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Oltipraz: A Laboratory and Clinical Review

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Abstract Oltipraz [5-(2-pyrazinyl)-4-methyl-1,2-dithiole-3-thione; RP 35972] is a synthetic, substituted 1,2-dithiole-3-thione previously used in humans as an antischistosomal agent. Cruciferous vegetables (*e.g.*, Brussels sprouts, cabbage) contain several agents, including dithiolethiones, which appear to inhibit carcinogenesis; however, it is unclear which dietary compounds produce the protective effects. Animal studies have demonstrated that oltipraz is a potent inducer of Phase II detoxification enzymes, most notably glutathione-*S*-transferase (GST). Laboratory evaluations have shown that dietary concentrations of oltipraz produce marked inhibition of aflatoxin B_1-induced hepatic tumorigenesis in rats. Levels of hepatic aflatoxin–DNA adducts, urinary aflatoxin–N^7-guanine, and serum aflatoxin–albumin adducts decreased when biliary elimination of aflatoxin–glutathione conjugants increased, thus providing predictive biomarkers that measured a chemopreventive effect. In other animal experiments, oltipraz was found to inhibit chemically induced carcinogenesis in bladder, colon, breast, stomach, and skin cancer models. In addition, oltipraz has been shown to be non-mutagenic, a radioprotector, and a chemoprotective agent against carbon tetrachloride and acetaminophen toxicity. More recent studies in rats suggest that unsubstituted 1,2-dithiole-3-thiones may more effectively inhibit aflatoxin-induced hepatic tumorigenesis and induce electrophile detoxification enzymes. Multiple human clinical trials have been conducted using 1.0–4.5 gram doses of oltipraz over 1–3 days for the treatment of schistosomiasis. Phototoxicity has precluded its use in tropical areas. More recently, a 6 month Phase I trial was completed in which patients with resected colon polyps, or females with first degree relatives with breast cancer, were given oral daily doses of oltipraz at 125 mg or 250 mg. The maximum tolerated dose of oltipraz was $\leq$125 mg daily. Grade I/II toxicities included photosensitivity/heat intolerance, GI and neurologic toxicity. Peak plasma concentrations were analyzed by HPLC with wide variability. In another Phase I study, a single oral dose of oltipraz was given to normal volunteers at dose levels of 125, 250, 375, and 500 mg. There was no significant difference in half-life ($t_{1/2}$) between the four dose levels nor in clearance at the 125 and 250 mg levels. Peak oltipraz levels $\geq$1.0 μg/mL were achievable with marked interpatient variability. A series of small trials evaluating single oral doses of oltipraz for up to 28 days (dosing range 1 mg/kg–3 mg/kg/day) also showed a short $t_{1/2}$ (4.1–5.3 hours), a sustained steady state without variation after a loading dose, and increased serum and urine concentrations with consumption of a high-fat diet. Future human clinical trials will include pharmacokinetic analyses of lower chronic dose administration of oltipraz with determination of intermediate endpoints in target tissues.

Key words: anti-carcinogenesis, antioxidants, chemoprevention, cancer prevention, dithiolethiones, oltipraz

Chemoprevention is a strategy to inhibit or delay the onset of neoplasia through endocrine, nutritional, or pharmacologic intervention prior to the clinical appearance of malignant lesions. Studies conducted in experimental animal

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models for human cancer have demonstrated that, in a number of tissues, carcinogenesis is suppressed by the administration of biological or chemical agents [1]. The Chemoprevention Branch of the National Cancer Institute is presently using a number of different target organs in animal tumor models to evaluate a multitude of potential chemopreventive (anticarcinogenic) compounds [2,3]. In addition, the Chemoprevention Branch is sponsoring a rapidly growing number of Phase I, Phase II and Phase III clinical trials utilizing chemoprevention agents [1,4]. Oltipraz [5-(2-pyrazinyl)-4-methyl-1,2-dithiole-3-thione; RP 35972], a synthetic, substituted, 1,2-dithiole-3-thione, is a Phase II metabolic enzyme inducer, *e.g.*, glutathione-*S*-transferase (GST), and is a particularly promising chemopreventive agent given its efficacy in animal screening models [3,5–7] (Fig. 1). The compound was described in 1977 and was used initially in humans as an antischistosomal agent [6,8–12].

Epidemiologic data suggest that the consumption of cruciferous vegetables (such as broccoli, cabbage, and Brussels sprouts) has a protective effect against some human cancers including cancer of the colon [13,14]. For example, in an evaluation of 576 patients with colon and rectal cancer compared with 1220 controls, a reduced risk of colon cancer was associated with eating cabbage, Brussels sprouts, and broccoli; rectal cancer risk, however, was not diminished [15]. A study in Japan also confirmed an inverse association between consumption of cabbage and the development of colon cancer, although there was no such association noted in Hawaii [16]. A more recent large project in Australia found that consumption of vegetables from the *Brassica* family provided protection against cancer of the colon [17].

Animal studies have demonstrated that consumption of cruciferous vegetables may produce a chemopreventive effect. A diet containing cauliflower was protective against the formation of aflatoxin–DNA adducts and decreased the levels of α-fetoprotein in rats exposed to aflatoxin [18,19]. Cabbage-supplemented diets protected against aflatoxin-induced liver tumors (50% reduction); a beet-supplemented diet was deleterious [20]. It has been suggested that the cancer protective effect was seen because *Brassica* species were inducers of enzymes associated with Phase I and/or Phase II drug and carcinogen biotransformations. *In vivo* anticarcinogenesis may be the result of increased metabolism and therefore elimination of carcinogens from the body [21]. The inductive effect of cabbage and Brussels sprouts on enzymes associated

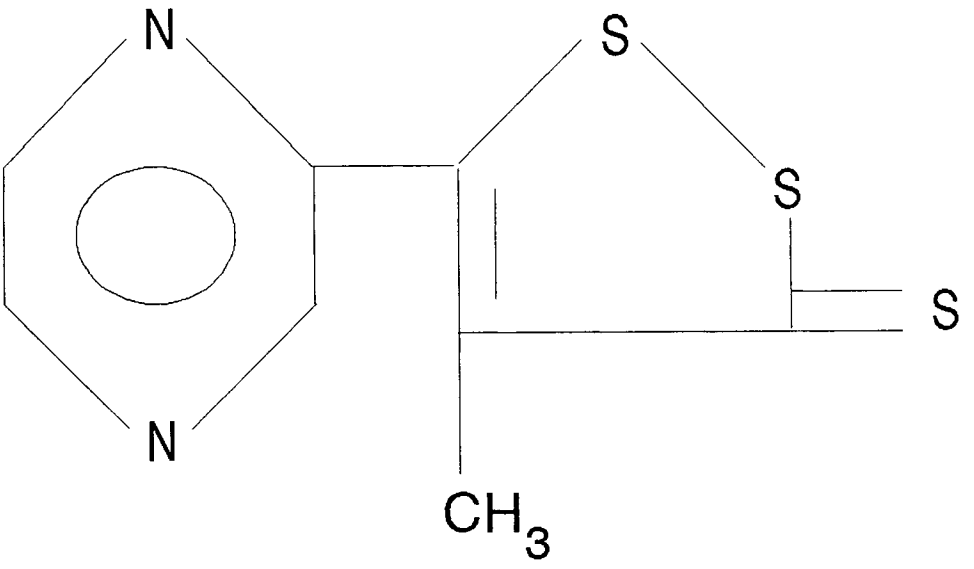

Fig. 1. Oltipraz [5-(2-pyrazinyl)-4-methyl-1,2-dithiole-3-thione].

with drug and carcinogen metabolism was later confirmed in humans consuming 1 or 2 servings of vegetables per day [22].

Three indole compounds have been identified which appear to be the greatest inducers of the aryl hydrocarbon hydroxylase system (Phase I induction) in the cruciferous vegetables [23]. Later studies evaluated the effects of indole-3-acetonitrile, indole-3-carbinol, and 3,'3-diindolylmethane plus cabbage and Brussels sprouts on intestinal drug metabolizing enzyme induction. The investigations demonstrated that the vegetables may contain other compounds capable of stimulating the activity of these enzymes since both cabbage- and Brussels sprouts-supplemented rat diets produced greater stimulation than the indoles alone [24].

Since the 1950s, it has been known that cruciferous vegetables contain dithiolethiones [25, 26] (Fig. 2). These and other thiono-sulfur derivatives are demonstrated chemoprotectors in rodent colon, forestomach, liver, lung, and mammary gland models against such compounds as polycyclic aromatic hydrocarbons [21]. These compounds produce elevated intracellular glutathione levels and induce carcinogen detoxification enzymes, including GSTs and NAD(P)H:quinone reductase (QR) in animal models [27]. Glutathione is an important cellular non-protein thiol and is significant for its ability to serve as a protector against oxidants, free radicals, and electrophilic intermediates of certain chemicals, including drugs [30]. The presence of 1,2-dithiole-3-thiones in cruciferous vegetables, however, may or may not contribute to a chemoprotective effect since these vegetables also contain a number of other chemoprotective compounds such as aromatic isothiocyanates, flavonoids, indoles, phenols, and protease inhibitors [13].

Additional uses of 1,2-dithiole-3-thiones include the ability to stimulate salivary secretion [27,28] and their utility as antioxidant additives in metals, commercial oils and greases, and rubber [29].

OLTIPRAZ ANIMAL INVESTIGATIONS

Over the past 10 years, a number of animal laboratory experiments have demonstrated oltipraz's effectiveness as an enhancer of epoxide hydrase, glucose-6-phosphate (G6P) dehydrogenase, glutathione reductase (GSSG), GSTs, QR, and UDP:glucuronyl transferase; as an inducer of glutathione (GSH); as an antihepatotoxic; and as a radioprotector [7]. A summary of the most significant observations is provided below (Table I).

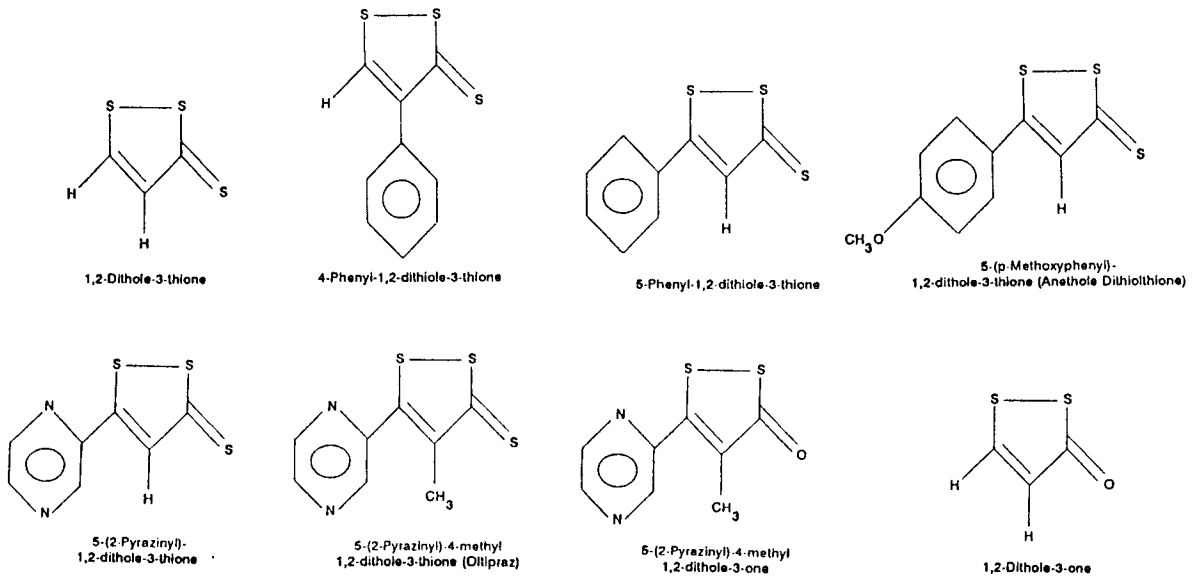

Fig. 2. Structures of 1,2-dithiole-3-thione analogs. Adapted from DeLong *et al.* [44].

TABLE I. Summary of Effects of Oltipraz on GST and GSH in Animal Tissue

Species	Oltipraz Dose	Liver GST	Liver GSH	Carcinogen	Reference
Rat	control	550*, 31$^+$	22		[31]
	0.1% × 14 d	1410*, 59$^+$ (nmol conjugate/min/mg)	46 (nmol/mg protein)		
Mouse (aged)	control	20	51.6		[41]
	0.05% × 14 d	38.5	83.4		
	0.10%	48.6 (μmol/min/mg protein)	103.1 (μmol/min/mg protein)		
Murine hepatoma cells	control		73		[44]
	30 μM (48 h)		124 (nmol/mg protein)		
Mouse	control	667*, 16.2$^+$	7.14		[27]
	0.1% × 14 d	2221*, 33.2$^+$ (nmol/min/mg protein)	10.9 (μmol/g)		
Rat	0.1% × 5 d	3.2*, 4.5$^+$-fold ↑			[40]
	0.02%	↑ 50%			
	0.01%	no effect			
	0.075%	2.7–3.5-fold ↑			
Rat	control	2.89			[51]
	300 ppm × 14 d	8.27		AOM	
	control	4.97			
	300 ppm × 14 d	22.7 (μmol/mg protein/min)		AOM	

GST: glutathione-*S*-transferase
GSH: reduced glutathione
AOM: azoxymethane
* 1-chloro-2,4-dinitrobenzene (CDNB) (substrate)
$^+$ 1,4-dichloro-2-nitrobenzene (DCNB) (substrate)

Kensler *et al.* [31–38,40] published a series of rodent experiments which further characterized the chemoprotective effects of oltipraz and related compounds. Aflatoxins, especially aflatoxin B_1 (AFB_1), are powerful naturally occurring toxins and hepatocarcinogens found in foods and feed products contaminated by molds. Hepatocellular carcinoma is a major world-wide health problem, especially in Africa and Asia. In an earlier study, Kensler *et al.* [31] evaluated the effects of dietary administration of four antioxidants including oltipraz (0.1%) on AFB_1 binding to DNA in male F344 rats treated with 1 mg/kg AFB_1. AFB_1 metabolite–DNA adducts were formed in the livers and kidneys [principal adduct: 8,9-dihydro-8-(N^7-guanyl)-9-hydroxyaflatoxin B_1]. Oltipraz reduced the covalent binding of AFB_1 to liver DNA by 76% and to kidney DNA by 64%, respectively. AFB_1 detoxification enzymes were all increased, including epoxide hydrase ($\leq$ 10-fold), glucuronyl reductase ($\leq$ 2.6-fold increase), and GSTs ($\leq$ 3.5-fold increase). Glutathione reductase and G6P dehydrogenase were also elevated.

Follow-up investigation further evaluated the effects of dietary oltipraz (0.01–0.1% for 4 weeks) on AFB_1 metabolism, DNA adduct formation, and hepatic tumorigenesis in male F344 rats from 1 week prior to and during exposure to AFB_1 [32–34]. Rats receiving 0.075% dietary oltipraz prior to AFB_1 exposure had reduced mortality (36% versus 83% control) and were also able to maintain their weight and growth rate [33]. Sections of liver were stained

for aflatoxin-induced γ-glutamyl transpeptidase (GGT) foci to determine oltipraz inhibitory effects on the development of these preneoplastic lesions. GGT-positive foci were markedly reduced at all doses of oltipraz. Furthermore, increasing doses of dietary oltipraz progressively reduced the AFB_1 bound to hepatic DNA by 40–80%. Results suggested that the chemoprotective effects of oltipraz may be secondary to its enhancement of electrophile detoxification pathways and modified oxidative metabolism of AFB_1. A dual effect was postulated since the low concentrations of oltipraz (0.01%) resulted in inductive effects only on cytochrome P-450 monooxygenases, while the higher doses of oltipraz (0.1%) increased the activity of GSTs by detoxifying 8,9-oxide, the electrophilic form of AFB_1. Other 1,2-dithiole-3-thione compounds demonstrated differences relative to induction of both glutathione-utilizing enzymes and Phase I and II enzymes and the ability to increase glutathione levels. Unsubstituted 1,2-dithiole-3-thione was most effective in increasing hepatic glutathione levels and in reducing AFB_1–DNA adduct levels. The unsubstituted compound had no effect on cytochrome P-450 levels nor on the activities of cytochrome P-450 reductase.

More recent work from Kensler's laboratory [39] has focused on previous investigations by Coles *et al.* [40] which have shown that aflatoxin detoxification through glutathione conjugation is principally catalyzed by one of the GST homodimers, YaYa. In F344 rats receiving dietary oltipraz (0.075%), GST activity was measured by conjugation with 1,2-dichloro-4-nitrobenzene or 1-chloro-2-4-dinitrobenzene. Levels of GST Ya protein were also evaluated. GST activity increased 1.5- to 3.2-fold with increases noted in steady-state mRNA levels for the GST subunit Ya and in the transcriptional activity of the GST Ya gene. After 5 days of the oltipraz diet, however, there was a decrease in the mRNA levels. The GST gene transcription activity returned to near control levels, although the GST enzymatic activities and GST Ya protein levels were persistently elevated. The regulation of GST Ya expression by oltipraz is therefore secondary to multiple mechanisms.

Recently published work, utilizing male F344 rats exposed to AFB_1 and fed a diet supplemented with 0.03% unsubstituted 1,2-dithiole-3-thione, has shown that levels of hepatic aflatoxin–DNA adducts, serum aflatoxin–albumin adducts and urinary aflatoxin-N^7-guanine adducts are potentially important as a measure of the chemoprotective efficacy of the compound [37]. The rats were fed the 1,2-dithiole-3-thione diet for 2 weeks and the reduction in the levels of hepatic DNA adducts, urinary aflatoxin-N^7-guanine and serum aflatoxin–albumin adducts were 76, 62, and 66%, respectively. Given the importance of determining biologic effect (intermediate endpoints) in human chemoprevention clinical trials, these biomarkers may become useful for individuals at risk for hepatocellular carcinoma secondary to aflatoxin exposure. Additional work with male F344 rats exposed to AFB_1 while receiving dietary 1,2-dithiole-3-thione (0.001–0.03%) confirmed that the higher the dietary concentration of the chemoprotector, the greater the reductions in the volume of liver occupied by GGT-positive foci and GST-P expression levels (greater than 98% reduction at the 0.03% concentration) [38].

Other investigators using laboratory models have contributed significant data on the effects of oltipraz [7,27,41–54]. One study evaluated aged mice to determine if the free radical processes associated with carcinogenesis and aging could be effectively hindered by an antioxidant diet [41,42]. The diets contained oltipraz (0.05% or 0.10%), anethole dithione (0.10%), butylated hydroxyanisole (0.10%), or 20% lyophilized cabbage. All of the diets resulted in decreases in lipid peroxidation (malondialdehyde content) and hepatic DNA damage (single strand breaks) along with increases in hepatic GSH content, glutathione reductase, and GST activities. Oltipraz and anethole dithione produced the most pronounced effects. Wattenberg and Bueding [43] evaluated the effect of oltipraz pretreatment on carcinogen-induced neoplasia in female ICR/Ha mice. The animals were given oltipraz by oral intubation at a dose of 500 mg/kg 48 hours before exposure to carcinogens including diethylnitrosamine (30 mg/kg), uracil mustard (1.2 mg/kg), or benzo(*a*)pyrene [B(*a*)P] (1.5 and 3.0 mg). Oltipraz effectively reduced the number of pulmonary adenomas in the diethylnitrosamine-and uracil mustard-treated mice and decreased both pulmonary adenomas and forestomach tumors in the B(*a*)P-exposed mice. Pepin *et al.* [54], however, failed to demonstrate

an antitumorigenesis effect when oltipraz (250 mg/kg diet) was given to strain A/J mice 2 weeks before exposure to tobacco-specific nitrosamine (4-methylnitrosamino-1)-(3-pyridyl)-1-butanone (NNK). In this same investigation, the non-steroidal, antiinflammatory drug sulindac reduced lung tumor multiplicity by 53%.

Utilizing Hepa 1c1c7 murine hepatoma cells in culture, 1,2-dithiole-3-thione and a series of substituted analogues including oltipraz were shown to induce QR activities. GSH levels were elevated without significant induction of cytochrome P-450 [44]. Ansher *et al.* [27] gave single intragastric doses of dietary dithiolethiones, including oltipraz, to mice (0.5% for 14 days) and rats (0.1% for 14 days). GSH and enzyme [GSTs, quinone (QT), GSSG, G6P, and 6-phosphogluconate dehydrogenase] activity levels increased in pancreas, upper jejunal mucosa, and in particular, liver and lung. The intragastric administration of oltipraz and other dithiolethiones caused no significant changes in GSH levels or enzyme activities in murine mammary adenocarcinoma transplants [27]. Oltipraz (30 mg/kg/day for 10 days), when given to rats treated with 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD), was shown to produce a 47% decrease in the incidence of DNA single strand breaks (liver nuclei) [45]. Helmes *et al.* [46] applied dimethylbenz(*a*)anthracene (DMBA) followed by 7,12-tetradecanoyl phorbol-13 acetate (TPA) to induce skin papillomas. Dietary oltipraz was given at levels of 0, 0.1%, or 0.2% beginning 1 week before initiation of carcinogen and continued either for 1 or 21 weeks after initiation. Alternatively, it was given 1 week after initiation and continued for 21 weeks. The most significant decrease in tumor incidence was seen in animals treated with 0.2% oltipraz from 1 week preinitiation until the end of the study at 21 weeks (tumor incidence decreased from 92% to 80%).

Mehta and Moon [48,61] utilized a DMBA or a DMBA/TPA mouse mammary gland model to evaluate a number of chemopreventive agents including oltipraz (10^{-8}–10^{-5} M concentrations). Oltipraz inhibited the development of mammary lesions when given prior to and during carcinogen treatment and is therefore described as an anti-initiator (35% inhibition at 10^{-7} M, 65% inhibition at 10^{-5} M). Moon [7] also employed a *N*-butyl-*N*-(4-hydroxybutyl) nitrosamine mouse urinary bladder carcinogenesis model to demonstrate that a combination of oltipraz (100 and 200 mg), difluoromethylornithine (DFMO, 0.64 and 1.28 g/kg), and *N*-(4-hydroxyphenyl)retinamide (4-HPR, 156 and 313 mg/kg) reduced tumor incidence by 43.9% (low dose) and 51.3% (high dose). The combination of oltipraz and DFMO also reduced tumor incidence; however, neither oltipraz alone nor in combination with 4-HPR altered urinary bladder carcinogenesis. High dose oltipraz and 4-HPR in combination produced excess mortality.

Another series of experiments evaluated the pharmacological and biochemical changes resulting from the inhibition of azoxymethane (AOM)-induced intestinal carcinogenesis by dietary oltipraz in male F344 rats [49,51]. The maximum tolerated dose (MTD) of oltipraz was first determined by feeding rats (ten per group) a diet containing 0, 32, 63, 125, 250, and 500 ppm oltipraz. After 6 weeks, excluding the animals receiving 500 ppm of oltipraz, body weights were comparable without clinical toxicity. The MTD of oltipraz was defined at 500 ppm since the body weights of the animals in this group decreased by an average of 8% compared to the control diet. Another group of animals at 5 weeks of age received experimental diets containing 200 ppm (40% MTD) and 400 ppm (80% MTD) of oltipraz. Oltipraz did not produce toxicity. At 7 weeks of age, animals were injected with the carcinogen AOM. Animals that received AOM and either the control diet or the 200 ppm oltipraz diet had comparable body weights. AOM-treated rats fed 400 ppm of oltipraz had a slight but significant decrease in body weight compared to those animals given a control diet (4.5–15% change in body weight). Since the animals that received the 400 ppm oltipraz diet had comparable weight to the animals on control diets, it appeared that the carcinogen was responsible for the body weight difference in animals fed 400 ppm oltipraz. The incidence of colon and small intestinal adenocarcinomas was significantly inhibited in animals given 200 and 400 ppm oltipraz without demonstration of a dose-response effect. There also was a significant inhibition in the multiplicity (number of tumors/animal) of colon adenomas and small intestinal adenocarcinomas (incidence in multiplicity of intestinal carcinogenesis inhib-

ited approximately 35% by oltipraz), with a slight inhibition in the number of adenocarcinomas of the colon.

Serum oltipraz levels obtained from animals receiving an oltipraz diet for 1, 8, 16, and 24 weeks were fairly constant, with increases shown for animals receiving 400 ppm oltipraz compared to those receiving 200 ppm (*e.g.*, 614 ng/ml versus 357 ng/ml). Prolonged feeding of oltipraz did not produce gross or histologic changes in the intestines, kidney, liver, lungs, or stomach. In this series of experiments, dietary oltipraz significantly inhibited AOM-induced O^6-methylguanine and 7-methylguanine adduct formation in the liver and colon of male rats. It is postulated that inhibition of AOM-induced intestinal carcinogenesis by oltipraz is mediated by GST carcinogen detoxification. Male F344 rats in other experiments received 300 ppm of oltipraz (60% MTD) 2 weeks prior to AOM exposure. Animals fed oltipraz had significant increases in liver weight for both the AOM- and saline-treated groups, with a similar trend in colonic mucosal weights. There was a 3–4-fold increase in liver GST activity for the oltipraz-fed animals whether or not carcinogen was administered. Significant inhibition was noted in the colonic mucosal AOM-induced ornithine decarboxylase (ODC) activity, with inhibition of AOM-induced hepatic tyrosine protein kinase (TPK) activity in cytosol and particulate fractions. DNA methylation was decreased by 2.5- to 3.0-fold for those animals receiving oltipraz. Both ODC and TPK were increased after AOM exposure.

A recent report described F344 female rats fed 5% or 20% freeze-dried savoy cabbage, green cabbage, or kale versus a control diet [50]. Hepatic GST activity was the greatest for those fed green cabbage; the glutathione levels were greater for those rats fed kale. Kale contained components identical to those from oltipraz on thin layer chromatograms; however, the components were not identified in cabbage. Mass spectral data and NMR identified one of the components as propionoic acid-3,3′-thiobisdidodecyl ester which is probably a breakdown product of dithiolethiones.

Thomas *et al.* [52] evaluated the effects of dietary piroxicam and oltipraz alone or in combination with DFMO relative to the prevention of urinary bladder carcinogenesis in mice induced by *N*-butyl-*N*-(4-hydroxybutyl) nitrosamine. Unlike piroxicam, oltipraz as a single agent did not reduce cancer incidence. Oltipraz (40% and 80% of the MTD) in combination with DFMO resulted in significant dose-related inhibition of bladder carcinogenesis.

Clapper *et al.* [53] have evaluated the response of GST isoenzymes (GSTμ and GSTπ) to oltipraz (1 g/kg) in mice. Maximum hepatic GST elevation (3-fold) was seen 4 days post-treatment; however, the increase (2-fold) persisted for 8 days compared to controls. Compared to controls, hepatic GSTμ and GSTπ were significantly increased after oltipraz exposure, although GSTα levels were unchanged 2 days post-treatment.

Finally, there have been reports discussing other characteristics of oltipraz. Limited animal data, including mutagenicity testing with Ames *Salmonella typhimurium* strains, suggest that there are no embryotoxic, teratogenic, or mutagenic effects of oltipraz, although these data are very preliminary [6,7,62]. Ansher [55] has demonstrated the effects of dithiolethiones, including oltipraz, against carbon tetrachloride and acetaminophen toxicity. In addition, thiol compounds have long been known to function as radioprotectors. The monoethyl ester of GSH may also have potential as a normal tissue protector [47]. Bueding [28] noted that oltipraz increased cellular thiol levels in mice. In the EMT6 mammary tumor cell line, 1,2-dithiole-3-thiones showed relatively low levels of cytotoxicity toward oxygenated and hypoxic cells, although oltipraz was 5-fold more toxic toward the hypoxic than the oxygenated cells. Oltipraz produced a radioprotection factor (RPF) of 1.1 [47].

OLTIPRAZ CLINICAL INVESTIGATIONS

There are additional data available related to pharmacokinetics in animals and humans after relatively brief exposures to oltipraz, namely distribution of metabolites and toxicity; however, there are few reports discussing chronic administration of oltipraz, particularly in humans (Table II). Rhone-Poulenc Industries (France), the manufacturer of oltipraz, has published data relative to the metabolism and pharmacokinetics of oltipraz [63,64]. At least 13 metabolites of oltipraz extracted from the urine

TABLE II. Comparison of Oltipraz Plasma (Serum) Levels and $t_{1/2}$ Across Species

Species	Oltipraz Dose	Plasma Level (Serum)	$t_{1/2}$ (h)	Reference
Mouse	250 mg/kg	14000 μg/L	4.5	[64]
Monkey	20 mg/kg	<100 μg/L	3.5–7.0	
Rat	50 mg/kg	1700 μg/L	2.5	
Rat	200 ppm	337 ng/ml		[49]
	400 ppm	597 ng/ml		
Human				[57]
(low-fat diet)	25 mg/kg	1.07 mg/L		
(high-fat diet)	25 mg/kg	1.39 mg/L		
Human	1 mg/kg	16 ng/ml	4.4	[58]
(single dose)	2 mg/kg	61 ng/ml	4.1	
	3 mg/kg	250 ng/ml	5.3	
Human	125 mg	282 ng/ml		[59]
(chronic dose)	250 mg	1451 ng/ml		
Human	125 mg	348 ng/ml	5.64	[60]
(single dose)	250 mg	1049 ng/ml	5.44	
	375 mg	3145 ng/ml	8.72	
	500 mg	4921 ng/ml	6.76	

of mice (^{14}C-labelled oltipraz as a single oral dose of 20 or 250 mg/kg), rats (20 or 50 mg/kg), monkeys (20 mg/kg), and humans (patients with schistosomiasis receiving a single dose of 0.50, 0.75, or 1 g) have been identified by GLC, TLC, and HPLC [63]. Structures of eleven of the compounds have been determined by spectroscopic evaluation, and six metabolites from human urine were available in quantities sufficient to determine the metabolic pathway of oltipraz (Fig. 3). The most abundant metabolites found in human plasma and urine were two diastereoisomers referred to as metabolite VII$_{1,2}$ ($C_{10}H_{12}N_2O_2S_2$), metabolite IX ($C_{10}H_{12}O_2N_2S_2$), VIII (the most abundant, $C_{10}H_{12}N_2O_2S_2$), a conjugated metabolite XIII ($C_{16}H_{20}N_2O_6S_2$) and metabolite II [4-methyl-5 (2-pyrazinyl)-1-2-dithiole-3-one]. Elimination of oltipraz metabolites is chiefly via the kidneys; less than 1% of unchanged oltipraz is excreted in the urine in all of the species investigated. In the feces, however, unchanged oltipraz is the principal compound excreted. The metabolism of oltipraz is similar in humans and monkeys.

In a subsequent study by Rhone-Poulenc Industries, ^{14}C-labeled oltipraz was given as a single dose to rhesus monkeys (20 mg/kg), rats (50 mg/kg), and female mice infected with *Schistosoma mansoni* (100 and 250 mg/kg) [64]. The absorption of oral oltipraz differed depending upon the animal species and the dose administered. Peak plasma and red blood cell oltipraz levels reached a maximum at 3 hours for the rat and monkey and at 12 hours for the mouse. Plasma oltipraz levels were similar to those of red blood cells except in the male monkey. Plasma elimination half-lives ($t_{1/2}$) were variable: 2.5 hours (rat), 4.5 hours (mouse), and 3.5–7.0 hours (monkey). Sex differences were noted in blood levels and elimination $t_{1/2}$ in monkeys. The absorbed oltipraz was highly metabolized with the majority of the radioactivity excreted in 3–4 days in the monkey, rat, and female mouse. Radioactive products were noted within 24 hours in the bile, gastrointestinal tracts, kidneys, and liver.

There have been a number of clinical trials investigating the acute toxicity of oltipraz in

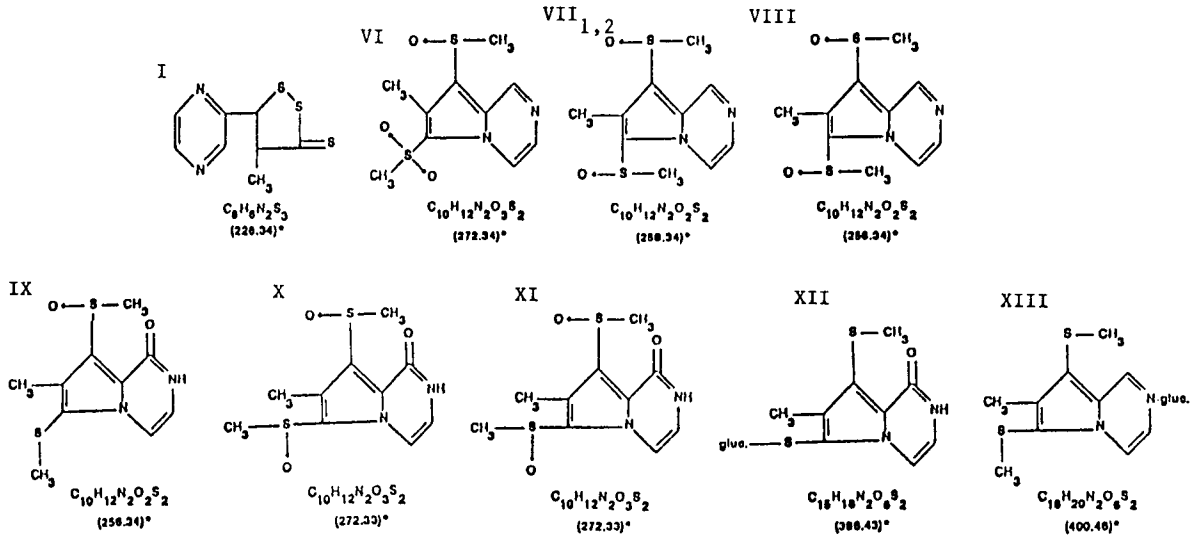

Fig. 3. Structures of significant oltipraz metabolites extracted from human urine with molecular weights adapted from Bieder *et al.* [63] and Heusse *et al.* [64].

humans with urinary tract or intestinal schistosomiasis [8–12,57,62]. Gentilini *et al.* [11] evaluated 321 patients with *Schistosoma haematobium* (Mali), *S. intercalatum* (Gaboon), and *S. mansoni* and *S. haematobium* (Paris). Variable doses were used, including 4.5 g in divided doses for 3 days, 1 g daily for 5 days, 7.5 g over 5 days twice a day, 3 g over 2 days twice a day, and 2 g in a single day in two doses. Cure rates were reported between 86% and 100%. The most frequent side effects were described as "minimal and transient" and included abdominal pain, nausea, vomiting, headache, and extremity paresthesias. The Paris patients experienced nausea and vomiting 25% of the time. There were no significant laboratory changes noted in blood chemistry and hematology.

Twenty-four patients from Brazil with *S. mansoni* were divided into groups of six and treated with a single dose of oltipraz (10, 20, 25, or 30 mg/kg) [62]. The percentage of patients cured was highest in the 25 and 30 mg/kg group (80% and 100%, respectively). Minimal toxicity was noted; patients receiving 30 mg/kg reported nausea and/or somnolence on the first day of drug ingestion. The only significant laboratory change was an increased eosinophil count at 1 month, which was later viewed as a secondary effect of schistosomicidal activity rather than oltipraz. A comparable study was performed in sixty-two male Sudanese patients with *S. mansoni* given two doses of either 25 mg/kg (group 1) or 35 mg/kg (group 2), one with breakfast, the other with supper [8]. The most significant toxicities included vomiting after the second dose (12% in group 1 and 24% in group 2, respectively) and mild abdominal pain (45% in group 1 and 61% in group 2, respectively). Other toxicities included nausea (7% and 6%, respectively), dizziness (3% in both groups), fatigue (16% and 19%, respectively) and headache (16% and 3%, respectively). The investigators suggested that the vomiting after the second dose was due to the drug's long $t_{1/2}$ of 7.5 hours. Blood chemistry and hematology were unremarkable.

In another trial 294 Sudanese children were given doses of 15 mg/kg, 20 mg/kg, and 25 mg/kg [10]. The most significant pre-treatment symptoms included abdominal pain (74%), blood in the stool (62%), and headache (1%). Within 24 hours of treatment, abdominal pain was noted but was greatly reduced; headache had increased. Significant treatment-related side effects included fatigue, vomiting, nausea, and dizziness; four children noted fingertip pain and 17 experienced blurred vision. All toxicities were transient. Additional data from Rhone-Poulenc

described photosensitivity with acute pain at the fingertips after a single dose of oltipraz and reports of photo-onycholysis [7]. Oltipraz is no longer marketed for treatment of schistosomiasis because of photosensitivity.

The bioavailability of oltipraz is increased when oltipraz is administered with food rather than while fasting [56,57]. Healthy male subjects were evaluated under fasting conditions. A single dose of oltipraz (25 mg/kg) was given with a low-fat meal (less than 5%) or a high-fat meal (24%). There were no appreciable plasma concentrations noted under fasting conditions; however, peak concentrations were 1.07 mg/L at 3 hours with the low-fat diet and 1.39 mg/L at 2 hours with the high-fat diet. The same investigators evaluated the effects of cysteine (150 mg) on oltipraz levels when administered to monkeys, along with a 50 mg/kg single dose of oltipraz. There was a 6- to 11-fold increase in peak serum concentrations when oltipraz and cysteine were given together [56].

In a more recent single dose pharmacokinetic study, 16 healthy volunteers in groups of four ingested 125 mg, 250 mg, 375 mg, or 500 mg of oltipraz after breakfast [60]. Plasma samples were collected beginning at 1 hour, with a final collection 24 hours after drug ingestion. Oltipraz and the ethyl analog of oltipraz (internal standard) were extracted from plasma and quantitated by HPLC. Two subjects reported diarrhea (one grade I and one grade II) and one subject each noted headache (grade II) and hunger (grade I). Peak concentration, $t_{1/2}$, area under the curve, and oral clearance are reported in Table III. At the 125 and 250 mg doses, peak concentration occurred between 1 and 3 hours with a $t_{1/2}$ between 5 and 6 hours. At 375 and 500 mg, peak concentrations occurred between 1 and 6 hours with a mean $t_{1/2}$ between 6 and 9 hours. The difference in the $t_{1/2}$ between the four dose levels was not considered significant nor was there a significant difference in clearance at the 125 and 250 mg levels.

There are only two reported studies evaluating the toxicity and pharmacology relative to the chronic administration of small doses of oltipraz. In one study of 27 healthy individuals (16 females, 11 males, ages 26–60 years) four separate scheduling groups were evaluated [58].

A single-dose kinetic arm involved nine subjects in three groups receiving a 35%-fat diet and 1, 2, or 3 mg/kg of oltipraz, given one-half hour after breakfast. Serum samples were collected up to 48 hours after dosing. In this project, the peak serum concentrations were dose-dependent and occurred between 2.5 and 4.0 hours with no detectable levels of oltipraz by 24 hours. The absorption and elimination constant and $t_{1/2}$ were also similar (mean $t_{1/2}$ = 4.1–5.3 hours). The area under the curve progressed with increasing dose.

The second arm included a loading dose of oltipraz (1.5 mg/kg) at 8 a.m. and 2 p.m. in three subjects followed by a 10 day maintenance dose of 1.5 mg/kg. A second cohort of three subjects received 2 mg/kg over the same time period on the first day followed by a 10 day maintenance dose of 2 mg/kg. Steady-state concentrations were noted after the second day of

TABLE III. Oltipraz Pharmacokinetics [60]

Single Dose (mg)	Peak (ng/ml)	$t_{1/2}$ (h)	AUC* (mg × min/L)	Clearance (L/min)
125	348 ± 135[1]	5.64 ± 4.18	50.6 ± 15.1	2.65 ± 0.83
250	1049 ± 806[1]	5.44 ± 1.61	262 ± 187	1.54 ± 1.19
375	3145 ± 2225[2]	8.72 ± 4.31	1690 ± 1664	0.39 ± 0.26
500	4921 ± 3428[2]	6.76 ± 2.68	1998 ± 1743	0.43 ± 0.30

* **AUC** = Area under the curve
[1] at 1–3 h
[2] at 1–6 h

drug administration with no detectable serum or urine oltipraz by day 12 (last dose was day 10). On day 3, two subjects experienced flatulence which resolved upon discontinuation of the drug.

The third arm included three subjects receiving a 3 mg/kg loading dose on day 1 followed by a maintenance dose of 1.5 mg/kg for 28 days, while three more subjects received a 4 mg/kg loading dose on day 1 followed by a 28 day maintenance dose of 2 mg/kg. Steady-state concentrations were obtained; however, there were substantial interindividual variations (1.2–9.3 *mg/ml in* group 1 and 1.2–15.3 mg/ml in group 2). Urinary oltipraz levels paralleled the pattern seen with serum concentrations; there was no detectable oltipraz 2 days after the last dose. One male subject experienced numbness and pain in his thumb tips by day 7 on a dose schedule of 1.5 mg/kg/day. This subject also noted small "purplish-black spots" on the thumbs after discontinuation of drug similar to those described in patients treated for schistosomiasis [8,57]. No other toxicities were noted and there were no laboratory changes including hematology and chemistry profiles and thyroid function tests.

The fourth arm involved three individuals on a low-fat diet (20% fat) for 5 days while taking oltipraz at 2 mg/kg/day. Two weeks after the last dose, the same individuals consumed a high-fat diet (53% fat with 2 mg/kg/day oltipraz). Interindividual variation was again noted; however, serum concentrations of oltipraz at 6 hours were higher for the individuals while consuming the high-fat diet compared to the low-fat diet. The effect did not persist over the ensuing 4 days and similar steady-state patterns were observed.

Finally, another Phase I trial of 24 subjects included ten patients with resected colon polyps and 14 healthy female subjects with breast cancer relatives, all of whom received a daily dose of oltipraz for 6 months [59]. Nine of 14 subjects completed the 6 month course of 125 mg/day oltipraz and four of ten subjects completed 6 months at 250 mg/day. Toxicity was frequent at the 250 mg/day dose, while 125 mg/day of oltipraz appeared to be near the MTD when given for an extended period of time. Table IV presents the grade I and grade II toxicities encountered. The most significant toxicities were marked photosensitivity characterized by fingertip and hand burning sensations when the hands were exposed to either warmth or sun. Some patients developed a tolerance which resolved the photosensitivity and paresthesias. The most common toxicities involved gastrointestinal discomfort, in particular diarrhea, nausea, and flatulence. Overall compliance by the participants was good. Monthly plasma samples obtained 2–3 hours post-oltipraz ingestion showed wide variability in peak concentrations. Mean oltipraz concentrations were 282 mg/ml (125 mg) and 1451 mg/ml (250 mg). Cruciferous vegetable intake was recorded on a monthly basis by each participant. The mean cruciferous vegetable intake was 6.5 servings/month (125 mg) and 7.2 servings/month (250 mg). There appeared to be an inverse relationship between cruciferous vegetable intake and plasma oltipraz level for

TABLE IV. Toxicities Secondary to Oltipraz When Taken for up to 6 Months at a Dose of 125 mg or 250 mg [59]

Symptom	Grade	No. of Patients
Diarrhea	1	5
	2	1
Nausea	1	7
	2	1
Flatulence	1	8
Constipation	1	1
Stomach Pain	2	1
Heartburn	1	1
Bloating	1	1
	2	1
Green Urine	1	1
Photosensitivity	1	4
	2	2
Dry Skin	1	1
Lines/Thumbnails	1	1
Rash	1	3
Acne	1	1
Taste Changes	1	1
	2	4
Headache	1	1
Paresthesia	1	3
Weakness	1	1
Fatigue	1	1

those receiving 125 mg oltipraz, while there was no relationship at the 250 mg dose level. The small sample sizes precluded definitive analyses.

Since the MTD of oltipraz during chronic administration is poorly understood, new Phase I clinical trials will include both a single dose and 6 month chronic dosing schedule of oltipraz at 20 mg, 50 mg, 100 mg, and 125 mg (Northwestern University, University of Chicago, and Loyola University). The pharmacokinetics of oltipraz at the four dose levels will be evaluated; lymphocytes and rectal biopsies will be obtained to explore the effect of oltipraz on GSH and GST. A third study will determine the toxicity associated with single doses of oltipraz ranging from 125–1000 mg/m^2 (Fox Chase Cancer Center). Pharmacokinetics and GSH levels in normal bowel mucosa and peripheral mononuclear cells also will be investigated.

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