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Effect of oltipraz, α-tocopherol, β-carotene and phenethylisothiocyanate on rat oesophageal, gastric, colonic and hepatic glutathione, glutathione S-transferase and peroxidase

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Four anticarcinogens (oltipraz, α-tocopherol, β-carotene and phenethylisothiocyanate [PEITC]) were studied with respect to their effects on oesophageal, gastric, colonic and hepatic (i) glutathione (GSH) content, (ii) glutathione S-transferase (GST) enzyme activity, (iii) GST isoenzyme levels, and (iv) glutathione peroxidase (GPx) enzyme activity in male Wistar rats. GST enzyme activity was significantly increased in oesophagus (1.9×) and colon (1.2×) by PEITC and in liver (1.4×) by oltipraz. GST Alpha was doubled in the liver by oltipraz, α-tocopherol and PEITC. GST Mu levels were increased by β-carotene and PEITC in stomach and liver, by oltipraz in liver and by α-tocopherol in stomach. PEITC induced colonic GST Pi levels (1.3×). GSH content was induced in liver by oltipraz (1.4×) and α-tocopherol (1.2×) and in colon by PEITC (1.6×). Each of the anticarcinogens tested increased GPx activity at one or more sites: Se-dependent and total GPx activities were induced in 31.3% and 37.5% of all possibilities, respectively. Major induction in total GPx was found in stomach by α-tocopherol (1.8×). In conclusion our data demonstrate that dietary administration of oltipraz, PEITC, α-tocopherol and β-carotene, may exert chemopreventive effects in the digestive tract of the rat by enhancing GST, GPx, and, to a lesser extent, GSH levels.

Introduction

There is considerable interest in identifying synthetic or dietary compounds which may possess anticarcinogenic properties (1). Vegetables and fruit contain a large number of inhibitors of carcinogenesis, including phenols, indoles, aromatic isothiocyanates, ascorbic acid, tocopherols and carotenes (2,3). Therefore it is recommended to daily consume a variety of fruits and vegetables. We are in search of a possible mechanism explaining the anticarcinogenic properties of some of these compounds, in particular those present in cruciferous vegetables.

Phenethylisothiocyanate (PEITC*) is a naturally occurring compound, present as its glucosinolate precursor in certain cruciferous vegetables, such as Chinese cabbage, Brussel sprouts, watercress and radishes (4,5). It is formed through hydrolysis of gluconasturtiin by myrosinase (6). PEITC was shown to inhibit the metabolism and carcinogenicity of nitrosamines and polycyclic aromatic hydrocarbons in various organs of the rat, such as lung, oesophagus and liver (3,7–9), and it reduced the incidence and size of adenomas and carcinomas (3,10,11).

α-Tocopherol, one of the carotenoids, is an antioxidant and free radical scavenger, but epidemiologic evidence for its chemopreventive action is inconsistent. α-Tocopherol reduced the production of carcinogenic *N*-nitroso compounds from nitrites and amides, and it is one of the most important antioxidants in lipid membranes. It inhibits the development of chemically induced tumours in experimental animals (12). Conclusions from the few cross-sectional studies are somewhat contradictory as reviewed by Knekt *et al.* (13). Several case-control studies showed that α-tocopherol levels in blood were inversely correlated with cancers of breast, lung, stomach, pancreas and urinary organs (14–17). In prospective studies, a similar correlation was found for liver and lung (18), while other studies do not show a significant difference between cases and controls (13,15,17).

β-Carotene is the most abundant of the carotenoids, which are found in dark-green leafy vegetables, carrots, and yellow or red fruits and vegetables. β-Carotene is a precursor of vitamin A, which plays a role in vision, growth, reproduction, and control of epithelial growth and differentiation. Epidemiological studies have provided strong evidence for a protective role of β-carotene against certain epithelial cancers, in particular those of the respiratory tract and oesophagus (19,20 and references therein). Daily consumption of vegetables with high levels of β-carotene was reported to diminish the risk of cancer of lung, colon, stomach, prostate and cervix (21–23). β-Carotene protected rats and mice against experimental tumours at various sites such as stomach, lung, colon, skin and buccal mucosa (12,24–26).

Oltipraz, 5-(2-pyrazinyl)-4-methyl-1,2-dithiole-3-thione, is a substituted dithiolthione which was used in humans as an antischistozomal drug (27). Dithiolthiones occur in cruciferous vegetables. Oltipraz was shown to protect against acute and chronic toxicities of structurally diverse agents in multiple target organs (for review, see 28). In addition, it was found to inhibit carcinogenesis induced by benzo[*a*]pyrene, diethylnitrosamine and uracil mustard in lung and forestomach of mice (29), and azoxymethane-induced colon cancer in rats (30,31).

The mechanism of action of the above mentioned inhibitors of carcinogenesis are poorly understood. One method of action may be an enhancing effect on carcinogen detoxification systems, such as glutathione S-transferases (GSTs) (for review, see 2). Animals fed diets containing high concentrations of cruciferous vegetables or other anticarcinogens showed elevated GST-activity (32,33). The soluble GSTs are a gene family of dimeric enzymes comprised of four classes: Alpha, Mu, Pi and Theta (34). They catalyze the binding of a large variety of electrophiles to the sulphydryl group of glutathione (GSH). Since the reactive ultimate carcinogenic forms of

*Abbreviations: CYP, cytochrome P450; GPx, glutathione peroxidase; GSH, glutathione; GST, glutathione S-transferase; PEITC, phenethylisothiocyanate; Se-GPx, selenium-dependent glutathione peroxidase; t-GPx, total glutathione peroxidase.

Table I. Daily food consumption, anticarcinogen intake and gain in body weight of male Wistar rats receiving diets supplemented with oltipraz, α-tocopherol, β-carotene or PEITC

Treatment group (n = 8)	Dose (% w/w)	Food consumption (g/day) intake	Total anticarcinogen (mg/day.kg body wt)	Gain in body wt (g/day)
Control	–	20.1±0.7	–	3.9±0.2
Oltipraz	0.030	18.9±0.5	28.5±0.8	2.9±0.3[a]
α-Tocopherol	0.020	18.5±0.2	18.5±0.2	3.4±0.3
β-Carotene	0.020	19.5±0.3	19.4±0.3	3.6±0.4
PEITC	0.045	17.9±0.3[a]	40.4±0.6	2.6±0.3[a]

Values given are means ± SEM. The one-tailed Wilcoxon rank sum test was used to assess statistical significance of differences between control and treated groups: [a]$P < 0.05$.

Table II. Effects of oltipraz, α-tocopherol, β-carotene or PEITC on oesophageal, gastric, colonic and hepatic glutathione S-transferase activity in rats

Treatment group (n = 8)	Glutathione S-transferase activity (nmol/min.mg protein)			
	Oesophagus	Stomach	Colon	Liver
Control	22±3	142±14	68±5	1277±113
Oltipraz	27±1	154±9	75±3	1760±109[c]
α-Tocopherol	25±4	148±8	74±7	1094±68
β-Carotene	27±3	138±12	74±7	1330±46
PEITC	41±3[c]	167±16	84±6[a]	1376±58

[c]$P < 0.005$.

Table III. Effects of oltipraz, α-tocopherol, β-carotene or PEITC on oesophageal, gastric, colonic and hepatic glutathione S-transferase Alpha levels in rats

Treatment group (n = 8)	Glutathione S-transferase Alpha level (ng/mg protein)			
	Oesophagus	Stomach	Colon	Liver
Control	ND	198±5	ND	8349±225
Oltipraz	ND	209±16	ND	16702±687[c]
α-Tocopherol	ND	196±10	ND	14583±1786[b]
β-Carotene	ND	198±6	ND	7921±473
PEITC	ND	199±12	ND	18724±988[c]

[b]$P < 0.01$ and [c]$P < 0.005$; ND, not detectable.

Table IV. Effects of oltipraz, α-tocopherol, β-carotene or PEITC on oesophageal, gastric, colonic and hepatic glutathione S-transferase Mu levels in rats

Treatment group (n = 8)	Glutathione S-transferase Mu level (ng/mg protein)			
	Oesophagus	Stomach	Colon	Liver
Control	2878±290	11934±735	2603±323	30769±781
Oltipraz	3068±622	13992±985	3290±228	42840±2021[c]
α-Tocopherol	3265±312	13857±863[a]	2807±255	34319±3390
β-Carotene	2956±411	13757±905[a]	3221±133	37586±2395[a]
PEITC	3523±313	13399±515[a]	2961±193	44980±905[c]

[a]$P < 0.05$, [b]$P < 0.01$ and [c]$P < 0.005$.

chemical carcinogens are electrophiles, GST takes considerable importance as a mechanism for carcinogen detoxification (34). Enhancement of the activity of this system by dietary anticarcinogens could result in a more efficient elimination of carcinogens, leading to cancer prevention.

Another parameter of importance in inhibiting carcinogenesis is prevention of oxidative damage by glutathione peroxidases (GPxs). GPxs are enzymes that catalyze the reduction of organic hydroperoxides and hydrogen peroxide (35). Two major types of GPx have been identified (35), a selenium-dependent form which is active with both organic hydroperoxides and hydrogen peroxide and a selenium-independent form which has no activity towards hydrogen peroxide and mainly comprises of GSTs (35).

The present study was designed to investigate the effects of dietary oltipraz, α-tocopherol, β-carotene and PEITC on

Table V. Effects of oltipraz, α-tocopherol, β-carotene or PEITC on oesophageal, gastric, colonic and hepatic glutathione S-transferase Pi levels in rats

Treatment group (n = 8)	Glutathione S-transferase Pi level (ng/mg protein)			
	Oesophagus	Stomach	Colon	Liver
Control	ND	984±55	1001±27	ND
Oltipraz	ND	1028±142	1036±17	ND
α-Tocopherol	ND	1033±80	1035±44	ND
β-Carotene	ND	1035±71	1024±48	ND
PEITC	ND	1018±90	1303±65[c]	ND

[c]$P < 0.005$; ND, not detectable

Table VI. Effects of oltipraz, α-tocopherol, β-carotene or PEITC on oesophageal, gastric, colonic and hepatic glutathione levels in rats

Treatment group (n = 8)	Glutathione (nmol/mg protein)			
	Oesophagus	*Stomach*	*Colon*	*Liver*
Control	10±2	18±3	7±1	47±3
Oltipraz	14±4	16±2	9±1	64±6[a]
α-Tocopherol	12±3	18±2	9±2	58±2[b]
β-Carotene	12±3	13±3	10±3	42±2
PEITC	16±3	20±2	11±2[a]	48±5

[a]$P < 0.05$ and [b]$P < 0.01$.

Table VII. Effects of oltipraz, α-tocopherol, β-carotene or PEITC on oesophageal, gastric, colonic and hepatic selenium-dependent glutathione peroxidase activity in rats

Treatment group (n = 8)	Selenium-dependent glutathione peroxidase activity* (nmol/min.mg protein)			
	Oesophagus	Stomach	Colon	Liver
Control	236±23	1574±165	208±11	1364±80
Oltipraz	321±31[a]	1855±28	244±7[a]	1598±121
α-Tocopherol	296±31	2098±294	248±20	1698±81[a]
β-Carotene	284±20	1738±202	206±17	1736±32[c]
PEITC	268±12	2403±212[c]	244±19	1548±82

*Hydrogen peroxide was used as substrate; [a]$P < 0.05$, [b]$P < 0.01$ and [c]$P < 0.005$.

Table VIII. Effects of oltipraz, α-tocopherol, β-carotene or PEITC on oesophageal, gastric, colonic and hepatic total glutathione peroxidase activity in rats

Treatment group (n = 8)	Total glutathione peroxidase activity* (nmol/min.mg protein)			
	Oesophagus	Stomach	Colon	Liver
Control	194±10	1213±115	216±16	1148±71
Oltipraz	261±7[a]	1360±114	239±8	1248±46
α-Tocopherol	221±11	2176±249[c]	254±14[a]	1278±56
β-Carotene	222±15	1646±229	218±16	1360±56[a]
PEITC	247±5[c]	2174±190[c]	220±23	1334±73

*t-Butyl hydroperoxide was used as substrate. [a]$P < 0.05$, [b]$P < 0.01$ and [c]$P < 0.005$.

glutathione S-transferases, glutathione peroxidase and glutathione in rat oesophagus, colon, stomach and liver.

Materials and methods

Animal treatment

Fourty male Wistar rats (178 ± 8 g; Central Laboratory Animal Centre, University of Nijmegen, The Netherlands) were housed in pairs on wooden shavings in macrolon cages, maintained at 20–25°C and 30–60% relative humidity. A ventilation rate of seven air cycles/h and a 12 h light/dark cycle were used. The rats were randomly assigned to one of the dietary treatment groups. All groups were fed powdered RMH-TM lab chow (Hope Farms, Woerden, The Netherlands). After acclimatization for 7 days the animals were fed either the basal diet (control group) or one of the four experimental diets. Food and water were available *ad libitum*. Food cups were replenished every 2–3 days. Food consumption and gain in body weight were recorded daily. After 2 weeks the rats were killed by decapitation.

Diets

Compounds and dose levels used were selected based on studies by others, showing antimutagenic and/or anticarcinogenic properties (9,30,36,37). The diets were prepared by supplementation with either 0.03% (w/w) oltipraz (kindly provided by Rhone Poulenc Rorer, Vitry, Alfortville, France), 0.02% (w/w) α-tocopherol (Sigma Chemical Company, St Louis, MO, USA), 0.02% (w/w) β-carotene (Sigma), or 0.045% (w/w) phenethylisothiocyanate (PEITC; Sigma). A food processor was used to obtain a homologous mixture of test compound and powdered lab chow.

Tissue preparation

All handlings were performed on ice. After decapitation the oesophagus, stomach, colon and liver were excised. Colon and stomach were slit longitudinally and the contents were removed by washing with cold buffer A (0.25 M saccharose, 20 mM Tris, 1 mM dithiothreitiol, pH 7.4). The organs were directly frozen in liquid nitrogen and stored at –20°C until use. For preparation of the cytosolic fraction the tissue was thawed quickly using cold running water. The mucosal surface of stomach, intestine and colon was collected by scraping with a scalpel and the mucosal scrapings were homogenized in buffer A (4 ml/g tissue) in a glass/glass Potter–Elvehjem tube. The liver was homogenized in buffer A (4 ml/g tissue) with ten strokes at 1000 r.p.m. of a motor-driven glass/Teflon homogenizer (Braun, Germany). The homogenate was centrifuged at 9000 *g* (4°C) for 30 min. The resulting supernatant fraction was transferred to an ultracentrifuge tube and spun at 150 000 *g* (4°C) for 60 min. The oesophagus was homogenized in 5 ml buffer A per gram tissue in a glass/glass Potter–Elvehjem tube. These homogenates were centrifuged at 150 000 *g* for 60 min (4°C). Aliquots of the 150 000 *g* supernatant, representing the cytosolic fraction, were frozen in liquid nitrogen and stored at –80°C.

Assays

Protein concentration was assayed in quadruplicate by the method of Lowry *et al.* (38) using bovine serum albumin as the standard. GST activity was determined in triplicate according to Habig *et al.* (39), using 1-chloro-2,4-dinitrobenzene (CDNB) as substrate. GST isoenzyme levels were determined as described before (33). In short, cytosolic fractions were subjected to sodium dodecyl sulfate polyacrylamide gel electrophoresis (11% acrylamide, w/v), subsequent to Western blotting, using a semi-dry blotting system (Novablot II, Pharmacia, Uppsala, Sweden). Western blots were incubated with monoclonal antibodies against human GST class Alpha, Mu, and Pi. Class Alpha antibodies react with rat GST subunit 1, class Mu antibodies recognize rat GST subunits 3 and 4, and class Pi antibodies react with rat GST subunit 7. The specific binding of the monoclonal antibodies to the isoenzymes was detected with 4-chloro-1-naphthol after incubation with peroxidase-conjugated rabbit anti-mouse immunoglobulin as second antibody (Dakopatts, Glostrup, Denmark). Staining intensity on the immunoblots was quantified using a laser densitometer (Ultroscan XL, LKB, Bromma, Sweden). Known amounts of purified GSTs were run in parallel with the experimental samples and served as standards for the calculation of the absolute amounts of these isoenzymes in the cytosolic fragments. Total glutathione was quantified by high performance liquid chromatography after reaction with monobromobimane, as described before (33). In this assay oxidized glutathione present is reduced by adding sodium borohydride to the reaction mixture. Glutathione peroxidase enzyme activity was measured with hydrogen peroxide and t-butylhydroperoxide (Sigma) as substrates, essentially as described by Howie *et al.* (40).

Statistical analysis

The Wilcoxon rank sum test with Bonferroni correction for multiple testing was used to assess statistical significance of differences between experimental and control groups: $^{a}P < 0.05$, $^{b}P < 0.01$ and $^{c}P < 0.005$.

Results

Daily food consumption, anticarcinogen intake and gain in body weight are given in Table I. In the PEITC group food consumption was significantly reduced, but total anticarcinogen intake was high. At termination of the experiment, the daily gain in body weight for the oltipraz and PEITC group was significantly lower as compared with the control group.

Table II shows the effects of the anticarcinogens on GST activity in the four organs investigated. PEITC induced GST activity in oesophagus and colon (1.9× and 1.2×, respectively) and oltipraz had an inducing effect in the liver (1.4×).

In Tables III, IV and V the effects of the anticarcinogens on GST class Alpha, Mu and Pi isoenzyme levels are given. In control animals GST Alpha (Table III) was undetectable in oesophagus and colon, low in stomach (198 ± 5 ng/mg protein) and high in liver (8349 ± 225 ng/mg protein). In oesophagus, stomach and colon, none of the diets significantly influenced the GST Alpha expression. In liver, on the other hand, increased levels of GST Alpha were found after feeding oltipraz, α-tocopherol or PEITC (2.0×, 1.7× and 2.2×, respectively) as compared with controls. GST Mu (Table IV) was expressed at high levels in all tissues examined, but only gastric and hepatic GST Mu levels were modulated by anticarcinogens. Gastric GST Mu levels were increased by α-tocopherol, β-carotene and PEITC (1.2×, 1.2× and 1.1×, respectively), and hepatic GST Mu levels were elevated by oltipraz, β-carotene and PEITC (1.4×, 1.2× and 1.5×, respectively). In control group GST Pi (Table V) was undetectable in oesophagus and liver, and low in stomach and colon (984 ± 55 and 1001 ± 27 ng/mg protein, respectively). Only in the colon PEITC induced GST Pi levels to 1303 ± 65 ng/mg protein.

Table VI shows the effect of the anticarcinogens on GSH content in the organs studied. Oesophageal and gastric GSH content was not significantly influenced by any of the anticarcinogens. Elevation of GSH content was seen by oltipraz and α-tocopherol (1.4× and 1.2×, respectively) in liver and by PEITC in colon (1.6×). No correlation between enhancement of GST and GSH levels was found.

In Tables VII and VIII, the data of glutathione peroxidase activity (GPx) are presented. Selenium-dependent (Se-GPx) and total GPx (t-GPx) were measured using hydrogen peroxide and t-butyl hydroperoxide as substrate, respectively. Interestingly, GPx activities are highest in stomach, slightly lower in liver and lowest in oesophagus and colon. The anticarcinogens tested all increased either one of the activities at one or more sites. Se-GPx activity (Table VII) was increased by oltipraz in oesophagus (1.4×) and colon (1.2×), by α-tocopherol and β-carotene in liver (1.2× and 1.3×, respectively) and by PEITC in stomach (1.5×). Total GPx activity (Table VIII) was increased by oltipraz in oesophagus (1.3×), by α-tocopherol in stomach and colon (1.8× and 1.2×), by β-carotene in liver (1.2×) and by PEITC in oesophagus (1.3×) and stomach (1.8×).

Discussion

Consumption of vegetables and fruit can significantly decrease the risk of human cancer (41,42). A relationship between vitamin A and human cancer was first reported in 1941 by Abels *et al.* (43), who documented low vitamin A levels in cancer patients, particularly in patients with gastrointestinal malignancies. The human diet contains a large number of both (pre)carcinogens as well as a variety of compounds that inhibit mutagenesis and/or carcinogenesis in laboratory models (44–46). This latter group consists of structurally diverse compounds, of which the protective mechanisms are generally unresolved. Although prevention of cancer may be due to multiple mechanisms, one way of action of anticarcinogens may be an enhancing effect on carcinogen detoxification systems, such as glutathione S-transferases (GSTs). Fruits and vegetables that elevate tissue phase II enzyme levels in rodents can effectively block experimental carcinogenesis and increase the clearance of drugs in humans (1,2,32,47).

In rats receiving a diet containing oltipraz or PEITC the daily gain in body weight was lower as compared to the control group. In the PEITC group (receiving 2.7 μmol/g diet) reduced food consumption coincided with reduced weight gain. Reduced weight gain after feeding rats 6 μmol PEITC per gram of diet has been reported before (9). The oltipraz group (receiving 300 p.p.m. in their diet) showed a lower daily gain in body weight, whereas there was a normal food intake. Rao *et al.* (31) did not find any effect on body weight of a somewhat lower dose of oltipraz (200 p.p.m. for 2 weeks). No effect of oltipraz on

food consumption has been reported before. During the course of the experiment no changes in behavioral pattern of the animals were observed. In addition to this, none of the organs studied showed any macroscopical sign of toxicity of the dietary additive at the end of the experiment. Therefore, we believe that the lower gain in body weight in the oltipraz group, despite a normal food intake may be the result of diminished absorption of macronutrients in the gastrointestinal tract. No effect of β-carotene on body weight gain has been reported (25) in accordance with our results.

At present, more information about the effects of dietary anticarcinogens on oesophageal, gastric, colonic and hepatic GST activity is becoming available. In our study, oesophageal and colonic GST activity were increased by PEITC that may be of significance in the protection against oesophageal and colonic cancer. We could not find any effect of PEITC on hepatic GST activity, in accordance with earlier results of Smith *et al.* (37), who found that dietary intake of PEITC (1 and 3 μmol/g diet) did not significantly induce GST activity in liver. This suggests that the biological action of PEITC is not due to its effect on hepatic GSTs under these experimental conditions. In male F344 rats, on the other hand, 0.25 mmol/kg base weight of PEITC resulted in a slight increase of liver GST activity after 24 h (48), while a single dose of 1 mmol/kg decreased liver GST activity by a factor 1.5 (49). These differences might be due to species-specific and/or concentration-dependent effects, which make it virtually impossible to extrapolate from one study to another. We have not found any reports on effects of PEITC on oesophageal and/or colonic GST activity. The inducing effect of dietary oltipraz on hepatic GST activity we noticed is in agreement with results reported by others (50,51).

In addition to the measurement of GST activity it may be important to study changes in levels of isoenzymes, which may have different substrate specificities. The isoenzymes showed a tissue specific distribution. Class Alpha GSTs are abundant in liver and small intestine, whereas class Pi GSTs are well expressed in stomach, small- and large intestine (33,34). In contrast, class Mu enzymes seem to be less organ specific since they were detected at high levels in a wide variety of tissues. In our study, GST Alpha was not detectable in oesophagus and colon, and GST Pi was undetectable in oesophagus and liver, and none of the anticarcinogens had any effect on this. At two sites where GST activity was induced a significant increase in one or more classes of GST was observed. The increase in colonic GST activity by PEITC was parallelled by a rise in GST Pi level in the same order of magnitude, and dietary administration of oltipraz resulted in an increase in hepatic GST activity and higher GST Alpha and GST Mu levels. Higher doses of oltipraz (0.075%) were reported to induce GST Alpha, Mu and Pi levels in livers of Sprague–Dawley rats (52). In human hepatocyte cultures, GST Alpha, but not Mu was selectively and specifically elevated by oltipraz (53). In our study, PEITC increased oesophageal GST activity, but had no effect on any of the isoenzymes in this organ. In addition, GST isoenzyme levels were induced at some sites without significant change in overall GST activity. Administration of PEITC induced hepatic GST Alpha and Mu, and gastric GST Mu levels; α-tocopherol increased gastric GST Mu and hepatic GST Alpha levels; and β-carotene increased gastric and hepatic GST Mu levels, although no effects on GST activity were notified.

Only few data on effects on GSH levels of the anticarcinogens tested have been reported before. GSH is a very important physiological nucleophile which forms conjugates with reactive electrophiles, including carcinogenic nitrosamines (54). We found an induction of GSH in only three out of 16 possibilities: (i) in colon by PEITC and (ii) in liver by oltipraz, which both parallelled increases in GST activity, and (iii) in liver by α-tocopherol. Oltipraz-induced hepatic GSH levels have been reported before by others (51).

Glutathione peroxidase (GPx), which utilizes GSH to catalyze the reduction of hydroperoxides, is a cellular defence system against the deleterious effects of hydroperoxides (55,56). Each of the four anticarcinogens tested in our study induced GPx activity at one or more sites: at five out of 16 (31%) sites for Se-dependent GPx and six out or 16 (38%) sites for total GPx. Therefore increase of GPx levels may significantly contribute to the overall effect of the compounds tested here. Again, there is little information available in the literature. Dietary α-tocopherol was reported to increase GPx activity in rat muscle but not in liver, lung, kidney and testes (57). In another study dietary α-tocopherol had no effect on GPx activity in rat liver and intestines (58).

Chemopreventive effects may be mediated by enzyme systems other than GSTs. Inhibition of enzymes involved in the bioactivation of pro-carcinogens may be equally important. The anticarcinogens tested in our study have previously been shown to be able to influence the phase I cytochrome P450 (CYP) enzyme system as well. PEITC efficiently prevented the ethanol-induced elevation of CYP2E1 apoprotein and mRNA levels in rat liver (59). α-Tocopherol significantly decreased cytochrome P450 in rats (60). β-Carotene inhibited the cytochrome P450-mediated benzo[*a*]pyrene metabolism in rat liver (61). Finally, inhibition by oltipraz of aflatoxin B1 metabolism in human primary cultures of hepatocytes was found to be mediated by inhibiting CYP1A2 and CYP3A4 (62).

It is therefore likely that these effects on cytochrome P450, in combination with enhancement of glutathione S-transferases, glutathione peroxidase and glutathione, are responsible for the chemoprotective character of the compounds tested in our study.

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