Effect of Oltipraz [5-(2-Pyrazinyl)-4-methyl-1,2-dithiol-3-thione] on Azoxymethane-Induced Biochemical Changes Related to Early Colon Carcinogenesis in Male F344 Rats (43228)

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Abstract. **Epidemiologic studies suggest that the consumption of cruciferous vegetables is associated with a reduced risk for several types of cancer including cancer of colon. Experimental studies indicate that dithiolthiones, naturally occurring substances in cruciferous vegetables, possess anticarcinogenic properties. 5-(2-Pyrazinyl)-4-methyl-1,2-dithiol-3-thione (oltipraz), a substituted dithiolthione, has been tested for its chemopreventive activity. We studied the effect of dietary oltipraz on liver and colonic mucosal enzymes and DNA adducts to evaluate the modulating role of this agent during the early period of azoxymethane (AOM)-induced carcinogenesis. At 6 weeks of age, groups of animals were fed the AIN-76A diet containing 0 and 300 ppm oltipraz. At 8 weeks of age, all of the animals except vehicle-treated animals were administered a subcutaneous injection of AOM (15 mg/kg body wt/week for 2 weeks). Animals intended for vehicle treatment were administered normal saline subcutaneously. Fifteen hours after the second AOM injection, six animals each from control and oltipraz diet groups were sacrificed and liver and colonic mucosa from each animal were used for DNA adduct analysis. Animals intended for liver and colonic mucosal glutathione S-transferase, tyrosine specific protein kinase (TPK), and ornithine decarboxylase (ODC) enzyme assays were killed 5 days after the second AOM or saline injection. The results of this study indicated that dietary oltipraz significantly increased liver ($P < 0.001$) and colonic mucosal ($P > 0.05$) weights, but had no effect on body weights ($P > 0.05$). In saline-treated animals, feeding of oltipraz significantly increased the cytosolic glutathione S-transferase ($P < 0.001$) and ODC ($P < 0.05$) activities in the liver and colon when compared with those fed the control diet. Although our unpublished results indicate an inhibitory role of oltipraz when fed during the initiation and postinitiation phases of intestinal carcinogenesis, the increased ODC activity may indicate a possible role of oltipraz in colon tumor promotion. Additional studies are indicated to test the antitumor properties of oltipraz administered during the postinitiation phases. AOM treatment significantly increased the TPK ($P < 0.0001$) and ODC ($P < 0.01$) activities in the liver and colon of animals fed the control diet. Dietary oltipraz significantly suppressed the AOM-induced TPK ($P < 0.001$) activities in liver and colon and ODC ($P < 0.01$) activity of colon. Analysis of nucleic acid bases, O^6-methylguanine, and 7-methylguanine revealed that dietary oltipraz significantly ($P < 0.05$) inhibited the AOM-induced adduct species. These results suggest that dietary oltipraz enhances the colonic and liver glutathione S-transferase activity and reduced the formation of DNA adducts. In addition, dietary oltipraz modulates liver and colonic ODC and TPK activities that have been shown to play a role in tumor promotion.** [P.S.E.B.M. 1991, Vol 197]

Epidemiologic evidence indicates that the consumption of cruciferous vegetables reduces the risk for development of several types of cancer including the cancer of colon in humans (1–3). Several natural substances such as phenols, indoles, aromatic isothiocyanates, and dithiolthiones, to cite a few, have been shown to have anticarcinogenic properties (1). Cabbage and other cruciferous vegetables contain dithiolthioles (4, 5).

Several substituted dithiolthiones such as 5-(2-pyrazinyl)-4-methyl-1,2-dithiol-3-thione (Oltipraz) are being used as antischitosomal agents. Pioneering studies by Bueding *et al.* (6) and further studies by Asher *et al.* (7) indicated that oltipraz and related dithiolthiones induce enzymes involved in electrophile detoxification, such as glutathione S-transferase (GST), epoxide hy-

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drase, and NAD(P)H-quinone oxidoreductase. Wattenberg and Bueding (8) demonstrated that the oral administration of oltipraz inhibited the occurrence of benzo(*a*)pyrine-induced pulmonary adenomas and tumors of forestomach in mice and to a lesser extent diethylnitrosamine- and uracil-induced pulmonary adenomas. Oltipraz has also been shown to inhibit aflatoxin-B_1-induced liver cancer and hepatotoxicity in rats (9) and the formation of aflatoxin-DNA adducts in target tissues of rat (10). This protective action appears to be due to elevation of GST activity, which facilitates the detoxification of aflatoxin (10).

With regard to colon carcinogenesis, our recent study indicated that oltipraz inhibits azoxymethane (AOM)-induced intestinal carcinogenesis in rats when administered before, during, and after carcinogen treatment (C. V. Rao *et al.*, unpublished data). During the initiation phase, AOM is metabolized in the liver to methylazoxymethanol (MAM), which is further metabolized in the liver and colon to a highly reactive electrophile, namely, methyl carbonium ion that methylates DNA (11). Since GST has been shown to play a role in carcinogen detoxification, it is possible that the inhibition of AOM-induced intestinal carcinogenesis by oltipraz could be also mediated through this enzyme system. Also, the formation of DNA adducts is an important step in the carcinogenic process (12); AOM forms two major adducts, namely, 7-methylguanine and O^6-methylguanine (13). Thus, it would be of interest to study the possible influence of oltipraz on colonic and liver GST and DNA adducts.

Increased levels of polyamines are commonly associated with normal, abnormal, and induced proliferative status (14, 15). Induction of polyamine biosynthetic activity as related to tumorigenesis of intestinal neoplasia is provided by studies with tumor promoters and carcinogens (16). Luk and Baylin (17) observed that the AOM-treated rat model of colonic carcinogenesis has a characteristic of a multistep process and that ornithine decarboxylase (ODC) activity is elevated in a fashion similar to that seen in clinical samples. In addition, tyrosine phosphorylation is now recognized as an important regulatory mechanism of tumor promotion and growth in response to a number of processes including the action of several growth factors, growth factor receptors, and oncogenes (18–23). Recent studies demonstrate a significant increase in tyrosine-specific protein kinase (TPK) enzyme activity in hyperproliferative and malignant tissues compared with normal tissues (24–26). These studies, thus, indicate that the mucosal ODC and TPK activities are associated with tumor promotion and suggest that the inhibitors of ODC and TPK might be used as anticancer agents.

In this study, we assessed the effect of dietary oltipraz on the liver and colonic mucosal activities of GST, ODC, and TPK and on the liver and colonic DNA methylation in male F344 rats treated with AOM.

Materials and Methods

Animals and Diets. Weanling male F344 rats were purchased from Charles River Breeding Laboratories (Kingston, NY) and maintained under conditions of controlled temperature, lighting, noise, and humidity. All ingredients of semipurified diet (modified AIN-76A; (27)) were purchased from Dyets, Inc. (Bethleham, PA). To determine the effective level of oltipraz to be used in the diet, the maximum tolerated dose (MTD) of oltipraz was determined in male F344 rats by feeding different concentrations of oltipraz for 10 weeks. MTD is the highest dose of test agent that does not affect weight gain compared with appropriate control groups, and does not produce mortality, clinical signs of toxicity, or pathologic lesions. We found that the MTD of oltipraz is 500 ppm in a modified AIN-76A diet. Since our preliminary results indicated an inhibition of AOM-induced intestinal carcinogenesis with 200 ppm (40% MTD) and 400 ppm (80% MTD) oltipraz, it was decided to use the average of these two dose levels (300 ppm) in the present study. Oltipraz was added to the modified AIN-76A diet at the expense of starch. The incorporation of oltipraz into a modified AIN-76A diet was done with a V-blender after oltipraz was premixed with a small quantity of diet in a food mixer to ensure uniform distribution of oltipraz.

Chemicals. AOM was obtained from Ash-Steven (Detroit, MI). Oltipraz was kindly provided by Rhone-Poulenc (Paris, France). O^6-Methylguanine was obtained from Chemsyn Science Laboratories (Lenexa, KA). 7-Methylguanine, 1-chloro-2,4-dinitrobenzene, poly(Glu:Tyr), and glutathione were obtained from Sigma (St. Louis, MO). [^{14}C]Ornithine and [^{32}P]ATP were purchased from Amersham (Arlington Heights, IL).

Experimental Procedure. Animals obtained at 4 weeks of age were quarantined and had access to an NIH-07 diet. At 5 weeks of age, all animals were fed the modified AIN-76A diet (27). Two weeks before AOM or vehicle treatment, i.e., at 6 weeks of age, animals were fed the modified AIN-76A diet containing 0 and 300 ppm (60% MTD) oltipraz. At 8 weeks of age, all of the animals except the vehicle-treated animals received AOM subcutaneously once weekly for 2 weeks at a dose rate of 15 mg/kg body wt/week. Animals intended for vehicle treatment were administered an equal volume of saline. After 15 hr of the second AOM treatment, six animals from each group were sacrificed by decapitation for DNA adduct analysis. The rationale for assaying DNA adducts at 15 hr after carcinogen administration has been based on the published results that these adducts persist up to about 30 hr following carcinogen treatment (28, 29) and reach maximum levels between 6 and 18 hr depending on the organ (13). Liver was removed, quick-frozen in liquid nitrogen, and stored at −80°C. Colon was rapidly removed,

rinsed in ice-cold normal saline, slit open longitudinally, and freed from all of the contents. It was laid flat on a glass plate and the mucosa was scraped with a microscope glass slide. The mucosa was immediately frozen in liquid nitrogen and stored at −80°C. Animals intended for enzyme assay were killed 5 days after the second AOM or vehicle treatment. liver and colonic mucosa were obtained and processed as described above. The rationale for measuring the enzyme activities during this time-frame has been based on earlier studies that showed that ODC and TPK activities reach their maximum on the fifth day following carcinogen treatment and reach basal levels at Day 7 (25, 30).

Glutathione S-Transferase Assay. All steps for the homogenization and preparation of cytosol were carried out at 0–4°C. Liver and colonic mucosa were homogenized in 0.1 *M* phosphate buffer. Cytosol fraction was prepared by centrifuging the homogenate at 9000*g* for 20 min, and the resulting supernatant was spun at 100,000*g* for 1 hr. GST activity was measured by using 1-chloro-2,4-dinitrobenzene as substrate by the method of Habig *et al.* (31). The extinction coefficient of 9.6 mM^{-1} was employed to calculate the activity.

Ornithine Decarboxylase Activity. A portion of liver from each rat and pooled mucosa from two animals were used for ODC assay. The samples were homogenized in 1.5 ml of homogenizing buffer (50 m*M* sodium phosphate buffer, pH 7.2, containing 5 m*M* dithiothreitol, 0.1 m*M* EDTA, 0.1 m*M* pyridoxal 5′-phosphate) using a Polytron homogenizer (Brinkman Instruments, Westbury, NY). The homogenates were centrifuged at 100,000*g*, 4°C, for 1 hr. The resulting cytosol fractions from liver or colon mucosa were used for determination of ODC activity and protein. ODC activity was determined using the modification method of Stanley and Kazarinoff (32). The reaction mixture containing [50 m*M* sodium phosphate, 2 m*M* pyridoxal phosphate, 1 m*M* dithiothreitol, 20 m*M* L-ornithine, and 0.25 μCi of DL-[^{14}C]ornithine (specific activity of 58 mCi/mmol)] in a final volume of 250 μl was incubated at 37°C. The reaction was stopped at 1 hr by adding 200 μl of 2 *N* sulfuric acid and the $^{14}CO_2$ released was collected for another 45 min. Radioactivity was counted in a Beckman scintillation counter model LS 6800.

Tyrosine Protein Kinase Activity. Liver and colon mucosa from individual animals were homogenized in 5 volumes of ice-cold homogenizing buffer (20 m*M* Tris-HCl, pH 7.4, containing 0.25 *M* sucrose, 1 m*M* $MgCl_2$, 1 m*M* EGTA, 1 m*M* EDTA, 50 μg/ml trypsin inhibitor, 10 μg/ml leupeptin, and 1 m*M* phenylmethylsulfonyl fluoride in a polytron homogenizer. The debris and nuclei were removed by centrifugation at 1000*g* for 10 min at 2°C, and the supernatant was filtered through glasswool to clear the fat. The filtrate was centrifuged at 100,000*g* for 1 hr at 4°C. The supernatant (cytosol) fraction was used for the assay of TPK activity. The resulting pellet (membrane fraction) was resuspended in 1.5 ml of solubilizing buffer (homogenizing buffer with 0.1% Nonidet P-40) and mixed in an automatic multi-tube vortex mixer for 4 hr at 4°C. It was then centrifuged at 80,000*g* for 20 min at 4°C, and solubilized membrane fraction was also used for TPK activity. The TPK activity was determined in the cytosol and membrane fractions using synthetic polymer substrate, poly(Glu-Tyr) 4.1:0.9, M_r 28,400. Phosphorylation of cytosolic and solubilized membrane proteins (5–10 μg/assay) were carried out at 24°C in a total volume of 50 μl reaction mixture, containing 50 m*M* Tris-HCl (pH 7.4), 20 m*M* $MgCl_2$, 0.02% Triton X-100, 30 μ*M* sodium *o*-vanadate, and 20 μg of Glu-Tyr polymer. The reaction was initiated with 50 μ*M* ATP, with a specific activity of 0.7 Ci/mmol. After 10 min, the reaction mixture samples were spotted on 2.3-cm^2 Whatman No. 3MM paper disks, precipitated, and washed several times in 10% trichloroacetic acid containing 10 m*M* sodium pyrophosphate. Paper disks were then rinsed with ethanol and petroleum ether, dried, and counted in 10 ml of scintillation cocktail. TPK activity was corrected for endogenous phosphorylation as described above in the absence of added substrate. Protein content was determined by the Bio-Rad method using bovine serum albumin as standard. The activity is expressed as pmol [^{32}P]ATP/mg protein/min. The TPK activity is linear as a function of time and protein up to 20 min and 15 μg of protein.

Analysis of DNA Methylation. DNA was isolated from liver and colonic mucosa by the phenol extraction method of Kirby as modified by Swann and Magee (33). DNA was hydrolyzed in 0.1 *M* HCl (5 mg/ml) for 30 min at 70°C to release the purines as free bases. Chromatographic separation of hydrolysate was carried out on Waters high-performance liquid chromatography using a Partisil strong cation exchange column (25 cm × 4.5 mm inner diameter; Whatman Inc., Clifton, NJ) and 0.2 *M* ammonium phosphate (pH 2) as mobile phase at a flow rate of 1.5 ml/min. Elution of fluorescing bases was monitored using a 280-nm excitation wavelength with a 366-nm emission interference filter in a Perkin Elmer LS-2 fluorescence detector. Quantitation was achieved using electronic integration calibrated with standard solutions of authentic guanine, 7-methylguanine, and O^6-methylguanine.

Statistical Analysis. Where applicable, data were analyzed using a two-way analysis of variance and Duncan's multiple range test taking $P < 0.05$ as the level of significance.

Results

General Observations. There were no significant differences in body weights of animals fed the control and oltipraz diets (Table I). However, the oltipraz diet significantly increased the liver weights in AOM- and

Table I. Effect of Oltipraz on Body, Liver, and Colonic Mucosal Weights

Experimental group	Body weight (g)	Liver weight (g)	Colonic mucosal weight (mg)
Saline-treated			
Control diet	229 ± 3.7[a]	8.51 ± 0.61	314 ± 4.2
Oltipraz diet	221 ± 4.2	9.17 ± 0.20[b,c]	331 ± 5.2
AOM-treated			
Control diet	230 ± 2.3	9.56 ± 0.31	366 ± 4.3
Oltipraz diet	228 ± 2.7	12.1 ± 0.30[d]	412 ± 13.1[c]
Statistical analysis (*P* values)[e]			
Oltipraz	0.43	0.001	0.42
AOM	0.93	0.001	0.02
Oltipraz × AOM interaction	0.89	0.001	0.03

[a] Mean ± SE ($n = 24$).
[b] Significantly different from its respective control diet group within saline-treated or AOM-treated groups.
[c] $P < 0.05$.
[d] $P < 0.001$.
[e] By two-way analysis of variance.

saline-treated groups (Table I). A similar nonsignificant trend was observed in the colonic mucosal weights among the various dietary groups (Table I).

Biochemical Observations. Table II summarizes the activities of GST, ODC, and TPK and DNA methylation in the liver. AOM treatment significantly increased the GST activity in the liver of animals fed the control and oltipraz diets. In the saline- and AOM-treated animals, feeding of oltipraz was associated with a marked increase (3- to 4-fold) in liver GST activity. There was also an increase in ODC activity in animals treated with AOM as compared with saline-treated animals. ODC activity was significantly increased by oltipraz feeding in saline-treated animals. The results also indicate that AOM treatment increased the hepatic TPK activity in cytosol and particulate fractions by more than 7-fold in animals fed the control diet. This increase in AOM-induced TPK activity in cytosol and particulate fractions was significantly inhibited by oltipraz. Feeding of oltipraz significantly inhibited both 7-methylguanine and O^6-methylguanine in liver. This decrease in DNA methylation by oltipraz may not be attributed to increased liver weight in this group, thus diluting the methylguanines with unaltered guanines, because the liver weight was increased by about 20% and the DNA methylation was decreased by 2.5- to 3.0-fold in oltipraz-fed animals.

Table III shows the activities of GST, ODC, and TPK and DNA methylation in colon. Colonic TPK (cytosolic and particulate) and ODC activities were markedly increased by the treatment with AOM. Colonic GST activity was increased slightly but significantly by AOM treatment. Dietary oltipraz increased the colonic mucosal GST activity in both saline- and AOM-treated animals. Also, oltipraz feeding significantly increased the colonic mucosal ODC activity in saline-treated animals. In addition, feeding of oltipraz significantly inhibited the AOM-induced ODC in cytosolic fraction and TPK in both cytosolic and particulate fractions. Also, dietary oltipraz reduced the DNA adducts, 7-methylguanine and O^6-methylguanine. The magnitude of inhibition of DNA adducts was demonstrated more with 7-methylguanine than with O^6-methylguanine.

Discussion

The purpose of this study, which is a part of a large scale investigation on the efficacy of several naturally occurring substances with a different mode of action as inhibitors of colon carcinogenesis, was to elucidate the modulating effect of oltipraz, a substituted dithiolthione, on colon carcinogenesis. Oltipraz and other dithiolthiones have been shown to have a broad spectrum of anticarcinogenic actions against several types of carcinogens (8, 10).

The results of the present study show that, in both AOM- and saline-treated animals, dietary oltipraz significantly enhanced the liver and colonic GST activity, which is accompanied by a decrease in DNA methylation (7-methylguanine and O^6-methylguanine) in the colon and liver. Although the significance of 7-methylguanine in tumor formation has been questioned, the formation and persistence of O^6-methylguanine in the DNA of target tissue is closely correlated with carcinogenicity (28, 29, 34). Many chemopreventive agents including several phenolic antioxidants have been shown to inhibit carcinogenesis by modulating the activities of detoxifying enzymes that are involved in the metabolism of carcinogens (10, 35, 36). It has also been reported that oltipraz elevates several enzymes, such as epoxide hydrase and NADP(H)-quinone reductase (10), that are involved in the inactivation of electrophilic species. *In vitro* studies also suggest that oltipraz elevates quinone reductase and glutathione levels in a dose-

Table II. Effect of Oltipraz on Liver Enzymes and DNA Methylation in Rats

Experimental group	Glutathione S-transferase (μmol/mg protein/min)	Tyrosine protein kinase (pmol [^{32}P]ATP incorporated/mg protein/min)		Ornithine decarboxylase (pmol $^{14}CO_2$ released/mg protein/hr)	7-Methyl guanine (μmol/mol guanine)	O_6-Methyl guanine (μmol/mol guanine)
		Cytosol	Particulate			
Saline-treated						
Control diet	2.89 ± 0.11[a]	7.73 ± 0.42	53 ± 3.8	3.36 ± 0.26		
Oltipraz diet	8.27 ± 0.27[b,c]	5.90 ± 0.41[d]	49 ± 3.0	6.25 ± 0.52[d]		
AOM-treated						
Control diet	4.97 ± 0.17	85.1 ± 7.7	333 ± 15	7.03 ± 0.76	6343 ± 667	728 ± 63
Oltipraz diet	22.7 ± 1.7[c]	44.2 ± 6.0[e]	146 ± 14[e]	7.21 ± 0.63	2185 ± 228[e]	306 ± 32[f]
Statistical analysis (P values)[g]						
Oltipraz	0.0001	0.001	0.0005	0.051		
AOM	0.001	0.0001	0.0001	0.038		
Oltipraz × AOM interaction	0.0001	0.002	0.006	0.46		

[a] Mean ± SE (n = 6).
[b] Significantly different from its control diet groups within saline-treated or AOM-treated groups.
[c] $P < 0.0001$.
[d] $P < 0.05$.
[e] $P < 0.001$.
[f] $P < 0.01$.
[g] By two-way analysis of variance.

dependent manner in Hepa 1c1c7 murine hepatoma cells (37). Whether these detoxifying enzymes are involved in the inactivation of AOM remains to be seen.

With regard to the activation of AOM to its highly reactive metabolite, AOM is first metabolized to MAM, a proximate carcinogen, in the liver by the microsomal mixed function oxidases, and MAM can be further metabolized in the liver to a DNA-alkylating species, presumably the methyldiazonium ion, which is considered to be an ultimate carcinogen, by microsomal mixed function oxidases or by cytosolic alcohol dehydrogenase (11, 38). Recent studies by Sohn *et al.* (39) and Fiala *et al.* (40) suggest that the MAM that is not metabolized by the enzymes in the liver enters the circulation and is carried to the colon, where it is activated further, probably by alcohol dehydrogenase. The activated electrophile, methyldiazonium ion, can methylate cellular nucleophiles, including DNA. Since the alkylation of nucleic acids is considered to be the initial step for induction of colon tumors by AOM, any changes in the metabolic activation of AOM leading to the formation of DNA-alkylating species in the target organ will influence the outcome of tumors (34). For example, several colon tumor inhibitors such as pyrazole, disulfiram, or its metabolites have been shown to interact with AOM metabolism and to inhibit DNA adduct formation and carcinogenicity (29, 41–43). Recently, Hamilton *et al.* (44) showed that AOM-induced colon carcinogenesis in male F344 rats was inhibited by dietary ethanol. This inhibition might have resulted from the interaction of ethanol with AOM metabolism by suppression of metabolic activation of AOM and subsequent reduced formation of O^6-methylguanine and 7-methylguanine adducts (44). Our recent experimental results indicate that dietary oltipraz inhibits intestinal tumors before, during, and after carcinogen treatment (C. V. Rao *et al.*, unpublished data). In the study presented here, although there is no experimental evidence, the possibility exists that dietary oltipraz alters the metabolism of AOM in the liver and colon mucosa by the microsomal AOM hydroxylase and MAM oxidase as well as by the cytosolic alcohol dehydrogenase. It is also possible that increased GST activity in the liver and colon of animals fed the oltipraz decreases the level of DNA adduct formation, by modulating AOM metabolism.

Previous studies in different cancer models indicated an important role of ODC in tumor promotion (45–47). In neoplastic disorders, the activity of ODC is typically increased by severalfold over that of normal surrounding tissue. Such is the case with liver and colon, in which ODC activity increases in a stepwise manner from the normal tissue to benign lesions to adenocarcinoma (15, 17). Our earlier studies have shown that dietary constituents, which enhance or inhibit colon carcinogenesis in animal models, respectively increase or decrease colonic mucosal ODC activity (27, 30). The present study demonstrates a distinct stimulation of colonic mucosal ODC activity by the administration of AOM and inhibition of this AOM-

Table III. Effect of Oltipraz on Colonic Mucosal Enzymes and DNA Methylation in Rats

Experimental group	Glutathione S-transferase (μmol/mg protein/min)	Tyrosine protein kinase (pmol [^{32}P]ATP incorporated/mg protein/min)		Ornithine decarboxylase (pmol $^{14}CO_2$ released/mg protein/hr)	7-Methyl-guanine (μmol/mol guanine)	O^6-Methyl-guanine (μmol/mol guanine)
		Cytosol	Particulate			
Saline-treated						
Control diet	0.63 ± 0.01[a]	4.92 ± 0.37	30 ± 3.2	6.93 ± 0.32		
Oltipraz diet	2.57 ± 0.12[b,c]	4.91 ± 0.22	36 ± 3.0	11.3 ± 1.3[d]		
AOM-treated						
Control diet	1.07 ± 0.08	61.9 ± 3.1	222 ± 13	40.5 ± 3.5	1567 ± 126	232 ± 24
Oltipraz diet	7.10 ± 0.20[e]	27.7 ± 3.0[c]	158 ± 8[f]	22.1 ± 3.4[f]	284 ± 23[e]	157 ± 11[d]
Statistical analysis (*P* values)						
Oltipraz	0.0001	0.007	0.013	0.041		
AOM	0.001	0.0001	0.0001	0.0001		
Oltipraz × AOM interaction	0.0002	0.0006	0.008	0.001		

[a] Mean ± SE ($n = 6$).
[b] Significantly different from its control diet group within saline-treated or AOM-treated groups.
[c] $P < 0.001$.
[d] $P < 0.05$.
[e] $P < 0.0001$.
[f] $P < 0.01$.

induced ODC activity by dietary oltipraz. A significant increase in ODC activity was also observed by oltipraz feeding in saline-treated animals. Although this may indicate a possible role of oltipraz in tumor promotion, no such experimental data on the tumor-promoting effect of oltipraz when administered during the postinitiation phase are available. Our unpublished results indicate that oltipraz given during the initiation and postinitiation phases of carcinogenesis inhibits intestinal carcinogenesis. In this connection, another naturally occurring compound, namely, indole-3-carbinol, has been shown to inhibit the aflatoxin-induced liver tumorigenesis when administered during the initiation phase but enhances liver carcinogenesis when given during the postinitiation phase (38). Additional studies are warranted to test the chemopreventive action of oltipraz when administered during the postinitiation phase of carcinogenesis.

Evidence from various studies indicates that the phosphorylation of proteins at tyrosine residues plays an important role in the regulation of cellular growth and differentiation (18, 49, 50). TPK activity is associated with cellular receptors for epidermal growth factor, platelet-derived growth factors, insulin receptor (49), and several oncogenes (18). Increased expression of TPK activity may be responsible for unlimited growth (51). Increased levels of TPK activity have been found in neoplastic tissues (24, 25, 51). Based on this, TPK inhibitors might be used as anticancer agents (52). In the present study, increased levels of TPK activity in the cytosol and membrane fractions of liver and colonic mucosa were significantly inhibited by oltipraz. The mechanism of TPK inhibition by oltipraz is unclear. There is, however, a possibility that the mechanism of inhibition by oltipraz might be similar to that of Genistein- and Erbstatin-like specific TPK inhibitors. These agents inhibit TPK activity by altering the DNA topoisomerase II activity and by acting at the level of the ATP site (52–54). Additional studies are warranted to test this hypothesis.

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