	No. of patients
Gastrointestinal	
Bloating	7
Abdominal cramps (1 additional patient had grade 3 abdominal cramps)	6
Flatulence	10
Loose stools	3
More frequent stools	1
Diarrhea	3
Constipation	5
Nausea/vomiting	8/1
Heartburn	1
Taste changes	1
Decreased appetite	3
Neurologic	
Paresthesia	4
Headaches (1 patient had grade 3 headaches)	5
Fatigue	1
Skin	
Sensitivity to sun/heat	6
Itching (no rash)	2
Rash	2
Breast tenderness	2
Cardiovascular	
Rapid heartbeat, chest pain	2
Other	7
1 dysuria	
1 lipoma	
1 calf cramping	
1 pedal edema	
1 dry mouth	
1 subconjunctival hemorrhage (occurred spontaneously)	
1 greenish urine	

[a] There was a total of 80 episodes in 26 patients.

Table 2 Severity of toxicity by dose and study completion status

Dose	Completed follow-up ($n = 17$)	Withdrew ($n = 9$)
20 mg/day	$n = 4$	$n = 2$
None	3	0
Mild	1	0
Moderate	0	0
Severe	0	2
50 mg/day	$n = 4$	$n = 2$
None	2	0
Mild	0	2
Moderate	2	0
Severe	0	0
100 mg/day	$n = 5$	$n = 2$
None	3	0
Mild	1	0
Moderate	1	2
Severe	0	0
125 mg two times per week	$n = 4$	$n = 3$
None	0	0
Mild	3	2
Moderate	1	1
Severe	0	0
Total[a]	$n = 17$	$n = 9$
None	8	0
Mild	5	4
Moderate	4	3
Severe	0	2

[a] $P = 0.025$ using Fisher's Exact test, indicating that there was less toxicity in those who completed follow-up compared with those who did not. Each patient was classified according to the most severe toxicity grade experienced by that patient.

ipants and headaches in 5, none of which interfered with activity and which resolved upon cessation of oltipraz. Less frequent symptoms included rash, pruritus, breast tenderness, chest pain, or rapid heartbeat. Of the 80 episodes, 61 were mild, 17 were moderate, and 2 were severe. Eighteen occurred at the 20-mg dose, 13 at the 50-mg dose, 21 at the 100-mg dose, and 28 at the 125-mg dose. Of note, for the 8 individuals who withdrew from the study because of toxicities, all but 1 experienced multiple symptoms.

A summary of toxicity by dose and study completion status is given in Table 2. Of 17 participants who completed the protocol, 8 had no toxicity, 5 had mild, and 4 had moderate toxicity. Of 9 who did not complete the protocol, 4 had mild, 3 had moderate, and 2 had severe toxicity, indicating a greater degree of toxicity than completers ($P = 0.025$ by Fisher's exact test). For each of the 26 patients, compliance was measured as the number of pills taken divided by the total number of pills that would be taken by a full complier over 6 months (expressed as a percentage). This calculation took into account the biweekly dose at the 125-mg level. Compliance ranged from 2 to 100% with a mean of 74% and a median of 94%.

Plasma Oltipraz Concentrations. At 2 weeks after the start of treatment, plasma oltipraz concentrations were observed before treatment was administered for that day and at 30, 60, 120, and 240 min after treatment administration (Fig. 1*A*). Eighteen of 24 persons had detectable levels during this 4-h period. Fifteen of 18 (83%) had their peak plasma oltipraz concentrations between 2 and 4 h.

Similarly, at 6 months after the start of treatment, plasma oltipraz concentrations were again observed over a 4-h period posttreatment administration. Fifteen of 24 persons had observable detectable levels during this period, and 8 of 15 (53%) had their peak level between 2 and 4 h.

Plasma oltipraz concentrations for each dose are given by time from 2 weeks to 6 months in Fig. 1*B*. There was a significant difference in mean oltipraz concentration across the four doses ($P < 0.001$). Detection at the 20-mg dose, however, was negligible. For each dose, there were no significant differences in mean oltipraz concentration over time.

Lymphocyte GSH Levels. Mean monthly lymphocyte GSH levels are summarized in Fig. 2 for a subset of 14 of 19 persons who had lymphocyte GSH measurements at the 20-, 50-, and 100-mg/day doses. There was no significant difference in mean levels across doses ($P = 0.13$). There was a significant difference across time for the 20-mg/day dose ($P = 0.02$).

Thirteen persons had lymphocyte GSH levels measured between 0 and 4 h immediately after treatment was initiated. Ten of these 13 (77%) had peak lymphocyte GSH levels between 2 and 4 h. Thirteen persons also had serial lymphocyte GSH levels measured between 0 and 4 h at 6 months after treatment initiation. Eleven of 13 (85%) had peak lymphocyte GSH levels between 2 and 4 h.

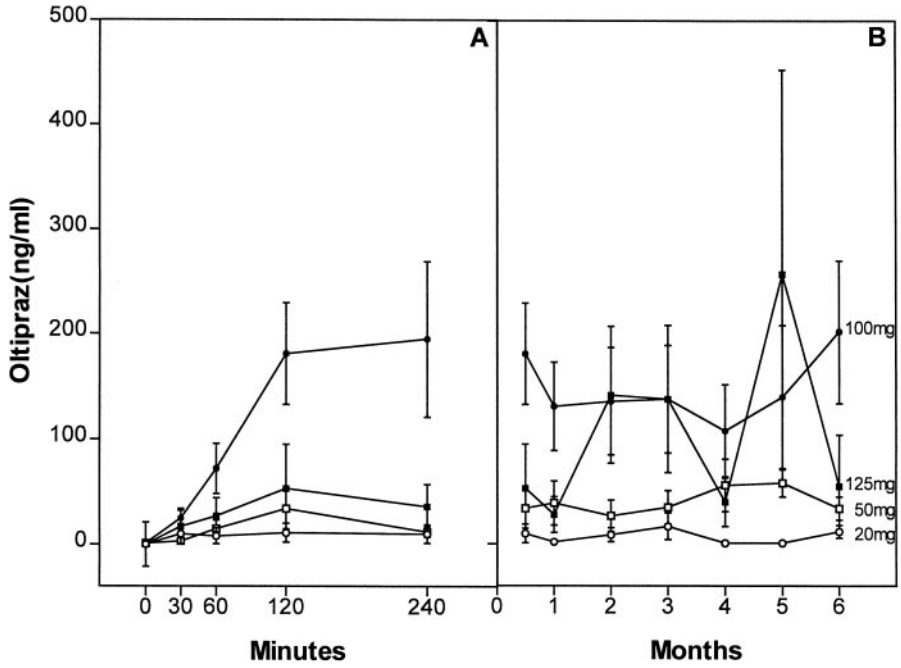

Fig. 1 *A*, oltipraz levels (ng/ml) by dose (○, 20 mg/day; □, 50 mg/day; ●, 100 mg/day; ■, 125 mg two times per week) and time (in minutes) from treatment administration, at 2 weeks after treatment initiation. Values reported are means; *bars*, SE. *B*, oltipraz levels (ng/ml) by dose (○, 20 mg/day; □, 50 mg/day; ●, 100 mg/day; ■, 125 mg two times per week) and time (in months) from 2 weeks through 6 months. Values reported are means; *bars*, SE.

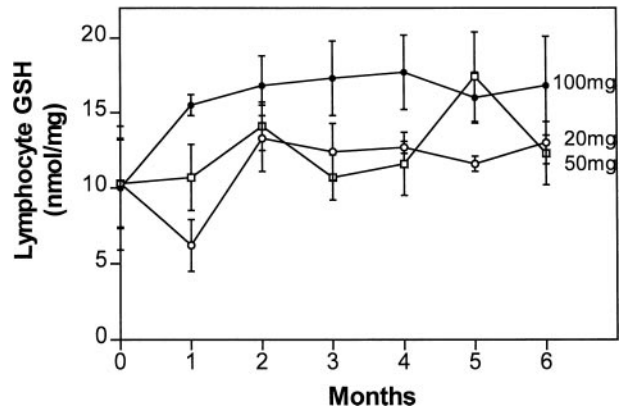

Fig. 2 Mean lymphocyte GSH (nmol/mg) by dose (○, 20 mg/day; □, 50 mg/day; and ●, 100 mg/day) and time, pretreatment through 6 months; *bars*, SE. Data available for a subset of 14 of 19 patients accrued to these doses.

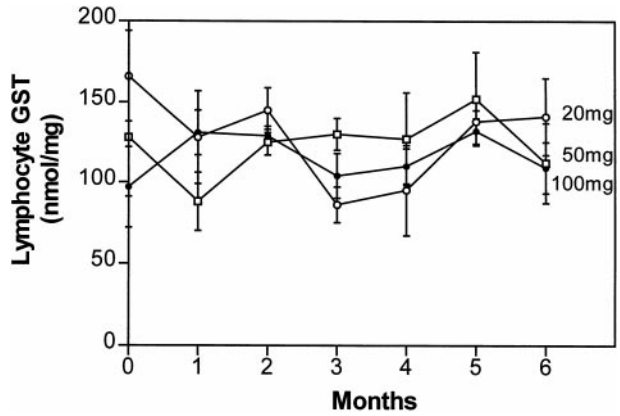

Fig. 3 Mean lymphocyte GST (nmol/min/mg) by dose (○, 20 mg/day; □, 50 mg/day; and ●, 100 mg/day) and time, pretreatment through 6 months; *bars*, SE. Data available for a subset of 14 of 19 patients accrued to these doses.

Lymphocyte GST Levels. Mean monthly lymphocyte GST levels are summarized in Fig. 3 for a subset of 14 of 19 persons who had lymphocyte GST measurements at the 20-, 50-, and 100-mg/day doses. There was no significant difference in mean levels across doses ($P = 0.74$). There were no significant differences over time for any dose.

Fourteen persons had lymphocyte GST levels measured between 0 and 4 h immediately before treatment was initiated. Nine of these 14 (64%) had peak lymphocyte GST levels between 2 and 4 h. Fourteen persons also had serial lymphocyte GST levels measured between 0 and 4 h at 6 months after treatment initiation. Nine of 14 (64%) had peak lymphocyte GST levels between 2 and 4 h.

Rectal Tissue GSH and GST Levels. Table 3 summarizes the rectal tissue GSH at baseline and at 3 months in a subset of 9 of 19 persons who had tissue GSH measurements at the 20-, 50-, and 100-mg/day doses, and 125-mg two times per week. Absolute change and percentage of change from baseline to 3 months is given. There was no association of tissue levels or change with dose. Four patients (20, 100, and 125 mg) had >50% increase in tissue GSH (55–263%). Table 4 gives similar data for tissue GST, and no dose association was found.

Correlation of Oltipraz Concentration with GST and GSH. Pearson correlation coefficients were calculated to relate plasma oltipraz concentration with lymphocyte and tissue GSH and GST levels. For lymphocyte GSH and GST, there

Table 3 Tissue GSH levels in nmol/mg at baseline and 3 months and plasma oltipraz levels in ng/ml at 3 months, by dose

Dose	Baseline	3-month	Change	% change	Plasma oltipraz
20 mg/day	11	17	6	55%	0.0
20 mg/day	10	5	−5	−50%	4.6
20 mg/day	11	12	1	9%	8.2
50 mg/day	8	5	−3	−38%	8.3
100 mg/day	5	0.4	−4.6	−92%	57.0
100 mg/day	8	29	21	263%	28.0
125 mg 2×/wk[a]	4	8	4	100%	129.0
125 mg 2×/wk	9	2	−7	−78%	264.0
125 mg 2×/wk	5	8	3	60%	Missing

[a] 2×/wk, two times per week.

Table 4 Tissue GST levels in nmol/min/mg at baseline and 3 months, and plasma oltipraz levels in ng/ml at 3 months, by dose

Dose	Baseline	3-month	Change	% change	Plasma oltipraz
20 mg/day	208	228	20	10%	0.0
20 mg/day	146	146	0	0%	4.6
20 mg/day	213	193	−20	−9%	8.2
50 mg/day	256	269	13	5%	8.3
100 mg/day	160	37	−123	−77%	57.0
100 mg/day	54	43	−11	−20%	98.0
100 mg/day	23	219	196	852%	28.0
125 mg 2×/wk[a]	120	79	−41	−34%	129.0
125 mg 2×/wk	175	164	−11	−6%	264.0
125 mg 2×/wk	103	108	5	5%	Missing

[a] 2×/wk, two times per week.

were 105 and 107 (respectively) pairs of lymphocyte and plasma measures combined over all doses and times. Lymphocyte GSH level was significantly related to plasma oltipraz concentration with a low Pearson correlation coefficient (r) of 0.26 ($P = 0.007$; $n = 105$). Using only postbaseline measures, the percentage of change from baseline in lymphocyte GSH levels was not related to the corresponding oltipraz concentration ($r = -0.03$, $P = 0.82$; $n = 73$). There were no significant correlations between plasma oltipraz concentration and lymphocyte GST level ($r = -0.16$, $P = 0.10$; $n = 107$) nor with percentage of changes in GST level ($r = 0.03$, $P = 0.79$; $n = 78$).

Using the data in Tables 3 and 4, there were no significant correlations between plasma oltipraz concentration and the percentage of change in tissue GSH or tissue GST between baseline and 3 months.

DISCUSSION

There is very limited investigation of the potential biological effects of oltipraz in humans. Our recent work evaluated 31 normal subjects who received a single dose of oltipraz at doses of 20–500 mg (32). Pharmacodynamic evaluation was conducted based on the percentage of elevation of GSH and GST levels over baseline in lymphocytes. There was intraindividual variability in the baseline levels of GSH and GST. Subjects with the highest maximum plasma concentration of oltipraz tended to have greater induction of GSH and GST, with peak elevations in lymphocytes occurring at 6 h after dosing. However, this direct relationship between oltipraz and GSH/GST was not observed for all patients. The maximum induction of GSH levels in the 100- and 125-mg subjects was 27 and 81%, respectively, whereas that observed in GST levels was 60 and 101%, respectively.

Our present trial evaluated four groups of patients with the first three groups receiving very low doses of oltipraz at 20, 50, or 100 mg daily for 6 months, whereas the fourth group received 125 mg twice weekly for 6 months. The data suggest that the greatest effect on lymphocyte GSH (Fig. 2) and GST activity (Fig. 3) was at the 100-mg/day dose. Although there is no definitive dose relationship, two of the three patients with change in rectal tissue GSH (100 and 125 mg) and the one patient with a change in tissue GST activity (100 mg) received the higher doses of oltipraz. These data are similar to other reports supporting the use of surrogate tissue to evaluate biological effect.

O'Dwyer *et al.* (33) observed increases in GST activity at lower dose levels, although these levels were still higher than those used in our study. Furthermore, they were among the first to demonstrate modulation of gene expression in humans by any chemopreventive agent. Twenty-four individuals at risk for colorectal cancer received a single oral dose of oltipraz (125, 250, 500, or 1000 mg/m^2). Subjects with positive GST transferase μ expression had higher GSH transferase catalytic activity in lymphocytes with a similar trend seen in colon mucosa. At the 125- and 250-mg/m^2 doses, there was an increase in mean GST activity in colon tissue. For example, at 125 mg/m^2, colon GST activity increased from 75.3 ± 7 nmol/min/mg to 102.2 ± 5.3 nmol/min/mg on day 8. Lymphocyte GST activity also increased but only at the 125-mg/m^2 dose level from 72.6 ± 9 nmol/min/mg to 82.4 ± 11 nmol/min/mg on day 3. No changes in GSH content were demonstrated. The most significant changes were seen in detoxicating enzyme gene expression in both lymphocytes and colon tissue. The lymphocyte and colon mRNA content for γ-glutamyl cysteine synthetase and DT-diaphorase increased with a peak on days 2–4 after treatment, returning to baseline by days 7–10. There was a 5.75-fold increase in induction of gene expression in colon mucosal for the γ-glutamyl cysteine synthetase and a 4.14-fold peak increase for DT-diaphorase at 250 mg/m^2 only. There was a good correlation between baseline and peak lymphocyte and colon mucosal levels of γ-GSH and DT-diaphorase. This investigation supported the use of surrogate markers for biological end points and also supported the use of intermittent dosing of oltipraz. In addition, there is the emphasis that the most pronounced changes were seen in detoxifying enzyme gene expression, an observation that was not evaluated in our trial.

Primiano *et al.* (31) also have emphasized that intermittent small doses of oltipraz may trigger enzyme induction response; however, the protracted pharmacodynamics of gene expression is perhaps the best reflection of biological activity, regardless of drug pharmacokinetics. Our previous single-dose trial suggested induction of GSH and GST activity in lymphocytes at 100- and 125-mg doses and also demonstrated the significant intrapatient variability in these measurements.

Although there are few human oltipraz pharmacology studies, our present data are consistent with previous reports, in-

cluding our own single-dose trial evaluating oltipraz at the same doses used in this report (13, 32, 33, 38–40, 42). There are insignificant plasma levels of oltipraz noted at the 20-mg dose. There was confirmation of the considerable intrasubject and intersubject variability in oltipraz levels at the 100- and 125-mg dose levels. The intermittent dosing at 125 mg produced plasma levels that were similar to those seen in our previous chronic dose trial (42). Peak median oltipraz levels between 2 and 4 h are consistent with the previous reports; however, mean oltipraz concentrations did not change over the 6-month dosing schedule. Lymphocyte GSH levels at various doses and times were significantly correlated with plasma oltipraz levels at the corresponding dose and time (Fig. 6). Plasma concentrations, however, were not related to lymphocyte GST activity nor percentage of change in lymphocyte and rectal tissue GSH and GST activity.

Likewise, the toxicity assessment was similar to previous investigations, including the high doses of oltipraz used to treat schistosomiasis and the lower doses used in other oltipraz chemoprevention trials (13, 32–39, 42). We have determined previously that the maximum tolerated dose of oltipraz is 125 mg daily (13, 42). Even at the doses $<$125 mg and at the twice weekly 125-mg dose schedule, similar toxicities are reported. In particular, gastrointestinal side effects, including mild nausea, bloating, cramps, increased flatus, and change in stool consistency, are most prevalent, followed by photo and/or thermal sensitivity. Although toxicities were noted, compliance was excellent for those who remained in the trial, and in some cases, mild side effects subsided over time.

A Phase II chemoprevention prevention trial of oltipraz was completed in Qidong, Jiangsu Province, People's Republic of China. (56). This study recruited 234 healthy adults, including those with hepatitis B virus, who were randomized to receive 125 mg of oltipraz daily, 500 mg of oltipraz weekly, or placebo with a subsequent 8-week follow-up. There were no significant differences between the oltipraz doses in symptom type or severity. However, an extremity syndrome, including numbness, tingling, and pain in the fingertips developing soon after treatment, did occur more frequently in the oltipraz patients (18.4 and 14.1%, respectively) compared with placebo (2.5%). There were 21.8% of individuals who reported toxicities. There were 132 subjects who maintained a dosing schedule without interruption, and $>$75% contributed all of the required urine and blood samples. Toxicities of the extremities were comparable in the Qidong study compared with our study, in that $\sim$15% of patients experienced such toxicities. A greater frequency of gastrointestinal problems was seen in our study. This could be related to the longer duration of treatment in our study.

Oltipraz remains an intriguing agent for the development of chemoprevention strategies. Other trials are being considered, including a randomized, double-blinded, placebo-controlled Phase II study of oltipraz for patients with stage I colon cancer or who have had one colon polyp 1 cm or greater. Patients with stage II or III colon cancer would be eligible, providing there has been a 5-year interval since the time of diagnosis. The proposed dose is 125 mg daily for 3 years. Further understanding of the biological effect after chronic intermittent doses of oltipraz is needed, including additional studies exploring modulation of gene expression.

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Chronic Daily Low Dose of 4-Methyl-5-(2-pyrazinyl)-1,2-dithiole-3-thione (Oltipraz) in Patients with Previously Resected Colon Polyps and First-Degree Female Relatives of Breast Cancer Patients

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