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Advances in Brief

Enhancement of Antioxidant and Phase II Enzymes by Oral Feeding of Green Tea Polyphenols in Drinking Water to SKH-1 Hairless Mice: Possible Role in Cancer Chemoprevention[1]

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Abstract

Following the oral feeding of a polyphenolic fraction isolated from green tea (GTP) in drinking water, an increase in the activities of antioxidant and phase II enzymes in skin, small bowel, liver, and lung of female SKH-1 hairless mice was observed. GTP feeding (0.2%, w/v) to mice for 30 days significantly increased the activities of glutathione peroxidase, catalase, and quinone reductase in small bowel, liver, and lungs, and glutathione *S*-transferase in small bowel and liver. GTP feeding to mice also resulted in considerable enhancement of glutathione reductase activity in liver. In general, the increase in antioxidant and phase II enzyme activities was more pronounced in lung and small bowel as compared to liver and skin. The significance of these results can be implicated in relation to the cancer chemopreventive effects of GTP against the induction of tumors in various target organs.

Introduction

In recent years, several studies from our laboratory as well as from others have shown that GTP,[5] WEGT, or EGCG, the major polyphenolic constituent present in GTP and WEGT, affords protection against UV B radiation-induced carcinogenesis in skin (1, 2) and chemical carcinogen-induced tumorigenesis at least in skin, colon, forestomach, esophagus, and lung in animal bioassay systems (3–8). The epidemiological studies related to the effects of green tea on human cancer, although inconclusive, have suggested a protective as well as an enhancing effect of tea ingestion on cancer risk (9). A case-control study by Kono *et al.* (10), however, has indicated that individuals consuming green tea tend to have a lower risk for gastric cancer. Studies by Oguni *et al.* (11) in Shizuoka Prefecture in Japan show that stomach cancer death rate in this tea producing and consuming area was lower than the national average.

Some studies suggest that the antioxidant properties of GTP, besides its protective effects against the tumor promoter-caused induction of ornithine decarboxylase (4, 5), play an important role against the chemical carcinogen-induced development of neoplasia (1–5, 12). Despite the fact that (*a*) GTP, WEGT, and epicatechin derivatives present in green tea (more specifically, EGCG) have shown significant protection against tumorigenesis in several animal tumor bioassay systems (1–8), (*b*) the depletion in the activities of antioxidant and phase II enzymes occurs during tumorigenesis (13, 14), and (*c*) cancer chemopreventive agents enhance the activity of these enzymes in target tissues (15), the role of GTP, WEGT, or EGCG administration on the levels of antioxidant defense and phase II enzyme activities has not yet been studied. Herein we report that oral feeding of GTP in drinking water to female SKH-1 hairless mice results in the enhancement of activities of antioxidant and phase II enzymes GSH-Px, catalase, GST, QR, and GSH-r in skin, small bowel, liver, and lung.

Materials and Methods

NADPH, flavin adenine dinucleotide, H_2O_2, 2,6-dichlorophenol-indophenol, oxidized and reduced glutathione, and horse radish peroxidase were obtained from Sigma Chemical Co. (St. Louis, MO). All other chemicals and reagents used were of the highest purity commercially available. GTP was prepared from green tea leaves by the method described earlier (5).

Female SKH-1 hairless mice (5–6 weeks old), purchased from Temple University Health Sciences, the Skin and Cancer Hospital (Philadelphia, PA), were used. Twenty animals were divided into two groups of ten each and fed with either normal drinking water (control group) or 0.2% GTP in water as the sole source of drinking water (experimental group); this defined feeding regimen was continued up to 30 days. The selection of dose of GTP was based on our previous studies where significant cancer chemopreventive effects were observed (1, 3). At the end of the feeding regimen, the animals were sacrificed, and whole skin, small bowel, liver, and lung were removed and immediately placed in ice-cold 0.1 M phosphate buffer, pH 7.4. Tissues were cleaned properly, minced and homogenized in the same buffer, and a 100,000 × *g* supernatant fraction was prepared as described earlier (5). GSH-Px and GSH-r activities were measured as described by Mohandas *et al.* (16). Catalase activity was determined by following the decomposition of H_2O_2 measured as a decrease in absorbance at 240 nm (17). GST activity was determined according to Habig *et al.* (18) using 1-chloro-2,4-dinitrobenzene as a substrate. QR activity was determined as described by Benson *et al.* (19) using 2,6-dichlorophenol-indophenol as an electron acceptor.

Results

The data in Table 1 show the activity of GSH-Px and catalase in the skin, small bowel, liver, and lungs of both GTP-fed experimental, and normal drinking water-fed control groups of mice, otherwise maintained under identical experimental conditions. The oral feeding of GTP resulted in a significant increase in the GSH-Px and catalase activities in small bowel, liver, and lung as compared to that observed in control group of animals. In general, a greater increase occurred in GSH-Px activity when compared to catalase activity. The increase in the

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[5] The abbreviations used are: GTP, polyphenolic fraction isolated from green tea; WEGT, water extract of green tea; EGCG, (−)-epigallocatechin-3-gallate; GSH-Px, glutathione peroxidase; GST, glutathione *S*-transferase; QR, quinone reductase; GSH-r, glutathione reductase.

activity of these two enzymes, however, was found to be markedly higher in lung and small bowel as compared to liver and skin (Table 1). The oral feeding of GTP also resulted in significant increase in the GST activity in small bowel and liver (Table 1) and QR activity in small bowel, liver, and lung (Table 1). Although the activity of these enzymes in skin was also found to be elevated, the levels were not statistically significant when compared with those obtained in the control group of animals (Table 1). Oral feeding of GTP in drinking water for 30 days, however, did not result in any significant change in GSH-r activity in skin, small bowel, and lung, whereas a significant increase in the activity of this enzyme was observed in the case of liver (Table 1). The dose of GTP used in the present study did not produce any apparent signs of toxicity, such as weight loss or reduced diet and water consumption, throughout the feeding regimen (data not shown).

Discussion

The generation of reactive oxidants in biological systems, either by normal metabolic pathways or as a consequence of exposure to chemical carcinogens, is extensively studied and contributes to the multistage process of carcinogenesis (13, 14). The collective action of both antioxidants and phase II enzymes such as GST and QR, besides small nonenzymatic water-soluble biomolecules, is to afford protection against the adverse effects of oxidants or reactive metabolites of precarcinogens (13, 14). In addition, the levels of antioxidant defense enzymes are also known to be lower in transformed cells and/or tumors (13, 14). Recently, Reiners *et al.* (20) have shown the depleted levels of antioxidant enzymes in 7,12-dimethylbenz(*a*)anthracene–12-*O*-tetradecanoylphorbol-13-acetate-treated skin and in skin tumors induced chemically. Depletion of these enzymes following exposure to carcinogens and/or tumor promoters is also known (13, 14). On the contrary, cancer chemoprevention studies have shown that following the administration of chemopreventive agents, the levels of antioxidant enzymes are elevated in various organs of the test animals (15). The significant enhancement in the activity of the antioxidant enzymes such as GSH-Px and catalase and phase II enzymes like GST and QR in the various organs of mice orally fed with GTP in drinking water suggests that it may contribute to the cancer chemopreventive effects observed with green tea (1–8).

The primary antioxidant enzyme catalase possesses a slow catalytic activity at low intracellular levels of its substrate H_2O_2, and under this condition, GSH-Px plays the predominant role in the detoxification of peroxides from the cells and/or tissues (21). The source of H_2O_2 in cells/tissues is mainly through superoxide dismutase-mediated dismutation of $\cdot O_2^-$ (14); the latter is generated in the cells/tissues by several endogenous enzyme systems as well as the nonenzymatic pathways (14). Several reports suggest the pronounced effects of peroxides as compared to $\cdot O_2^-$ in producing cytotoxicity/genotoxicity in the cellular systems (13, 14). Besides, the highly reactive $\cdot OH$, generated from H_2O_2 via the Haber-Weiss-like Fenton reaction (14), is known to damage macromolecules, specifically DNA, to produce the pathological alterations (13, 14). In view of these facts, the enhancement in the activity of both GSH-Px and catalase in various organs by oral feeding of GTP in drinking water suggests that such a treatment could protect the cells/tissues against the cytotoxic/genotoxic effects of peroxides and $\cdot OH$. The statistically insignificant effects of GTP on GSH-r activity in lung, small bowel, and skin indicate that the increase in the activity of other enzymes such as GSH-Px is not a result of generation of oxidative stress by GTP, since the induction of GSH-r during such conditions to regenerate reduced glutathione from oxidized glutathione for the efficient detoxification of H_2O_2 by GSH-Px is very well documented (22).

The two-electron reduction of the metabolic products of polycyclic aromatic hydrocarbons such as quinones, catalyzed by QR, has been considered to be a detoxification pathway, since the resulting hydroquinones may be conjugated and excreted

Table 1 *Effect of oral feeding of GTP (0.2%, w/v) in drinking water to female SKH-1 hairless mice on antioxidant and phase II enzyme activities in skin, small bowel, liver, and lung*[a]

Treatments	Skin	Small bowel	Liver	Lung
GSH-Px activity (nmol NADPH oxidized/mg protein/min)				
Control	236 ± 5	393 ± 8	14 ± 0.5	7 ± 0.5
GTP-fed	258 ± 6	893 ± 16[b]	26 ± 0.6[b]	16 ± 0.6[b]
% of control	109	227[b]	186[b]	229[b]
Catalase activity (nmol H_2O_2 consumed/mg protein/min)				
Control	457 ± 10	297 ± 7	25 ± 0.9	200 ± 7
GTP-fed	483 ± 11	471 ± 8[b]	48 ± 0.9[b]	349 ± 9[b]
% of control	107	159[b]	192[b]	175[b]
GST activity (nmol 1-chloro-2,4-dinitrobenzene conjugate formed/mg protein/min)				
Control	244 ± 6	904 ± 18	2244 ± 37	427 ± 11
GTP-fed	242 ± 7	1156 ± 20[c]	2917 ± 46[c]	425 ± 12
% of control	99	128[c]	130[c]	99
QR activity (nmol 2,6-dichlorophenol-indophenol reduced/mg protein/min)				
Control	531 ± 22	1740 ± 82	580 ± 27	261 ± 14
GTP-fed	568 ± 27	2662 ± 93[b]	887 ± 33[b]	446 ± 20[b]
% of control	107	153[b]	153[b]	171[b]
GSH-r activity (nmol NADPH oxidized/mg protein/min)				
Control	74 ± 3	395 ± 7	117 ± 2	33 ± 0.5
GTP-fed	70 ± 3	395 ± 8	137 ± 3[c]	32 ± 0.4
% of control	95	100	118[c]	97

[a] Data represent mean ± SE of five values obtained by assaying each in duplicate from pooled tissues of two animals. For statistical significance, Student's *t* test was used between normal water-fed control and GTP-fed experimental groups.
[b] Statistically significant *versus* control; $P < 0.001$.
[c] Statistically significant *versus* control; $P < 0.05$.

through mercapturic acid pathways (23). These quinones, in addition to electrophilic characteristics, are well-known oxidants (23). The semiquinones, the product of one-electron reduction of quinones via microsomal NADPH-cytochrome P-450 reductase, may be toxic *per se* or react with molecular oxygen, forming $\cdot O_2^-$ and regenerating the parent quinones, which is then available for rereduction and thereby undergoes a futile redox cycling (23). The net result of such a redox cycling is an oxidative stress resulting from the disproportionate consumption of cellular reducing equivalent and the generation of $\cdot O_2^-$, H_2O_2, and $\cdot OH$, the reactive oxygen species (13, 14). A phase II enzyme such as GST not only catalyzes the conjugation of both hydroquinones and epoxides of polycyclic aromatic hydrocarbons with reduced glutathione for their excretion, but also shows low activity toward organic hydroperoxides for their detoxification from cells/tissues (24). It is, therefore, reasonable to assume that increased activities of GST and QR in various organs of GTP-fed mice may play an important role in relation to the cancer chemopreventive effects of green tea (1–8).

In summary, our data indicate an elevation in the activities of both antioxidant and phase II enzymes in various organs of mice orally fed with GTP in drinking water and suggest that such an affect may be one of the possible mechanisms of cancer chemopreventive effects associated with green tea in several animal tumor bioassay systems.

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