Research Article

Cancer Prevention Research

A Phase II Randomized, Double-blind, Presurgical Trial of Polyphenon E in Bladder Cancer Patients to Evaluate Pharmacodynamics and Bladder Tissue Biomarkers

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Abstract

We performed a phase II pharmacodynamic prevention trial of Polyphenon E [a green tea polyphenol formulation primarily consisting of epigallocatechin gallate (EGCG)] in patients prior to bladder cancer surgery. Patients with a bladder tumor were randomized to receive Polyphenon E containing either 800 or 1,200 mg of EGCG or placebo for 14 to 28 days prior to transurethral resection of bladder tumor or cystectomy. The primary objective was to compare the postintervention EGCG tissue levels in patients receiving Polyphenon E as compared with placebo. Secondary objectives included assessments of tissue expression of PCNA, MMP2, clusterin, VEGF, p27, IGF-1, IGFBP-3; correlation of tissue, plasma, and urine levels of EGCG; and EGCG metabolism by catechol-O-methyltransferase and UDP-glucuronosyltransferase pharmacogenomic mutations. Thirty-one patients (male:female, 26:5; mean age, 67.2 years) were randomized and 29 (94%) completed the study. There was not an observed significant difference ($P = 0.12$) in EGCG tissue levels between two Polyphenon E dosage groups combined versus placebo. However, a dose–response relationship for EGCG levels was observed in both normal ($P = 0.046$) and malignant bladder tissue ($P = 0.005$) across the three study arms. In addition, EGCG levels in plasma ($P < 0.001$) and urine ($P < 0.001$) increased and PCNA ($P = 0.016$) and clusterin ($P = 0.008$) were downregulated in a dose-dependent fashion. No pharmacogenomic relationship was observed. EGCG levels in plasma, urine, and bladder tissue followed a dose–response relationship, as did modulation of tissue biomarkers of proliferation and apoptosis. Despite the limitations of this pilot study, the observed pharmacodynamics and desirable biologic activity warrant further clinical studies of this agent in bladder cancer prevention. *Cancer Prev Res; 10(5); 298–307. ©2017 AACR.*

Introduction

Bladder cancer is the second most common malignancy of the genitourinary tract. During the year 2016 alone, approximately 76,960 new cases will be diagnosed with an estimated 16,390 deaths occurring in the United States as a result of this disease (1). Muscle-invasive bladder cancer, a major contributor to morbidity and mortality, is initially diagnosed in as many as 30% of patients. These patients generally undergo radical cystectomy, and often neoadjuvant or adjuvant chemotherapy, for locally advanced disease. However, the remaining 70% of patients have noninvasive papillary bladder tumors (Ta), carcinoma *in situ* (CIS/Tis), and tumors that invade through the lamina propria (T1). These tumors are often multifocal and recurrent, and may appear anywhere in the bladder over relatively long intervals, thereby requiring long-term surveillance (2, 3). Repeated examination entails regular cystoscopic evaluation and monitoring of urine cytology and/or other diagnostic markers. As such, this closely monitored group of patients at risk for tumor recurrence and progression represents an ideal cohort for the evaluation of chemopreventive agents and an important patient population that could benefit from effective secondary chemoprevention.

Green tea consumption has been associated with a reduction in bladder cancer risk. Green tea has a higher content of catechins than black tea, and its effectiveness against cancer has been attributed to the presence of these polyphenolic antioxidants, and particularly epigallocatechin gallate (EGCG; refs. 4, 5). Early mechanistic investigations into the biological activities of green tea focused on the ability of catechins to regulate antioxidant and free radical scavenging activity, to prevent mutagenicity and genotoxicity, to regulate phase I and II enzymes (modulation of carcinogen activation and detoxification), and to inhibit markers of tumor initiation and promotion (6). More recently, research has focused on the ability of tea catechins to target multiple signaling pathways involved in carcinogenesis, angiogenesis, metastasis, and migration (7).

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In terms of preclinical *in vivo* studies specifically related to bladder cancer, Kemberling and colleagues (8) employed an orthotopic bladder cancer model in which intravesical instillation of EGCG 200 μmol/L inhibited tumorigenesis. Furthermore, Sato described the efficacy of green tea in reducing bladder tumor growth induced by N-butyl-N-(4-hydroxybutyl)-nitrosamine in rats (9). Corresponding *in vitro* studies have evaluated the effects of EGCG in bladder cancer cell lines in which dose-dependent growth inhibition has been observed (8, 10). In prostate cancer, other potential markers of EGCG activity have been studied. Work by the Mukhtar laboratory established that green tea polyphenols inhibit prostate tumorigenesis with a marked reduction in proliferating cell nuclear antigen (PCNA; ref. 11). Caporali and colleagues found that inhibition of prostate tumorigenesis by green tea catechins in TRAMP mice was associated with accumulation of the glycoprotein clusterin (12). Furthermore, inhibition of growth with induction of apoptosis was observed in human bladder cancer cells treated *in vitro* and *in vivo* with an antisense clusterin oligodeoxynucleotide (13). Clusterin has therefore been found both to inhibit apoptosis and to inhibit proliferation in bladder cancer as well as prostate cancer and was determined to be an indicator of chemopreventive activity (12, 13). In addition, significant inhibition of serum IGF-1 levels and a corresponding increase in serum insulin-like growth factor binding protein-3, a major IGF-1 binding protein, which suppresses the mitogenic action of IGF-1, has been described in association with green tea polyphenol–induced inhibition of prostate carcinogenesis in TRAMP mice (11).

Polyphenon E (Mitsui Norin Co., Ltd.) is derived from a hot water extract of green tea leaves (*Camellia sinensis* species of the Theaceae family) containing 85%–95% total catechins; the main component is EGCG, which comprises 56%–72% of the material. NCI, DCP has sponsored four phase I pharmacokinetics and safety studies with Polyphenon E (14–17). These studies have established no difference in EGCG pharmacokinetics (C_{max}, AUC, $t_{1/2}$) between Polyphenon E and EGCG (14) as well as the lack of significant accumulation in healthy human subjects with 4-week administration of 800 mg EGCG (15). Subsequently, Chow and colleagues determined the effect of fasting on pharmacokinetics in healthy adults taking 400, 800, or 1,200 mg EGCG as Polyphenon E (16). Plasma levels of free EGCG were dramatically higher when taking Polyphenon E as a single daily dose, and also with fasting as compared with the fed state for all three dose levels. The most common adverse events were mild gastrointestinal complaints, which were more common with increasing dose and under fasting conditions. Some subjects also experienced headaches and fatigue, possibly related to study products, but not clearly associated with dose level. More recently, the safety and effects of repeated administration of Polyphenon E (800 mg EGCG every day for four weeks) on cytochrome P450 and glutathione-S-transferase (GST) activities were studied (17). Chronic Polyphenon E administration significantly increased GST enzyme activity and GSTπ enzyme levels in individuals with low baseline values, whereas a minimal effect on four major cytochrome P450 (CYP) isozymes was observed. On the other hand, alterations in EGCG metabolism could potentially affect EGCG efficacy. UDP-glucuronosyltransferases (UGT) are membrane bound, microsomal phase II enzymes localized in the endoplasmic reticulum of liver and extrahepatic tissues which participate in EGCG metabolism. EGCG is extensively glucuronidated by UGT 1A1 to an active metabolite (-)-EGCG-4"-O-glucuronide. Given the primary role of glucuronidation in preparing endogenous and exogenous compounds for elimination via the biliary and urinary tracts, this could potentially significantly affect the half-life of EGCG. In addition, catechol-containing tea polyphenols are very rapidly *O*-methylated by human catechol-O-methyltransferase (COMT). These genes are polymorphic, and therefore, individuals with variant UGT or COMT enzymes may have altered metabolism, efficacy, and toxicity (18, 19).

On the basis of these early phase I/II clinical studies of EGCG, in addition to promising preclinical studies of EGCG supporting its role as a potentially safe and effective cancer preventive agent in bladder cancer, we evaluated the tolerability, tissue accumulation, and biologic effects of Polyphenon E in a randomized, double-blind, placebo-controlled phase II presurgical trial in patients with bladder cancer.

Materials and Methods

Study objectives

The primary objective of the study was to assess the nonmalignant bladder tissue levels of EGCG. Secondary objectives included comparison of EGCG levels in nonmalignant versus malignant tissue; examination of the dose-dependent modulation of intermediate endpoint biomarkers (PCNA, MMP2, clusterin, VEGF, p27, IGF-1, IGFBP-3) in malignant and nonmalignant bladder tissue; correlation of tissue, plasma and urine levels of EGCG; examination of the levels of other catechins (epicatechin, epicatechin gallate, and epigallocatechin) in tissue, plasma, and urine; metabolism of EGCG in tissue, plasma, and urine by COMT and UGT in relation to pharmacogenomic mutations.

Study enrollment

This was a multicentered, randomized, double-blind, placebo-controlled study with a planned enrollment of 33 participants, to be randomized on a 1:1:1 basis to Polyphenon E in doses of 800 mg EGCG, 1,200 mg EGCG, or placebo orally once daily for 14–28 days prior to transurethral resection of bladder tumor (TURBT) or cystectomy. Patients meeting study inclusion criteria were males and females 18 years of age and older with an office cystoscopy diagnostic of a bladder tumor. Enrollment must have occurred within 60 days of the prestudy cystoscopy. Patients could have no evidence of distant metastasis. Patients were also candidates for either subsequent TURBT or complete or partial cystectomy and the tumor could represent either an initial primary tumor or recurrent disease of any clinical stage. Histologic diagnosis was not required for enrollment. Pre-enrollment diagnostic cystoscopy had to occur at least 90 days after treatment of the bladder with other agents such as BCG.

Patients were excluded from this study if they had any treatment for superficial or invasive bladder cancer between the diagnostic cystoscopy and curative surgery. Furthermore, patients with any prior intravesical therapy or adjuvant chemotherapy within 30 days before baseline procedures or any prior surgery to the bladder within 30 days of baseline procedures were excluded. (Biopsies were not considered surgeries.) Evidence of other cancers (excluding nonmelanoma skin cancer) or metastatic disease, prior pelvic radiation for any other reason, or concurrent systemic chemotherapy for any other cancer, excluding nonmelanoma skin cancer were also exclusion criteria. Furthermore, participants could not take NSAIDs, with the exception of $\leq$81 mg aspirin per day, during study participation, whereas acetaminophen was

permitted for pain relief. Consumption of any green tea supplements or more than 2 cups (16 oz) of green tea in the 24 hours prior to baseline procedures was not permitted. Patients receiving any other investigational agents, or with any history of allergic reactions attributed to compounds of similar chemical or biologic composition to Polyphenon E or any of the inactive ingredients in Polyphenon E capsules were also excluded from the study. Uncontrolled intercurrent illness including, but not limited to, ongoing or active infection, symptomatic congestive heart failure, unstable angina pectoris, cardiac arrhythmia, or psychiatric illness/social situations that would limit compliance with study requirements were also contraindications to study enrollment.

Study conduct

Study visits included a baseline and presurgery visit as well as weekly phone contact. At the baseline visit, eligibility and safety labs were obtained, as well as urine pharmacokinetics, plasma pharmacokinetics, serum biomarkers, and pharmacogenetics markers. Patients were then randomized to one of the three study groups (Polyphenon E in doses of 0 mg in placebo, 800 mg EGCG, or 1,200 mg EGCG). Patients were given instructions to take the study drug with food, and to refrain from consuming additional green tea, either from dietary sources or through nutritional supplementation for the duration of the study. At the time of surgery, fresh tissue was harvested. In addition, paraffin-embedded blocks were requested after processing, as well as serum specimens for safety labs, urine pharmacokinetics, plasma pharmacokinetics, and serum biomarkers.

Study agent

Polyphenon E (Mitsui Norin Co., Ltd.) is a botanical drug substance containing a mixture of catechins originating from the leaves of green tea (*Camellia sinensis*). To manufacture Polyphenon E, a hot water extract of green tea is extracted further with ethyl acetate. The resulting crude extract is dissolved in methanol and purified by affinity column fractionation. Once dried, the final product contains 85%–95% total catechins; the main component is EGCG, which comprises 56%–72% of the material. Other catechins present in Polyphenon E include epicatechin (EC), epicatechin gallate (ECG), epigallocatechin (EGC), gallocatechin gallate (GCG), gallocatechin (GC), catechin gallate (CG), and catechin. Polyphenon E may also contain small quantities of caffeine (<1.0%), theobromine (<1.0%), and gallic acid (<0.5%).

The investigational product is a dark green, opaque, size 0 hard gelatin capsule containing enough Polyphenon E to deliver 200 mg EGCG per capsule. Polyphenon E capsules are manufactured, stored, distributed, and evaluated for stability under contract to NCI, DCP using current good manufacturing procedures (cGMP) as outlined in the United States Code of Federal Regulations. A placebo capsule containing 0 mg EGCG is also utilized in our study.

Tissue harvesting

Patients undergoing a cystectomy. From the excised bladder tissue, approximately 0.5 cm^2 each of normal and neoplastic urothelium was harvested. Detailed freezing instructions were included in the patient research kit. Once the specimens were frozen, the NUNC tubes were labeled (ID, time, and date of collection) and the specimens were kept in a −80°C freezer until shipping.

Patients undergoing a TURBT. Once enough of the bladder tumor was excised for pathologic analysis, two pieces of tumor and normal urothelium, approximately 0.5 cm^2 each, were harvested for research purposes. Once the specimens were frozen, the NUNC tubes were labeled (ID, time, and date of collection) and specimens were kept in a −80°C freezer until shipping.

EGCG levels and biomarker assays

HPLC assay for EGCG. Polyphenon E is a mixture of catechins originating from the leaves of green tea, the main component is EGCG, which comprises 56%–72% of the material. Each capsule delivers 200 mg of EGCG. Other catechins present in Polyphenon E include epicatechin (EC), epicatechin gallate (ECG), epigallocatechin (EGC), gallocatechin gallate (GCG), gallocatechin (GC), catechin gallate (CG), and catechin. Polyphenon E may also contain small quantities of caffeine (<1.0%), theobromine (<1.0%), and gallic acid (<0.5%).

Urine and plasma samples were obtained at baseline and at the end of the study during the presurgical visit. Tissue samples (both tumor and normal) were obtained at cystectomy or TURBT, depending on the patient's procedure. Plasma, urine, and EGCG, ECG, EGC, and EC were analyzed by validated liquid chromatography-mass spectrometry methods (14, 15). The lower level of quantitation is 3.12 ng/mL, and the lowest level of detection (signal-to-noise ratio, 3:1) was 0.78 ng/mL. Values lower than the level of quantitation are reported, and have CVs in excess of 15%, typically 20%–25%.

LC-MS protocol. Two-hundred microliters of patient plasma or urine was transferred into a glass tube containing 10 μL internal standard (IS) and 10 μL 20% vitamin C solution. Ethyl gallate (Sigma-Aldrich) was used as internal standard of which a stock solution was prepared in MeOH containing 100 μg/mL ethyl gallate. The resultant mixture of plasma or urine with IS and vitamin C solution was extracted with 3 mL of methyl *tert*-butyl ether (MTBE) by vortex-mixing for 10 minutes and centrifuging at 2,500 rpm for 10 minutes. The upper organic phase was transferred into another glass tube and evaporated to dryness under a gentle N_2 stream at room temperature. The residue was reconstituted in 100 μL of a 10% MeOH aqueous solution with 0.1% formic acid in an orbital shaker for 10 minutes. Five microliters of the supernatant was injected onto the mass spectrometer for analysis.

LC analysis was performed by using an Agilent 1100 HPLC (Agilent Technologies), equipped with a quaternary pump and a refrigerated autosampler. For chromatographic separation, a Kinetex PFP column (2.6 μm, 50 × 2.10 mm) (Phenomenex) was used. A gradient mobile phase was used, composed of 0.1% formic acid and MeOH with 0.1% formic acid at a constant flow rate of 500 μL/minute.

A triple quadruple mass spectrometer, Agilent 1100 (Agilent Technologies) equipped with a TurboIonSpray source, was used to obtain LC-MS data. TurboIonSpray source settings were as follows: ion spray voltage (IS) -5,000 V, nebulizer gas (GS1) 40, heater (TEM) 600°C, heater gas (GS2) 40, curtain gas (CUR) 20. Collision gas (CAD) was set on 6.

IHC staining. Tissue biomarkers assayed by immunostaining included PCNA, MMP2, Clusterin, VEGF, and p27. For the immunostains, paraffin sections were cut from the blocks, approximately 4-μm thick each, and deparaffinized. After antigen

retrieval, the slides were loaded onto automated IHC stainers (Lab Vision Autostainer 360 XT and Benchmark, Ventana Medical Systems, Inc.). Endogenous peroxidase quenching was done on-instrument with kit reagents (Biocare Medical) and the slides were stained at a constant incubation temperature of 42°C according to predetermined protocols: The slides were incubated with the first antibody of optimal dilution for 32 minutes at 42°C. After the washing step, the slides were incubated with a Mach 3 species-specific probe and Mach 3 HRP polymer detecting system (Biocare Medical) incorporating betazoid diaminobenzidine (DAB). Then, the immunostained slides were counterstained with hematoxylin on the instrument. Upon completion of the staining protocols, the slides were removed from the instrument and dehydrated through a series of graded alcohols, cleared in xylene, and coverslipped manually on a Tissue-Tek automated coverslipper (Sakura).

IHC scoring. From stained slides, the IHC score was reported for normal and malignant tissue separately. The slides were scanned and the biomarker expression levels were quantitated by a Vectra system (Perkin Elmer). This quantitative system provides a continuous score of mean optical density (OD) per pixel area of IHC chromogenic response. This system greatly reduces inter- and intra-observer variation and provides accurate and reproducible measurement (20).

ELISA assay for IGF-1 and IGFBP3. IGF-1 and IGFBP3 were evaluated by a commercially available sandwich immunoassay (Quantikine human, R&D Systems).

Genotyping by pyrosequencing for UGT and COMT. To compare the metabolism of EGCG by COMT and UGT1A1 in relation to pharmacogenetic polymorphisms in COMT and UGT1A1 genotyping by pyrosequencing (21) was performed as described previously.

Statistical considerations

Sample size. The sample size for each group was based on comparing the EGCG levels between the placebo group (0 mg) and the combined group of those on 800 mg and 1,200 mg of Polyphenon E. The sample size was estimated to detect the effect size (defined as the difference in the means divided by the SD) in the range of 1.12–1.30 with power 0.8–0.9 given equal sample size for each group, according to the two-sided level $\alpha = 0.05$ Student t test. To account for the anticipated dilution of treatment effect due to up to 10% of the subjects being noncompliant with treatment, the sample size was inflated to 33 subjects in total, or 11 subjects on each treatment arm.

Statistical analysis

The primary analysis was a comparison of nonmalignant bladder tissue levels of EGCG between the placebo group and the Polyphenon E groups combined using Student t test. In the case of violation of normality assumptions, an appropriate transformation of the data, such as logarithm, was considered or a nonparametric test such as Wilcoxon rank-sum test was used for comparison. A dose–response relationship between nonmalignant bladder tissue levels of EGCG and the dose (0, 800, and 1,200 mg) was tested using the Jonckheere–Terpstra test. For secondary endpoints and safety data, the following analyses were used. To test for dose–response relationships for continuous data, such as levels of catechins and other intermediate endpoint biomarkers, the Jonckheere–Terpstra test was performed. To test for dose–response relationships for dichotomous data, such as the percentage of patients that had occurrence of individual adverse events, the Cochran–Armitage test was performed. To test for correlations between tissue, plasma, and urine levels of EGCG, we used Spearman rank correlation coefficients. Wilcoxon signed rank tests were used for within treatment comparison between baseline and presurgery.

Table 1. Patient characteristics

Characteristics	Placebo ($n = 11$)	800 mg EGCG ($n = 10$)	1,200 mg EGCG ($n = 10$)
Sex			
Female	2 (18)	1 (10)	2 (20)
Male	9 (82)	9 (90)	8 (80)
Race			
White	11 (100)	10 (100)	9 (90)
Black	0 (0)	0 (0)	1 (10)
Ethnicity			
Not Hispanic/Latino	8 (73)	8 (80)	8 (80)
Hispanic/Latino	3 (27)	2 (20)	2 (20)
Performance status (ECOG)			
0	9 (82)	8 (80)	9 (90)
1	2 (18)	2 (20)	1 (10)
Age (yrs)	70.0 ± 7.0	65.2 ± 9.5	66.2 ± 9.6
Weight (kg)	86.0 ± 18.8	89.6 ± 17.8	91.1 ± 19.6
BMI, kg/m^2	30.4 ± 7.17	30.4 ± 6.54	30.8 ± 6.01
Blood pressure (mm/Hg)			
Systolic	134 ± 16.5	132 ± 11.1	136 ± 15.8
Diastolic	72.6 ± 7.47	78.6 ± 6.95	79.0 ± 8.67

NOTE: Data are expressed as mean ± SD or number of patients (%).

Results

Accrual and baseline characteristics

A total of 31 subjects (11 to placebo, 10 to low dose, 10 to high dose) were randomized with subject accrual from September 2008 through March 2012.

The mean age at enrollment was 67.2 years (SD 8.7). Twenty-six (84%) were males, and 5 (16%) female. Twenty-six (84%) were ECOG performance status (PS) 0, and five (16%) ECOG PS 1. Seven (23%) were considered "hispanic or latino"; 24 (77%) were not. Thirty (97%) were white, while one (3%) was African-American. The mean BMI was 30.5 (SD 6.4). Patient demographics are summarized in Table 1. The distribution of baseline demographics and vital signs across the three treatment arms appears consistent with randomization.

Study conduct

Two subjects dropped from the study, of which one withdrew consent. Of the 29 (94%) patients who completed study, tissue was not obtained from one, and another did not take study medication for the entire duration of the study. Altogether four patients (13%) were noncompliant.

Adverse events

Table 2 provides an overview of adverse events (AE) with information on the severity of reported AEs according to the NCI Common Terminology Criteria for Adverse Events (CTCAE v. 3.0) grading scale for each event. For each AE, a patient was assigned to

Table 2. Adverse events

	Placebo ($n = 11$)				800 mg EGCG ($n = 10$)				1,200 mg EGCG ($n = 10$)			
Toxicity	**All (%)**	**1**	**2**	**3**	**All (%)**	**1**	**2**	**3**	**All (%)**	**1**	**2**	**3**
Head/headache[a]	0 (0)	0	0	0	1 (10)	1	0	0	4 (40)	3	1	0
Abdomen nos	0 (0)	0	0	0	1 (10)	1	0	0	1 (10)	0	0	1
Muscle	0 (0)	0	0	0	0 (0)	0	0	0	2 (20)	2	0	0
Nausea	0 (0)	0	0	0	0 (0)	0	0	0	2 (20)	2	0	0
Anxiety	0 (0)	0	0	0	0 (0)	0	0	0	1 (10)	0	1	0
Back	0 (0)	0	0	0	0 (0)	0	0	0	1 (10)	0	0	1
Hyperglycemia	1 (9)	0	1	0	0 (0)	0	0	0	0 (0)	0	0	0
Kidney	0 (0)	0	0	0	1 (10)	0	1	0	0 (0)	0	0	0
Tinnitus	0 (0)	0	0	0	0 (0)	0	0	0	1 (10)	0	1	0
Urinary tract nos	1 (9)	0	1	0	0 (0)	0	0	0	0 (0)	0	0	0

NOTE: Data are expressed as the number of patients (%). Patients are categorized into worst CTCAE grade v4.0. Adverse events shown here have a frequency of 20% or higher in any treatment arm or have at least one patient with grade of 2 (moderate) or greater.

[a]$P = 0.036$ for dose–response relationship for all head/headache events.

a single severity grade category based on the AE with the worst severity within that system.

For the overall summary, the maximum AE severity was severe (grade 3) for one (3%) patient, moderate (grade 2) for 5 patients (16%), mild (grade 1) for 8 (26%) patients, and no AE for 17 patients (55%). Tests for dose–response relationship with severity were significant for pain ($P = 0.009$) and gastrointestinal ($P = 0.026$) body systems. The most common adverse event was headache, followed by nausea and muscle ache. A significant ($P = 0.036$) dose–response relationship was observed for all head/headache events.

EGCG, ECG, EGC, and EC tissue levels

Nonmalignant bladder tissue levels (mean values) of EGCG were 0, 0.50, and 1.72 ng/mL for the placebo, low-dose, and high-dose Polyphenon E arms, respectively. The corresponding tumor tissue levels of EGCG were 0, 0, and 2.54 ng/mL. These data are displayed in Fig. 1. For normal tissue level of EGCG, 4 of 25 (16%) had nonzero values (are not "ND"), one of which is detectable but below the lower limit of quantitation ("NQ"). Our primary comparison, between the placebo group and the Polyphenon E groups combined, was not significant ($P = 0.12$). However, in our analysis supplemental to the primary analysis, despite the number of patients with "ND" values, we did find a dose–response relationship for normal ($P = 0.046$) and tumor tissue ($P = 0.005$).

The mean differences between tumor and normal tissue levels of EGCG by treatment group were −0.57 and 0.57 ng/mL for the low-dose and high-dose arms, respectively. There is no significant dose–response relationship ($P = 0.49$). Normal tissue levels of EGCG were significantly correlated with tumor tissue levels ($r = 0.53$, $P = 0.005$) and end of study plasma levels ($r = 0.48$, $P = 0.025$); also, tumor tissue levels were correlated with urine levels ($r = 0.56$, $P = 0.007$). Levels of EGCG in tissue, plasma, and urine are summarized in Table 3. Almost all of the tumor and normal tissue levels of ECG, EGC, and EC are zero; none of these catechins showed even a trend for differences in tissue.

EGCG plasma and urine levels

As expected, plasma EGCG levels were significantly higher after daily administration of Polyphenon E than at baseline by the Wilcoxon signed-rank test ($P < 0.02$ for each treated arm). Baseline plasma levels of EGCG were 2.66 ± 4.14 ng/mL, 1.6 ± 2.74 ng/mL, and 2.15 ± 3.58 ng/mL in the placebo, low-dose, and high-dose groups, respectively, compared with 2.94 ± 3.40 ng/mL, 78.09 ± 81.24 ng/mL, and 87.52 ± 77.46 ng/mL at the presurgical visit; there was an ordered relationship between dose and plasma concentrations by Jonckheere–Terpstra test ($P < 0.001$). While urine concentrations were less than 5% of corresponding plasma levels, urine EGCG levels showed a similar relationship and were also significantly higher after administration ($P < 0.02$ for each treated arm). Baseline urine levels of EGCG were 0.00 ± 0.00 ng/mL, 0.22 ± 0.59 ng/mL, and 0.94 ± 2.08 ng/mL in the placebo, low-dose, and high-dose groups, respectively, compared with 0.00 ± 0.00 ng/mL, 2.60 ± 1.93 ng/mL, and 4.32 ± 2.27 ng/mL at the presurgical visit; there was a dose relationship for urine concentrations ($P = 0.001$). Similar results were observed for plasma EGC and ECG, as well as EGC in urine with significant differences between baseline and presurgery and a dose–response relationship. Overall, catechins had plasma and urine concentrations consistent with a dose–response relationship, suggesting that increased doses lead to higher plasma and urine concentrations. Plasma EGCG levels at baseline and presurgery are shown in Fig. 1.

Tissue biomarkers

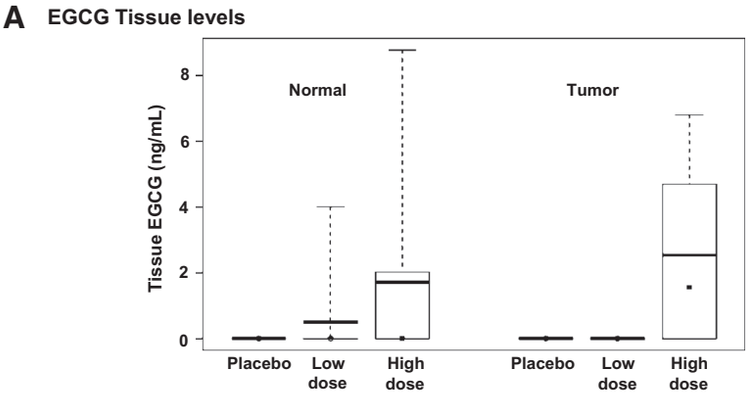
Measured levels of expression (mean values) were reported as mean optical density per pixel area for the placebo, low-dose, and high-dose Polyphenon E arms as follows: PCNA (0.41, 0.38, 0.35); MMP-2 (0.16, 0.18, 0.16); clusterin (0.074, 0.061, 0.046); VEGF (0.011, 0.021, 0.013); p27 (0.36, 0.35, 0.31). Negative dose–response relationships were observed with PCNA ($P = 0.016$) and clusterin ($P = 0.008$). A nonsignificant reduction in p27 expression ($P = 0.15$) was also observed. Biomarker tissue expression findings are shown in Fig. 2 and Table 4.

Serum IGF-1/IGFBP-3

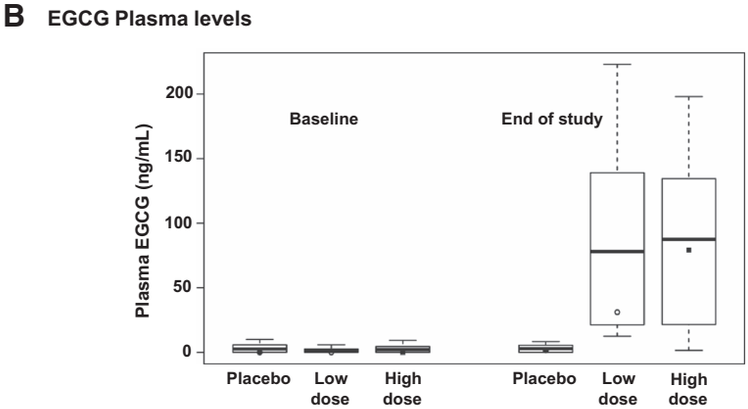
The presurgery levels (mean values) for the placebo, low-dose, and high-dose Polyphenon E arms were as follows: IGF-1 (131.86, 124.22, 132.27 ng/mL), IGFBP-3 (2,020.81, 1,800.08, 1,624.03 ng/mL), and IGF-1/IGFBP-3 (0.043, 0.035, 0.054). We found no dose–response relationships for IGF-1 ($P = 0.266$), IGFBP-3 ($P = 0.533$), or the IGF-1/IGFBP-3 ratio ($P = 0.197$).

Pharmacogenomics of EGCG by UGT and COMT

There were no significant differences found in baseline levels of plasma and urine EGCG, although there was a trend for difference between G/G and A/G ($P = 0.068$) for baseline plasma EGCG by COMT. Furthermore, no significant relationships were observed for genotype for presurgery levels of EGCG in urine or plasma.



Treatment		N	Mean	SD	*P*
Normal	Placebo	9	0.00	0.00	0.046
	Low dose	8	0.50	1.42	
	High dose	8	1.72	3.11	
Tumor	Placebo	10	0.00	0.00	0.005
	Low dose	7	0.00	0.00	
	High dose	7	2.54	2.92	



Treatment		N	Mean	SD	*P*
Baseline	Placebo	9	2.66	4.14	0.908
	Low dose	7	1.60	2.74	
	High dose	10	2.15	3.58	
End of study	Placebo	9	2.94	3.40	0.001
	Low dose	8	78.09	81.24	
	High dose	10	87.52	77.46	

Figure 1.
A, Nonmalignant and malignant bladder tissue levels of EGCG for the placebo, low-dose, and high-dose Polyphenon E arms with a significant dose–response relationship for normal ($P = 0.046$) and tumor tissue ($P = 0.005$). **B,** Baseline and presurgical plasma levels of EGCG for the placebo, low-dose, and high-dose groups with an ordered relationship between dose and plasma concentrations ($P < 0.001$).

Discussion

Limited epidemiologic studies have implied regular consumers of tea may have a lower risk of bladder cancer (22, 23). A study by Bianchi and colleagues suggests that individuals consuming greater than 5 cups of tea daily may have a decreased incidence of bladder cancer, although these findings are tempered somewhat by borderline significance and a lack of a dose–response relationship (24). Nevertheless, consistent with these data, geographic areas with relatively high consumption of green tea in Japan have a lower incidence of bladder cancer as compared with the United States and western European countries. Interestingly, Japanese families who immigrate to the United States have twice the incidence of bladder cancer as those families that reside in Japan (25). Epidemiologic and laboratory studies provide ample justification for the further study of green tea and EGCG in bladder cancer patients, although to date efficacy in a randomized trial has not been demonstrated. To our knowledge, this is the first clinical trial to

Table 3. Levels of EGCG in tissue, plasma, and urine

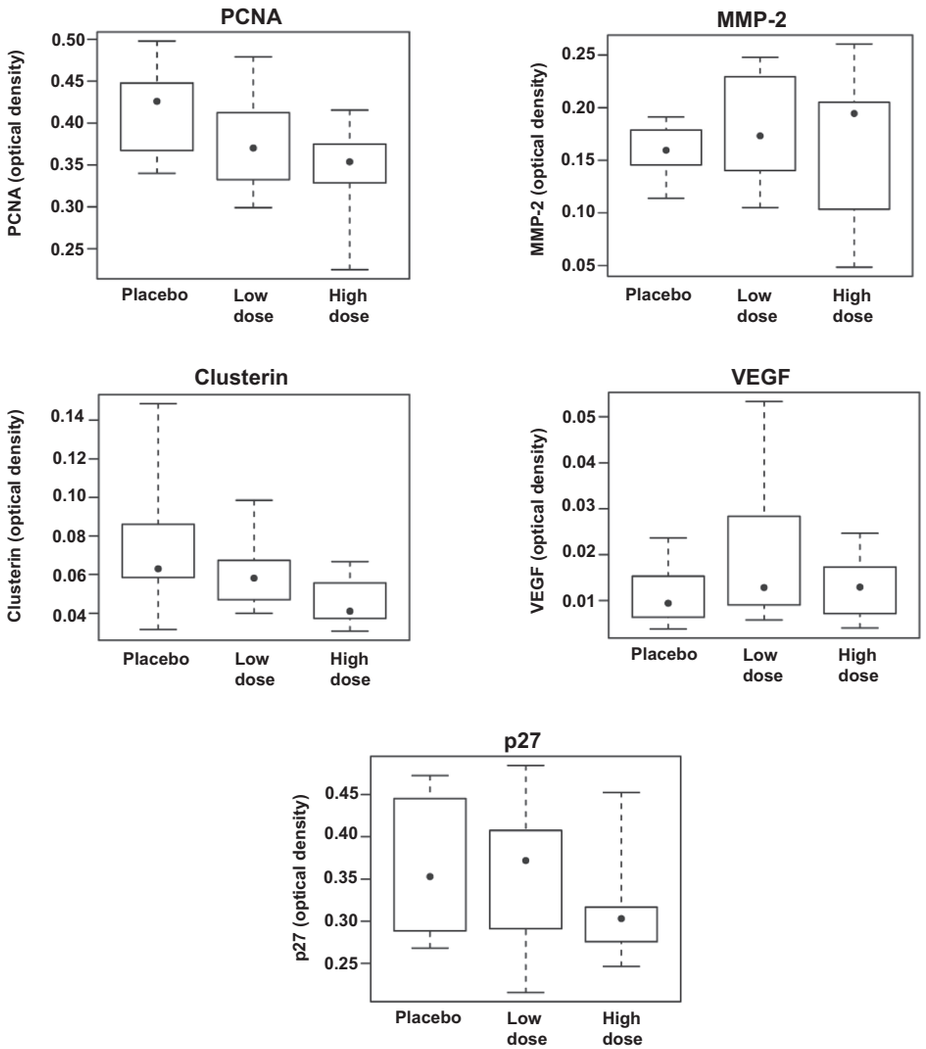
Sample type	Tissue type or timepoint	EGCG Treatment arm	*N*	Mean ± SD	Median	Range
Tissue	Normal	Placebo	9	0 ± 0	0	(0[a]–0)
		800 mg	8	0.501 ± 1.42	0	(0[b]–4.01)
		1,200 mg	8	1.72 ± 3.11	0	(0[c]–8.78)
	Tumor	Placebo	10	0 ± 0	0	(0[a]–0)
		800 mg	7	0 ± 0	0	(0[a]–0)
		1,200 mg	7	2.54 ± 2.92	1.56	(0[d]–6.80)
Plasma	Baseline	Placebo	9	2.66 ± 4.14	0	(0[e]–10.1)
		800 mg	7	1.60 ± 2.74	0	(0[c]–5.90)
		1,200 mg	10	2.15 ± 3.58	0	(0[b]–9.32)
	Presurgery	Placebo	9	2.94 ± 3.40	1.56	(0[d]–8.33)
		800 mg	8	78.1 ± 81.2	31.1	(12.5–223)
		1,200 mg	10	87.5 ± 77.5	79.2	(1.56–198)
Urine	Baseline	Placebo	9	0 ± 0	0	(0[a]–0)
		800 mg	7	0.223 ± 0.590	0	(0[e]–1.56)
		1,200 mg	10	0.943 ± 2.08	0	(0[f]–6.00)
	Presurgery	Placebo	8	0 ± 0	0	(0[a]–0)
		800 mg	8	2.60 ± 1.93	1.56	(1.56–5.74)
		1,200 mg	10	4.32 ± 2.27	4.74	(1.56–8.61)

As many observations are ND (not detected and considered to be 0), mean ± SD as well as median (range) are presented to better characterize distributions.
[a]All patients had values of ND.
[b]Seven patients had values of ND.
[c]Five patients had values of ND.
[d]Three patients had values of ND.
[e]Six patients had values of ND.
[f]Eight patients had values of ND.

explore both tissue accumulation and biologic efficacy of EGCG in patients with bladder cancer.

Our primary objective of this study was to evaluate tissue levels of EGCG in nonmalignant bladder tissue. The demonstration of tissue accumulation in our study implies the bioavailability of EGCG to confer biologic activity locally, to induce biologic change in both premalignant and malignant tissue. In this study, we were able to demonstrate statistically significant tissue accumulation in a dose-dependent fashion, in both nonmalignant and malignant bladder tissue, whereas no significant difference in EGCG accumulation between normal and tumor tissue was seen. While the finding of EGCG accumulation in nonmalignant bladder urothelium suggests that our primary study objective was achieved, an important caveat is that a low rate of detectability of EGCG in tissue was observed, in that only 4 of 16 specimens from patients treated with EGCG (and 4 of 25 specimens overall) demonstrated detectable levels of EGCG. The reasons for this are not clear, but could be related to study logistics. In particular, the timing between the last dose of EGCG and surgery, with harvesting of tissue, certainly varies patient to patient, and is not easy to control as it depends on surgical scheduling, time for anesthesia induction, and the operation itself which depending on the patient and tumor type can vary in technical difficulty. One potential advantage in our study is that the vast majority of patients underwent TURBT, a shorter procedure in which viable tissue can be resected and almost immediately frozen/processed, as compared with radical cystectomy, a longer operation in which tissue is gradually devitalized as the specimen is removed. Indeed the half-life of EGCG was only determined to be 2–4 hours in phase I testing (14), whereas patients who undergo surgery are required to maintain NPO status (no dietary intake) from midnight prior to surgery and therefore tissue harvesting would occur a minimum of 8–10 hours following their last dose of Polyphenon E. Given these logistic limitations, perhaps it is remarkable that we were able to detect EGCG tissue accumulation and biologic activity in any of the patients, and with a statistically significant dose–response relationship as well. However, were tissue harvesting to have occurred within 2–4 hours subsequent to the last dose of EGCG, it is possible that we would have observed a higher rate of detectability of EGCG in tissue. The lower detectability of EGCG in tissue in this study, as compared with plasma, seems to suggest a more rapid clearance or metabolism of EGCG in tissue. Other means of more immediate tissue acquisition, that is, needle biopsy to circumvent logistical challenges with tissue acquisition, would not be in accordance with standard of care and therefore timing of tissue acquisition remains a limitation to consider in the design of future trials.

Even more encouraging than the observed evidence of dose-related tissue accumulation, was evidence of favorable biologic activity. Specifically, both PCNA and clusterin were downregulated in a statistically significant, dose-dependent manner in study participants taking Polyphenon E. Inhibition of bladder cancer cell growth by EGCG has been demonstrated *in vitro* in multiple studies (8), with downregulation of cyclin D1, cyclin-dependent kinase 4/6, and inhibition of Rb phosphorylation in association with G_0–G_1 cell-cycle arrest (10). Furthermore, a reduction in the activity of ornithine decarboxylase, an enzyme responsible for polyamine production with biological significance in bladder cancer, has been observed with EGCG (26–28). We evaluated PCNA expression in our study, as PCNA has been established as a marker of cellular proliferation by the Mukhtar laboratory, in that green tea polyphenols were found to inhibit PCNA expression with corresponding tumor formation in the TRAMP model of prostate cancer (11). Similarly, downregulation of clusterin by EGCG with induction of cellular apoptosis has been described in laboratory studies (12, 13). In other preclinical studies, induction and accumulation of the cell-cycle mediator p27 has also been associated with cell-cycle arrest mediated by EGCG induction of p27/Kip1 (29, 30). While our findings pertaining to p27 as a function of EGCG administration were not statistically significant,



		PCNA		MMP2		Clusterin		VEGF		p27	
		P = 0.016		*P* = 0.574		*P* = 0.008		*P* = 0.574		*P* = 0.154	
Treatment	N	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Placebo	11	0.41	0.052	0.16	0.026	0.074	0.032	0.011	0.0065	0.36	0.080
Low dose	8	0.38	0.061	0.18	0.054	0.061	0.020	0.021	0.0173	0.35	0.090
High dose	10	0.35	0.052	0.16	0.069	0.046	0.013	0.013	0.0066	0.31	0.057

Figure 2.
Measured levels of expression reported as mean optical density per pixel area for the placebo, low-dose, and high-dose Polyphenon E with negative dose–response relationships observed for PCNA ($P = 0.016$) and clusterin ($P = 0.008$).

a nonsignificant reduction in p27 expression was observed. Taken together, the favorable biomarker changes observed in this study have been associated with anticarcinogenic effects in numerous studies across different tumor types including bladder cancer. While follow-up biologic studies of effect on growth and apoptosis are needed to further corroborate our observations, these EGCG dose-dependent tissue biomarker findings, coupled with similar dose-dependent tissue and plasma pharmacokinetic results, strongly support the potential bladder cancer preventive properties of Polyphenon E.

We also explored variability in the metabolism of EGCG according to polymorphisms in UGT and COMT. Our rationale for exploring pharmacogenomic relationship between EGCG and UGT and COMT polymorphisms is based on known population

Table 4. Intermediate endpoint biomarker expression

Biomarker	Placebo ($n = 11$)	800 mg EGCG ($n = 8$)	1,200 mg EGCG ($n = 10$)	*P* (J-T)
PCNA	0.43 (0.37–0.45)	0.37 (0.33–0.41)	0.35 (0.33–0.37)	0.016
MMP2	0.16 (0.15–0.18)	0.17 (0.14–0.23)	0.19 (0.10–0.20)	0.574
Clusterin	0.063 (0.058–0.086)	0.058 (0.047–0.067)	0.041 (0.037–0.056)	0.008
VEGF	0.0094 (0.0064–0.0150)	0.0128 (0.0090–0.0280)	0.0129 (0.0072–0.0170)	0.574
p27	0.45 (0.35–0.47)	0.41 (0.37–0.48)	0.32 (0.30–0.45)	0.154

NOTE: Data are expressed as median and interquartile range (IQR).

prevalent altered metabolic enzymatic activity. For instance, EGCG is extensively glucuronidated by UGT 1A1 to an active metabolite (-)-EGCG-4"-O-glucuronide (18). An increase in dinucleotide repeats within the TATA box results in reduced UGT1A1 enzyme activity (31). Therefore, individuals with variant UGT enzymes could potentially have altered metabolism, efficacy, and toxicity (19). However, significant differences in EGCG metabolism were not determined in our study in relation to either UGT or COMT polymorphisms.

Conclusion

We demonstrate in a phase II pilot study tissue accumulation of EGCG in nonmalignant bladder urothelium which follows both plasma and urine levels in a dose-dependent fashion. Furthermore, dose-dependent changes in the intermediate endpoint biomarkers, PCNA and clusterin, were observed consistent with potential chemopreventive efficacy. Acknowledging the limitations of this pilot study, we feel these findings indicate Polyphenon E administration results in definable tissue accumulation and more importantly desirable biologic activity which warrant further clinical studies assessing the effect of Polyphenon E on actual bladder tumor recurrence and progression.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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Writing, review, and/or revision of the manuscript: J.R. Gee, D.R. Saltzstein, K. Kim, J.M. Kolesar, W. Huang, T.C. Havighurst, B.W. Wollmer, T. Downs
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Study supervision: H.H. Bailey
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