Modulation of Human Glutathione *S*-Transferases by Polyphenon E Intervention

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Abstract

Purpose: **Green tea consumption has been associated with decreased risk of certain types of cancers in humans. Induction of detoxification enzymes has been suggested as one of the biochemical mechanisms responsible for the cancer-preventive effect of green tea. We conducted this clinical study to determine the effect of repeated green tea polyphenol administration on a major group of detoxification enzymes, glutathione *S*-transferases (GST).**

Methods: **A total of 42 healthy volunteers underwent a 4-week washout period by refraining from tea or tea-related products. At the end of the washout period, a fasting blood sample was collected, and plasma and lymphocytes were isolated for assessment of GST activity and level. Following the baseline evaluation, study participants underwent 4 weeks of green tea polyphenol intervention in the form of a standardized Polyphenon E preparation at a dose that contains 800 mg epigallocatechin gallate (EGCG) once a day. Polyphenon E was taken on an empty stomach to optimize the oral bioavailability of EGCG. Upon completion of the intervention, samples were collected for postintervention GST assessment.**

Results: **Four weeks of Polyphenon E intervention enhanced the GST activity in blood lymphocytes from 30.7 ± 12.2 to 35.1 ± 14.3 nmol/min/mg protein, $P = 0.058$. Analysis based on baseline activity showed that a statistically significant increase (80%, $P = 0.004$) in GST activity was observed in individuals with baseline activity in the lowest tertile, whereas a statistically significant decrease (20%, $P = 0.02$) in GST activity was observed in the highest tertile. In addition, Polyphenon E intervention significantly increased the GST-π level in blood lymphocytes from 2,252.9 ± 734.2 to 2,634.4 ± 1,138.3 ng/mg protein, $P = 0.035$. Analysis based on baseline level showed that this increase was only significant ($P = 0.003$) in individuals with baseline level in the lowest tertile, with a mean increase of 80%. Repeated Polyphenon E administration had minimal effects on lymphocyte GST-μ and plasma GST-α levels. There was a small but statistically significant decrease (8%, $P = 0.003$) in plasma GST-α levels in the highest tertile.**

Conclusions: **We conclude that 4 weeks of Polyphenon E administration resulted in differential effects on GST activity and level based on baseline enzyme activity/level, with GST activity and GST-π level increased significantly in individuals with low baseline enzyme activity/level. This suggests that green tea polyphenol intervention may enhance the detoxification of carcinogens in individuals with low baseline detoxification capacity.** (Cancer Epidemiol Biomarkers Prev 2007;16(8):1662–6)

Introduction

Metabolism is the major route of elimination of xenobiotics, including drugs and carcinogens, from the body. The most common pathways of xenobiotic metabolism are oxidation, reduction, and conjugation. Oxidation and reduction are commonly referred to as phase I metabolism, with cytochrome P450 (CYP) 1, 2, and 3 families encoding the phase I metabolizing enzymes involved in the majority of drug biotransformation. Conjugation is considered phase II metabolism, which is mediated by multiple enzyme superfamilies, including, but not limited to, UDP-glucuronosyltransferases, glutathione *S*-transferases (GST), sulfotransferases, and *N*-acetyltransferases (NAT). Phase I metabolism typically activates procarcinogens to carcinogenic electrophilic intermediates, and phase II metabolism is mostly responsible for detoxification of such products. As a consequence, the balance between the bioactivation and detoxification reactions plays a central role in an individual's susceptibility to chemical carcinogens.

The family of GSTs represents a major group of phase II detoxification enzymes. GSTs catalyze the reaction of glutathione with electrophiles, resulting in the elimination of potentially carcinogenic chemicals (1). GSTs also exhibit glutathione peroxidase activity and catalyze the reduction of organic hydroperoxides to their corresponding alcohols (1). Results from both animal models and clinical investigations continue to suggest that this family of enzymes plays a pivotal role in cancer susceptibility and cancer prevention. Individuals deficient in the protection afforded by this family of enzymes are at increased risk of cancer (2-4). Induction of these enzymes by either naturally occurring or synthetic agents represents a promising chemopreventive strategy (5-8). Green tea or green tea polyphenols have been shown to modulate various xenobiotic metabolizing enzymes, including GSTs, in animal studies and in *in vitro* systems (9-14). Phase II enzyme induction by green tea polyphenols is generally believed to involve the activation of the mitogen-activated protein kinase pathway via the electrophilic-mediated stress response, resulting in the activation of transcription factors that bind to antioxidant/electrophile response element located on many phase II genes (15). We conducted a clinical study to determine whether repeated green tea polyphenol administration would affect the phase I and II enzymes in humans. Repeated green tea polyphenol

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administration was found to have minimal effects on human CYP enzyme activities (16). This report summarizes the findings on human GSTs.

Materials and Methods

Study Drugs. Polyphenon E is a green tea catechin-enriched and defined product produced by Mitsui Norin, Ltd. It contains 80% to 98% total catechins by weight with epigallocatechin gallate (EGCG) as the main component accounting for 50% to 75% of the material. Other catechins, including epicatechin, epigallocatechin, epicatechin gallate, and gallocatechin gallate, are present in levels ranging from ~2% to 12% each. Polyphenon E contains small quantities of caffeine (~0.5% w/w) and can be considered a decaffeinated product. This study used Polyphenon E oral capsules standardized to contain 200 mg EGCG per capsule, supplied by the Chemoprevention Agent Development Research Group, National Cancer Institute. The study capsules were stored at room temperature and protected from environmental extremes.

Study Participants. Forty-two nonsmoking healthy men and women ≥18 years of age participated in the study. The participants had normal liver and renal function. Participants were excluded if they were pregnant or breast feeding, had invasive cancers within the past 5 years, had uncontrolled severe metabolic disorders or other serious acute or chronic diseases, consumed more than three drinks of alcohol per week on average, consumed tea regularly, had known hypersensitivity to green tea or CYP metabolic probe drugs (caffeine, dextromethorphan, losartan, or buspirone), were taking medications/supplements that are known P450 enzyme inducers or inhibitors, or had participated in other clinical research studies within the past 3 months. The study was approved by the University of Arizona Human Subjects Committee. Written informed consent was obtained from all participants.

Study Design. During the initial clinical visit, study participants completed a medical history form and underwent a brief physical examination. A fasting blood sample was collected and subjected to a complete blood count with differential leukocyte count and a comprehensive blood chemistry analysis. Eligible subjects underwent a 4-week washout period in which they were required to refrain from tea or its related products and herbal/botanical supplements and to minimize the consumption of cruciferous vegetables. At the end of the washout period, a fasting blood sample was collected into heparinized vacutainer tubes. Plasma was separated, and lymphocytes were isolated using the Ficoll-Hypaque medium (Amersham Biosciences) for assessment of GST activities and levels. Following the fasting blood sample collection, study subjects underwent baseline CYP enzyme activity assessment. Clinical procedures for CYP enzyme activity assessment are detailed elsewhere (16).

Following the completion of the washout period and baseline enzyme activity determination, study participants were provided with a 4-week supply of Polyphenon E. They continued to complete a daily diary of any adverse reactions and an intake calendar for recording the daily intake of the study agent and any nonroutine medications. Study participants were instructed to take four Polyphenon E capsules everyday in the morning on an empty stomach. Breakfast could be consumed 1 h after Polyphenon E dosing. Participants continued to refrain from tea or its related products and herbal/botanical supplements and to minimize the consumption of cruciferous vegetables.

A fasting blood sample was collected the day after completing the 4-week Polyphenon E treatment period. Plasma and lymphocytes were separated for measurement of postintervention GST activities and levels. A fasting blood sample was also collected for a complete blood count and comprehensive blood chemistry. Following the fasting blood collection, study participants underwent postintervention CYP enzyme activity assessment.

Measurements of GST Activities and Levels. Cell lysates were prepared by sonicating the lymphocyte pellets twice at 15-s intervals. The lysed cells were centrifuged at 10,000 × *g* at 4°C for 30 min. The supernatant was collected and stored at −70°C before the analysis. Total GST activity in lymphocyte lysates was determined using a GST assay kit (Cayman Chemical) by measuring the conjugation of 1-chloro-2,4-dinitrobenzene with reduced glutathione. This GST-mediated conjugation reaction is accompanied by an increase in absorbance at 340 nm. GST activity was expressed as nanomoles per minute per milligram of protein, with protein concentrations assayed by the Bio-Rad protein assay kit.

GST-π concentrations in lymphocyte lysates were measured using an enzyme-linked immunoassay kit (Human pi GST EIA assay, Biotrin International). The assay procedure is based on sequential addition of diluted samples or standards, anti–GST-π immunoglobulin G (IgG) conjugated with horseradish peroxidase, and substrate to microassay wells coated with anti–GST-π IgG. The assay range is 3.12 to 100 ng/mL. Standards of known concentrations were included in every run, and the enzyme levels were calculated from a standard curve. GST-π concentrations were normalized to protein concentrations and expressed as nanograms per milligram of protein.

GST-μ levels in lymphocyte lysates were determined semiquantitatively using Western blotting. Briefly, proteins (20 μg total protein per sample) were separated by gel electrophoresis and transferred onto polyvinylidene difluoride membranes (NEN Life Science Products) as previously described (17). For immunodetection, membranes were incubated and washed thrice with 5% bovine serum albumin fraction V in PBS with 0.5% Tween 20 before probing with 1:500 dilution of anti-GST Yb1 (GST-μ) antibodies (Biotrin). These antibodies react with GST-M1 and GST-M2 subunits. Proteins were detected by incubating with a 1:2,000 dilution of horseradish peroxidase–linked anti-rabbit immunoglobulin antibody (GE Healthcare) and visualized by chemiluminescence (Renaissance Western Blot Chemiluminescence Reagent Plus; NEN Life Science Products). Gel bands were quantified using ID gel analysis software (Kodak) and a ScanJet 4c scanner (Hewlett-Packard).

Plasma GST-α concentrations were measured using an enzyme-linked immunoassay kit (High-sensitivity alpha GST EIA assay, Biotrin International). The assay procedure is based on sequential addition of diluted samples or standards, anti–GST-α IgG conjugated with horseradish peroxidase, and substrate to microassay wells coated with anti–GST-α IgG. The assay range is 0.0625 to 2 ng/mL. Standards of known concentrations were included in every run, and the enzyme levels were calculated from a standard curve and expressed as nanograms per milliliter of plasma.

Data Analysis. The effects of tea polyphenol intervention on the enzyme activity/level were analyzed using a conditional change model, in which baseline activity/level was included as a covariate. The model is written as

$$y_1 - y_0 = \beta_0 + \beta_1 y_0 + e$$

where y_0 and y_1 are baseline and posttreatment values for the outcome, and e is the error term. The primary effect measure was assessed after centering the data by testing the statistical significance of β_0. The data were further divided into tertiles based on baseline activity/level. The significance level of the

Table 1. GST activity in blood lymphocytes before and after 4 wks of daily Polyphenon E administration

	GST activity (nmol/min/mg protein)*		*P*
	Baseline	Posttreatment	
All participants (N = 42)	30.7 ± 12.2	35.1 ± 14.3	0.058
Baseline activity in the lowest tertile (n = 14)	19.6 ± 3.7	35.6 ± 16.2	0.004
Baseline activity in the middle tertile (n = 14)	29.2 ± 3.0	34.8 ± 15.1	0.160
Baseline activity in the highest tertile (n = 14)	43.5 ± 11.8	34.8 ± 12.3	0.020

*Mean ± 1 SD.

intervention effect within each tertile was also determined by a conditional change model, this time centering the data within tertile. A $P < 0.05$ was considered statistically significant.

Results

Table 1 summarizes the GST activity determined in peripheral blood lymphocytes before and after 4 weeks of daily Polyphenon E administration. Polyphenon E intervention was found to increase GST activity from 30.7 ± 12.2 to 35.1 ± 14.3 nmol/min/mg protein (borderline significant, $P = 0.058$), and the effect was significantly dependent on baseline GST activity ($P < 0.001$). The tertile analysis showed that individuals with baseline activity in the lowest tertile exhibited a more than 80% increase in mean GST activity ($P = 0.004$). Individuals with baseline activity in the highest tertile showed a small (20%) but statistically significant decrease in mean GST activity ($P = 0.02$).

Because GST-π is the major GST isozyme present in blood lymphocytes (18), we have determined whether Polyphenon E intervention affected GST-π enzyme levels in blood lymphocytes, and the data are summarized in Table 2. Polyphenon E intervention was found to significantly increase GST-π enzyme levels in blood lymphocytes from 2,252.9 ± 734.2 to 2,634.4 ± 1,138.3 ng/mg protein ($P = 0.035$), and the effect was dependent on the baseline GST-π enzyme level ($P = 0.005$). The tertile analysis showed that the increase in GST-π enzyme level was only significant in individuals with baseline level in the lowest tertile, with a mean increase of ~80% ($P = 0.003$). Figure 1 illustrates the relationship between posttreatment changes in GST activity and GST-π enzyme levels in blood lymphocytes. Posttreatment changes in GST activity correlated significantly with changes in GST-π enzyme level ($r^2 = 0.35$, $P < 0.0001$).

Lymphocyte GST-μ protein levels in 41 of the 42 participants (insufficient sample in one participant) were determined semiquantitatively using Western blotting. The antibodies

Table 2. GST-π enzyme level in blood lymphocytes before and after 4 wks of daily Polyphenon E administration

	GST-π level (ng/mg protein)*		*P*
	Baseline	Posttreatment	
All participants (N = 42)	2,252.9 ± 734.2	2,634.4 ± 1,138.3	0.035
Baseline level in the lowest tertile (n = 14)	1,505.7 ± 434.9	2,754.5 ± 1,456.8	0.003
Baseline level in the middle tertile (n = 14)	2,254.1 ± 144.3	2,561.8 ± 762.3	0.160
Baseline level in the highest tertile (n = 14)	2,998.8 ± 538.2	2,587.0 ± 1,166.1	0.174

*Mean ± 1 SD.

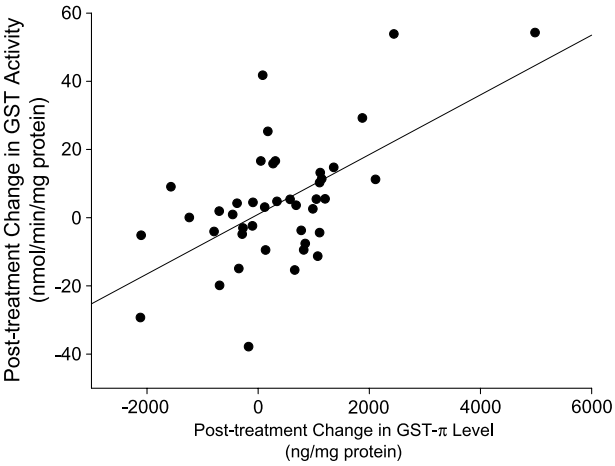

Figure 1. Relationship between posttreatment changes in GST activity and in GST-π enzyme level in blood lymphocytes. A regression fit of the data is shown as the solid line ($r^2 = 0.35$, $P < 0.0001$).

used in the assay react with GST-M1 and GST-M2 subunits. Expression of GST-μ was not detectable in 23 of the 41 participants (56%). Polyphenon E intervention did not result in a significant change in lymphocyte GST-μ levels in those who express detectable levels of the protein; posttreatment levels were 113 ± 31% of baseline levels ($n = 18$; $P = 0.127$).

Table 3 summarizes plasma GST-α levels obtained before and after Polyphenon E intervention. The average baseline plasma GST-α concentration was 7.46 ng/mL, with values ranging from 2.51 to 40.13 ng/mL. Following 4 weeks of daily Polyphenon E administration, the average plasma GST-α concentration was not different from those determined at baseline ($P = 0.770$). The tertile analysis showed that individuals with baseline levels in the highest tertile had a small (8%) but statistically significant ($P = 0.003$) decrease after the Polyphenon E intervention.

Baseline and posttreatment changes in blood lymphocyte GST activity and GST-π levels were compared between those who express GST-μ and those who do not express this protein (Table 4). There were no statistically significant differences in baseline in blood lymphocyte GST activity and GST-π levels between GST-μ expressors and nonexpressors. Posttreatment changes in blood lymphocyte GST activity and GST-π levels were not statistically significantly different between GST-μ expressors and nonexpressors, although a trend toward a more pronounced posttreatment increase in GST-π levels was observed in GST-μ expressors.

Discussion

This study showed that 4 weeks of Polyphenon E intervention resulted in differential effects on human GST activity and levels. Analysis based on baseline activity/level showed that GST activity and GST-π levels in peripheral blood lymphocytes increased significantly in individuals with low baseline activity or level (~80% increase in both measurements). Individuals with baseline activity or levels in the highest tertile exhibited a small but statistically significant decline in lymphocyte GST activity (~20%) and plasma GST-α concentrations (~8%) following 4 weeks of Polyphenon E intervention. The decline in the highest tertile is likely due to regression to the mean rather than as a result of biological activity because unusually high values at baseline (these values would fall in the highest tertile) are more likely to be lower in repeated measures due to random natural fluctuations, absent any intervention effect (19, 20). Likewise, values that are unusually

low at baseline (these would fall in the lowest tertile) are likely to increase in repeated measures due to random fluctuations. It is plausible that the increase in lymphocyte GST activity and GST-π seen in individuals with low baseline activity/level could be due, in part, to regression to the mean, but we believe that this increase was mostly attributed to the intervention effect because the overall effect reveals an increase.

The most abundant cytosolic GST enzymes in humans are encoded by three distinctly related classes; α, μ, and π (1). These isozymes are differentially expressed in hepatic and extrahepatic tissues. GST-π is the major GST isozyme present in human blood lymphocytes (18) and is also the major GST isozyme in human colon and rectum (21), which may explain the correlation observed between total GST activities measured in blood lymphocytes and those determined in colon mucosa (22, 23). GST-π expression has been associated with preneoplastic and neoplastic changes. Human prostate cancer is characterized by an early and near-universal loss GST-π expression due to hypermethylation of a CpG island (24). Restoration of GST-π expression with inhibitors of CpG methylation or GST inducers has been suggested as a rational prostate cancer prevention approach (7). We showed that green tea catechin intervention significantly increased GST-π protein levels in blood lymphocytes in individuals with low baseline levels. Further studies are needed to determine the human tissue GST-π expression after green tea catechin intervention. Expression of GST-μ is also evident in human blood lymphocytes (25). We have measured GST-μ levels in lymphocyte lysates semiquantitatively. A total of 56% of the study participants do not express detectable levels of this protein. This proportion is similar to that observed in the general population (26, 27). Polyphenon E did not result in a significant change in GST-μ levels in individuals who express this protein ($n = 18$). However, the sample size may be limited for such an evaluation. GST-α is the major GST isozyme found in human liver (28). Plasma GST-α has been shown to reflect induction of hepatic GST-α levels in mice when fed with phase II enzyme inducers (29) and has been used to assess the effect of intervention with vegetable diets in the clinical setting (6, 30, 31). We showed that repeated green tea catechin administration had minimal effects on plasma GST-α levels. This tends to suggest that human GST isozymes may be differentially induced by green tea catechins, which may lead to tissue-specific induction of human GST activity.

We have shown that induction of GST activity and GST-π levels with repeated tea polyphenol intervention is affected by baseline enzyme activity and GST-π levels, with significant effects observed in individuals with low baseline activity or GST-π levels. Changes in lymphocyte GST activity and GST-π levels are not affected by lymphocyte GST-μ expression. Recent studies have shown that modulation of GSTs with dietary constituents can be affected by genotypes or phenotypes of these enzymes. Lampe et al. (6) illustrated that botanically defined vegetable diets can modulate GST isozyme level or activity, and the modulation effects are differentially affected by *GST-M1* genotype. The brassica vegetable diets resulted in a significant increase in serum GST-α levels in the *GST-M1* null individuals. The induction of lymphocyte GST-μ activity by both brassica and allium vegetable diets were only observed in *GST-M1+* women. Pool-Zobel (18) showed that induction of lymphocyte GST-π levels by vegetable consumption is more pronounced in individuals with low baseline GST-π levels or in *GST-M1+* individuals. It is plausible that there may be a threshold in phase II enzyme expression; thus, induction of these detoxification enzymes is more pronounced in individuals with low baseline activity or level. It is also possible that specific phase II enzyme genotypes/phenotypes may differentiate individuals with varied systemic green tea catechin exposure, which may lead to different degrees of enzyme induction. The observations of phenotype/genotype-dependent induction of phase II enzymes suggest the potential of selecting individuals with low baseline enzyme activity for cancer prevention trials of naturally occurring or synthetic agents that target the phase II enzymes.

In this study, Polyphenon E intervention at a daily bolus dose of 800 mg EGCG was taken on an empty stomach. This dosing condition was implemented because taking Polyphenon E on an empty stomach has been shown to significantly enhance the oral bioavailability of EGCG (32). The intervention was generally well tolerated, with most adverse events reported as mild and transient events. The most common adverse events were gastrointestinal in nature, with 19 subjects reported at least one episode of nausea during the 4 weeks of intervention (15). Monitoring serum liver enzymes showed no signs of liver toxicity. Because the magnitude of enzyme induction may be dependent on the exposure level and the duration of exposure, it is not known whether a different dosing regimen or dosing condition would result in the same magnitude of induction with 4 weeks of exposure period.

We conclude that 4 weeks of Polyphenon E administration resulted in differential effects on GST activity and level based on baseline enzyme activity or level, with GST activity and GST-π level increased significantly in individuals with low baseline enzyme activity/level. This suggests that green tea polyphenol intervention may enhance the detoxification of carcinogens in individuals with low baseline detoxification capacity.

Table 3. Plasma GST-α concentration before and after 4 wks of daily Polyphenon E administration

	GST-α concentration (ng/mL)*		*P*
	Baseline	Posttreatment	
All participants ($N = 42$)	7.46 ± 6.54	7.59 ± 6.91	0.770
Baseline level in the lowest tertile ($n = 14$)	3.32 ± 0.54	5.17 ± 4.19	0.136
Baseline level in the middle tertile ($n = 14$)	5.52 ± 0.66	5.09 ± 1.65	0.334
Baseline level in the highest tertile ($n = 14$)	13.5 ± 8.47	12.5 ± 9.53	0.003

*Mean ± 1 SD.

Table 4. Baseline and posttreatment changes in blood lymphocyte GST activity and GST-π levels based on GST-μ expression

	GST-μ expression*		*P*
	Nonexpressors ($n = 23$)	Expressor ($n = 18$)	
Baseline lymphocyte GST activity (nmol/min/mg protein)	29.4 ± 9.6	32.2 ± 15.3	0.470
% Posttreatment increase in GST activity	29.2 ± 73.3	36.8 ± 78.3	0.753
Baseline lymphocyte GST-π levels (ng/mg protein)	2,337.8 ± 689.8	2,125.1 ± 806.3	0.368
% Posttreatment increase in GST-π levels	19.1 ± 56.6	46.4 ± 67.0	0.166

*Mean ± 1 SD.

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