Research Article

Cancer Prevention Research

A Presurgical Study of Lecithin Formulation of Green Tea Extract in Women with Early Breast Cancer

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Abstract

Epidemiologic data support an inverse association between green tea intake and breast cancer risk. Greenselect Phytosome (GSP) is a lecithin formulation of a caffeine-free green tea catechin extract. The purpose of the study was to determine the tissue distribution of epigallocatechin-3-O-gallate (EGCG) and its effect on cell proliferation and circulating biomarkers in breast cancer patients. Twelve early breast cancer patients received GSP 300 mg, equivalent to 44.9 mg of EGCG, daily for 4 weeks prior to surgery. The EGCG levels were measured before (free) and after (total) enzymatic hydrolysis by HPLC-MS/MS in plasma, urine, breast cancer tissue, and surrounding normal breast tissue. Fasting blood samples were taken at baseline, before the last administration, and 2 hours later. Repeated administration of GSP achieved levels of total EGCG ranging from 17 to 121 ng/mL in plasma. Despite a high between-subject variability, total EGCG was detectable in all tumor tissue samples collected up to 8 ng/g. Median total EGCG concentration was higher in the tumor as compared with the adjacent normal tissue (3.18 ng/g vs. 0 ng/g, $P = 0.02$). Free EGCG concentrations ranged from 8 to 65.8 ng/mL in plasma (P between last administration and 2 hours after <0.001). Free EGCG plasma levels showed a significant positive correlation with the Ki-67 decrease in tumor tissue ($P = 0.02$). No change in any other biomarkers was noted, except for a slight increase in testosterone levels after treatment. Oral GSP increases bioavailability of EGCG, which is detectable in breast tumor tissue and is associated with antiproliferative effects on breast cancer tissue. *Cancer Prev Res; 10(6); 363–70. ©2017 AACR.*

Introduction

Catechin (C), epicatechin (EC), epigallocatechin (EGC), and epigallocatechin-3-gallate (EGCG) are the four major constituents of green tea, with EGCG accounting for more than 50% of total green tea polyphenols (1). Preclinical data (2) have shown strong chemopreventive and possibly cancer chemotherapeutic effects of green tea polyphenols against breast cancer, although epidemiologic studies are conflicting (3). A recent ancillary study using archived blood/urine from a phase IB randomized, placebo-controlled dose escalation trial of an oral green tea extract in breast cancer patients suggested potential mechanistic actions of tea polyphenols in growth factor signaling, angiogenesis, and lipid metabolism (4). However, the optimal dose, duration, and formulation of green tea have yet to be determined. Studies in healthy humans have shown that EGCG when formulated with phosphatidylcholine [Greenselect Phytosome (GSP)] improves its systemic availability compared with EGCG (5). We recently demonstrated that the polyphenolic flavonolignan silybin when formulated with phosphatidylcholine can deliver high blood concentrations of silybin, which selectively accumulate in breast tumor tissue (6). Using the same model, we carried out a pilot window-of-opportunity trial in women with early breast cancer who were candidates to surgery to assess the pharmacokinetic profile and the pharmacodynamic effects of GSP on malignant as well as surrounding normal tissue. The primary outcome measure was the determination of EGCG levels in plasma, urine, breast cancer tissue, and surrounding normal tissue. Because of the heterogeneous effects of EGCG on cellular signal transduction events, we also assessed several biological endpoints, including cell proliferation/angiogenesis (7), oxidative stress (8), chronic inflammation (9), and adiposity-related endocrine mechanisms (10).

Materials and Methods

Study design

The study was a pilot "window-of-opportunity" presurgical trial. Twelve consecutive patients with a diagnosis of breast cancer scheduled to undergo lumpectomy or mastectomy were recruited

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into the trial. Patients received GSP, 300 mg daily for 4 weeks prior to surgery. The primary objective was to determine the bioavailability of green tea polyphenols in breast cancer tissue, adjacent normal breast tissue, plasma, and urine after GSP intervention. The secondary objectives were to explore the possible effect of GSP on cell proliferation in breast cancer tissue and the modulation of insulin resistance biomarkers in serum, including insulin growth factor-I (IGF-I), insulin, homeostasis model assessment–estimated insulin resistance (HOMA-IR) index, high-sensitivity C-reactive protein (hs-CRP), sex hormone–binding globulin (SHBG), testosterone, adiponectin, VEGF, and nitric oxide (NO). This study (R621-IEO661/511) was approved by the local Institutional Review Board and conducted in accordance with the Declaration of Helsinki. All patients provided written informed consent prior to participation.

Study product

Greenselect Phytosome (GSP) is a lecithin formulation of a caffeine-free green tea catechin extract. Its active constituents are mainly represented by a group of compounds having a flavan-3-olic structure and generally defined as green tea catechins: epigallocatechin, catechin, epigallocatechin-3-O-gallate, gallocatechin-3-O-gallate, epigallo-3-O-methylgallate, and epicatechin-3-O-gallate. The active constituents of green tea leaves are this group of polyphenols. GSP was formulated in granules (sachet) to be suspended in drinkable water provided by Indena S.p.A. Each sachet contained 300 mg of GSP, the soy lecithin delivery form standardized to contain $\geq$ 19% $\leq$ 25% of catechins, at least 13% of the main constituent EGCG and $\leq$0.1% of caffeine as by high-performance liquid chromatography (HPLC). Each sachet of the clinical batch contained a total of 44.9 mg of EGCG and 65.12 mg of catechins. The study sachets were stored at room temperature and protected from environmental extremes. The dose was chosen based on the high tolerability observed in clinical studies following repeated administration to healthy volunteers (5, 11).

Study population

Patients with biopsy-confirmed breast cancer not eligible for neoadjuvant treatment and candidate to surgical lumpectomy or mastectomy were enrolled onto the study. To be eligible, patients must have had biopsy-proved breast cancer, not received other therapies for their breast cancer, be at least 18 years old, had no history of chemotherapy and/or radiotherapy for their breast cancer or other malignancy in the previous 5 years, had a good performance status, and adequate normal biochemical function tests. Patients were excluded if they drank tea regularly (more than 6 servings of hot tea per week) within 2 weeks of enrollment, were receiving other investigational agents, had a history of allergic reactions attributed to compounds of similar chemical to GSP, or had uncontrolled intercurrent illness.

Study procedures

During the initial visit, participants underwent eligibility evaluation. Each participant underwent an interview and brief physical examination to obtain medical history, performance status, height, weight, blood pressure, and pulse. Urine and morning fasting blood for serum and plasma analysis were drawn at baseline. Blood samples were collected for complete blood count with differentials, comprehensive metabolic panel, and systemic research endpoints. Upon determination of eligibility, participants were instructed to take a single sachet of GSP drinkable suspension once daily for 4 weeks prior to surgery, in fasting conditions (30 minutes before eating, at least 2 hours after the previous meal). Supplementation was continued until the day of surgery. Date and time of the last intake were recorded. Participants were also required to keep an intake calendar and adverse event diary throughout the study participation. Participants returned to the clinic within the day of surgery to undergo assessment of adverse events and compliance, and blood collection. The NCI Common Terminology Criteria for Adverse Events (CTCAE) version 3.0 was used for adverse event description and grading. Agent intervention compliance was evaluated by sachet count and intake calendar. Participants were considered compliant if they had taken at least 75% of their assigned study doses.

Specimen collection

Morning fasting blood for serum and plasma analysis was drawn at baseline, the day of surgery immediately before the last administration of GSP, and 2 hours later. Samples were stored at −80°C. Urine samples were taken at baseline and the day of surgery, before the last administration of GSP. Breast cancer tissue was collected by tru-cut biopsy at baseline and from surgical resections at treatment conclusion (tumor and distant surrounding noncancerous tissue), snap frozen on liquid nitrogen, and stored at −80°C.

Analysis of EGCG

Blood samples were collected in heparinized tubes. After centrifugation, 1 mL of each plasma sample was mixed with 20 μL of an ascorbate–EDTA solution (0.4 mol/L NaH_2PO_4 buffer containing 20% ascorbic acid-0.1% EDTA, pH 3.6) as a preservative, and the mixture was stored at −80°C until analysis. The total volume of each urine sample was recorded. Aliquots of 20 mL of each urine sample were transferred into plastic tubes that contained 20 mg of ascorbic acid and 0.5 mg of EDTA. The urine samples were stored at −80°C until analysis (12).

The green tea polyphenols are extensively modified by phase II human enzymes. EGCG levels were first measured as free (unconjugated) EGCG (free EGCG), and thereafter through enzymatic hydrolysis as total (free and conjugated) EGCG (total EGCG), by HPLC-MS/MS in all types of biological specimens (plasma, urine, breast cancer, and surrounding normal tissue). The methodology was developed by Kymos Pharma Services starting from literature references (12, 13). EGCG from plasma and urine (0.2 mL) was determined by HPLC-MS/MS after liquid–liquid extraction with ethyl acetate (Scharlau). Ethyl gallate (Sigma-Aldrich) was used as internal standard. The calibration ranges for plasma and urine were 0.5 to 204.1 ng/mL and 0.5 to 210.5 ng/mL, respectively. For total EGCG measurements, β-glucuronidase from *Helix pomatia* (β-glucuronidase and sulfatase activities, BBI Solutions) was added prior to run, whereas for the determination of free EGCG, the enzymatic process was omitted. Samples were analyzed in batches; free EGCG and total EGCG (for plasma or urine). Each batch included standards and human plasma or urine controls. The concentrations of three plasma controls were 1.5, 10.2, and 153.1 ng/mL, and of three urine controls 1.6, 10.5, and 157.9 ng/mL, respectively. The chromatographic method

API 4000QTRAP (A438-MS/A565-XL) was applied for plasma and urine.

EGCG from breast tissue (50 mg) was determined by HPLC MS/MS after liquid–liquid extraction with ethyl acetate. Ethyl gallate was used as internal standard. There were samples weighting less than 50 mg, thus insufficient to measure free EGCG. As for plasma and urine, the enzymatic hydrolysis step was added to determinate total EGCG. Tissue samples were analyzed in one batch for free EGCG and one batch for total EGCG determination. Each batch included standards (range, 1–40 ng/g) and controls (3, 6, and 32 ng/g). The lower limit of quantification (LLOQ) was 1 ng/g except for samples with insufficient tissue (<50 mg), for which the LLOQ was recalculated taking into account the weight of sample available. A subrogate matrix of pig muscle (Clinobs) was used for the preparation of standards and controls. The chromatographic method API 4000 (A438-MS/A565-XL) was used.

Acceptance criterion between chromatographic batches for calibration curves was that the correlation coefficient should not be lower than 0.99. Accuracy of the back-calculated concentrations was $\leq$15% for all standard concentrations except for the lower limit of quantitation, for which the accuracy should be $\leq$20%. At least 75% of the calibration standards met the above criteria, including the LLOQ and the highest standard. A calibration standard was dropped if this criterion was not achieved. At least 67% of the quality controls (at least 50% at each concentration level) were within $\pm$15% of the nominal value. The EGCG concentrations of samples were determined by interpolation from the corresponding calibration curve using Analyst software (1.5.1. version). Samples were analyzed in single and the concentrations of breast tissue samples were expressed in ng/g.

Serum and urine biomarkers

Biological marker endpoints included IGF-I, insulin, HOMA-IR index, testosterone, adiponectin, C-reactive protein, SHBG, VEGF, and NO.

Serum concentrations of IGF-I (ng/mL) and testosterone were determined by chemiluminescent immunometric assays (Diasorin SpA) with the automatic instrument LIAISON. The sensitivities of the tests were 0.8 nmol/L and 0.05 ng/mL, respectively; interassay coefficients of variation (CV) of our in-house pooled serum control sample were 4.1% and 10.8%, respectively.

Serum glucose, insulin, SHBG and hs-CRP were measured by the automated ARCHITECT system (Abbott Laboratories), according to the manufacturer's instructions. The glucose and insulin assays have a detection limit of 2.5 mg/dL and 1.0 μU/mL, respectively. The interassay CV of a glucose test control sample was 0.9% (mean 0.88 mg/dL) and our in-house serum pool CV was 4.4% (mean 5.3 μU/mL) for insulin. The analytic sensitivity of SHBG is 0.02 nmol/L and hs-CRP has a detection limit of 0.01 mg/dL. The interassay CV of our in-house serum pool was 6.2% (mean 93 nmol/L) for SHBG and 10.9% for hs-CRP (mean 0.026 mg/dL).

Serum adiponectin and VEGF were determined by ELISA. The methods have a detection limit 0.246 ng/mL and 9.0 pg/ml, respectively. The interassay CV of our in-house serum pool were 11% (mean 13 μg/mL) and 10% (mean 385 pg/mL), respectively.

The urine concentrations of NO were determined as previously described tissue (6). The results were normalized by urinary creatinine concentrations.

Tissue biomarkers

Following histologic diagnosis and IHC profiling, 5-μm thick sections of the invasive tumors were prepared from each formalin-fixed and paraffin-embedded block of interest, that is, those obtained upon diagnostic biopsy procedures and after surgical interventions. For invasive tumors, the histologic subtype and grade as well as the presence of peritumoral vascular invasion were annotated. Immunostaining was performed using anti-ER, PgR (PharmDX), HER2 (HercepTest), and Ki-67 antibodies, as previously reported (14–16).

Statistical analysis

We presented median values and interquartile ranges (IQR) of free EGCG and total EGCG in plasma and urine by time (before last administration and 2 hours after last administration) and tissue by type: cancer and surrounding normal tissue. We evaluated changes in time and differences between types of tissue using Wilcoxon signed rank test for paired differences. We also evaluated the association of free EGCG and total EGCG with the change in time of several biomarkers, including Ki-67, adjusting for tumor size and baseline values, through multivariate models. We verified the normal distribution of residuals of the full model. The median values and IQRs of free EGCG and total EGCG in plasma, urine, breast cancer tissue, and surrounding normal tissue are reported. Changes in time and differences between types of tissue using nonparametric tests (Wilcoxon rank tests) were evaluated. We did not adjust for multiple comparisons analyses on secondary endpoints because they are exploratory in nature and should therefore be considered hypothesis generating rather than definitive. We reported two-sided *P* values. We set the criterion for statistical significance at 5%. Data were analyzed using the SAS System Software for Windows, release 9.2. (SAS Institute).

Results

Twelve consecutive breast cancer patients were enrolled in this study. Table 1 describes the patient and the tumor characteristics at surgery. Women were mostly premenopausal (58%). Ten breast cancers were estrogen receptor positive (mainly luminal-B phenotype), one pure HER2 positive, one basal like. They were mostly unifocal and with no vascular invasion. All patients were fully compliant and completed the treatment program. GSP was well tolerated with minimal adverse events, and no withdrawals from the study were noted. The only adverse event occurring in 3 subjects was grade 1 headache, sporadic in two cases (reported only one day). Serum, plasma, and urine samples were collected during all time points in 10 of 12 patients (in two cases, the date of surgery was anticipated with a short notice), whereas fresh-frozen tissue for EGCG determination was collected in 8 of 10 patients (2 patients where scheduled for surgery very late in the evening). Free EGCG and total EGCG were undetectable in all compartments (plasma, urine, breast tissue) at baseline. A large degree of interpatient variability in the EGCG blood levels was noted at all time points. The median (IQR) free EGCG and total EGCG concentrations after supplementation are shown in Table 2. The median total EGCG plasma level was 20.3 ng/mL (IQR, 14.7–30.7) before the last administration (22–27 hours from the penultimate intake, depending on time of hospitalization), and 89 ng/mL (IQR, 50.9–111.4) 2 hours after the last intake of GSP. Free EGCG was measurable

Table 1. Main patient and tumor characteristics (at surgery)

Patients characteristics	***N***	**%**
Age (years)		
<50	7	58
≥50	5	42
Menopausal status		
Premenopausal	7	58
Postmenopausal	5	42
Body mass index (kg/m^2)		
<25	6	50
≥25	6	50
Tumor characteristics	***N***	**%**
Molecular phenotype		
Luminal A	3	25
Luminal B	7	59
HER2 like	1	8
Basal like	1	8
TN Stage		
Tis	0	0
pT1	4	34
pT2	6	50
pT3	1	8
pT4	1	8
pN0-Nx	3	25
pN1	4	34
pN2	3	25
pN3	2	16
Vascular invasion		
Yes	5	42
No	7	58
Focality		
Unifocal	7	59
Multifocal	3	25
Multicentric	2	16

only in plasma samples collected 2 hours after the last intake of GSP (median, 30.9 ng/mL; IQR, 16.6–38.6).

After 4 weeks of GSP administration, EGCG reached the breast organ and was detectable in all tumor tissue samples collected ($n = 8$). The median time interval between the last administration of GSP and surgery was 5 hours. Total EGCG concentration was higher in the tumor as compared with the adjacent normal tissue. Median total EGCG was 3.18 ng/g (IQR, 2.76–4.58) in breast tumor tissue and under the limit of detectability in adjacent normal tissue (minimum, 0 ng/g; maximum, 2.85 ng/g; *P* value for difference in medians = 0.02).

Free EGCG concentrations were under the limit of detectability in tumor tissue but present in adjacent normal breast tissue (median = 1.07 ng/g; IQR, 0–1.25; $P = 0.004$; Table 2).

Change in circulating and tissue biomarkers of activity is summarized in Table 3. IGF-I, insulin, HOMA index, testosterone, adiponectin, hs-CRP, SHBG, VEGF, and NO were measured in serum before and after the administration of GSP. No clear trend to a change in any of the circulating biomarkers was noted, except for an increase in testosterone levels after treatment. Notably, we reported a borderline significant positive association between free EGCG plasma levels and the change in tumor Ki-67 between biopsy and surgery. Figure 1 represents the association between free EGCG plasma levels after supplementation and the change in Ki-67 between baseline and surgery. The higher the plasma concentration of free EGCG, the greater was the decrease of Ki-67 after adjustment for baseline Ki-67 and tumor diameter ($P = 0.05$). Conversely, when free EGCG was below the limit of quantitation, the Ki-67 change increased considerably. Shown at the top left is a patient with undetectable free EGCG levels, triple-negative phenotype, and approximately 30% increase of Ki-67; at the bottom right, a patient with the highest level of plasma concentration of free EGCG (65.8 ng/mL), a luminal B HER2-negative phenotype, and a decrease of 20% of the proliferation index between baseline and surgery.

Discussion

In this study, we found that breast tissue bioavailability of green tea polyphenols is possible with oral GSP and that EGCG reaches breast tumor tissue. Circulating and tissue biomarkers did not differ between baseline and surgery except for testosterone levels, and a positive association between plasma free EGCG concentration and a decrease of Ki-67 in tumor tissue was noted. GSP intervention was well tolerated with minimal adverse events.

Our findings suggest that the formulation with lecithin can improve the absorption of EGCG without compromising safety. Phytosome is a biocompatible and biodegradable delivery system. By embedding the natural compounds into the environment of phospholipids, these are shielded from water-triggered degradation while, at the same time, the rapid exchange of phospholipids between biological membranes and

Table 2. Total and free EGCG levels in plasma, urine, breast cancer, and adjacent unaffected breast tissue in patients who received Greenselect Phytosome for 28 days

	Time	**Median**	**Q1**	**Q3**	**Min**	**Max**	***P***
Total EGCG (plasma) ng/mL	Baseline	0	0	0	0	0	
	Before last administration	20.3	14.7	30.7	1.32	41.43	
	2 hours after last administration	89	50.9	111.4	17.4	121.2	<0.008[a]
Free EGCG (plasma) ng/mL	Baseline	0	0	0	0	0	
	Before last administration	0	0	0	0	0	
	2 hours after last administration	30.9	16.6	38.6	0	65.8	<0.001[a]
Total EGCG (urine) ng/mL[b]	Baseline	0	0	0	0	0	
	Surgery	4.57	3.32	5.38	2.05	6.38	0.002
Free EGCG (urine) ng/mL[b]	Baseline	0	0	0	0	0	
	Surgery	0	0	0	0	0	1.0
Total EGCG (tissue) ng/g	Tumor	3.18	2.76	4.58	1.18	8.56	0.02
	Normal breast tissue	0	0	2.34	0	2.85	
Free EGCG (tissue) ng/g	Tumor	0	0	0	0	0	0.004
	Normal breast tissue	1.07	0	1.25	0	1.46	

[a]*P* for the difference between before last administration and 2 hours after last administration.
[b]Creatinine normalization; end: before the last administration; end-2h: 2 hours after the last administration.

Table 3. Biomarker results of women with breast cancer before and after GSP supplementation

Biomarker	Time	Median	Q1	Q3	P^a
Glucose	Baseline	97	89	103	1.00
mg/dL	Surgery	92.5	83	99	
IGF-I	Baseline	181.5	142	205	0.75
ng/mL	Surgery	181.5	149	205	
SHBG	Baseline	93.15	48.2	116.3	0.34
nmol/L	Surgery	72	47.7	115.6	
Insulin	Baseline	8.5	5	14.5	0.34
µU/mL	Surgery	5.1	3.7	12.7	
Testosterone	Baseline	0.165	0.09	0.26	0.02
ng/mL	Surgery	0.225	0.19	0.29	
Adiponectin	Baseline	12.75	8.9	15.7	0.75
µg/mL	Surgery	12.7	9.9	14.6	
C-reactive protein	Baseline	0.105	0.04	0.27	1.00
mg/dL	Surgery	0.135	0.05	0.37	
NOS	Baseline	214.5	133.3	680.6	1.00
µmol/L	Surgery	223.8	127.4	541.4	
VEGF	Baseline	401.5	347	712	0.11
pg/mL	Surgery	456.5	338	720	
HOMA	Baseline	2.1	1	3.7	0.34
	Surgery	1.15	0.8	3.1	
Tumor Ki-67	Biopsy	33.5	20	39	0.39
%	Surgery	32.5	17	50	

[a]Nonparametric test for paired data.

the extracellular fluids can shuttle them into biological membranes, boosting its cellular capitation (17). Unique physicochemical properties of phospholipids confer phytosomes an amphiphilic nature that improves the bioavailability of compounds with extremely hydrophilic or lipophilic properties and protect phytochemicals from destruction by digestive enzymes and gut bacteria (18). To our knowledge, this is the first time GSP pharmacokinetics has been evaluated in cancer patients. For this reason, we compared our results with the ones obtained using polyphenon E (Poly E), one of the most tested green tea polyphenol mixture in cancer trials (13, 19–21). Poly E is a standardized botanical extract obtained from green tea leaves and contains a defined mixture of catechins (80%–98% total catechins by weight, 50%–75% EGCG, and other catechins $\leq$12%). A dose of Poly E equivalent to EGCG 800 mg/die administered for 3 to 6 weeks before surgery in prostate cancer patients showed an average plasma peak concentration of approximately 68 ng/mL (146 pmol/mL) with low to undetectable prostate tissue bioaccumulation levels (13). In our trial, a daily dose of 300 mg of GSP (~EGCG 45 mg/die) was associated with a median concentration of total EGCG of 89 ng/mL 2 hours after the last GSP intake and 20.3 ng/mL after 22 to 27 hours from the penultimate intake, suggesting a higher bioavailability potential, even taking into account the difference between the fasting state of our trial and the fed state of the prostate study. The fasting state has been associated with a maximum 3- to 5-fold increase in plasma levels (22), so our findings of an approximately 20-fold increase confirm the high absorption of GSP in relation to the administered dose. In our pilot trial, free EGCG was not detectable in plasma samples taken before the last administration (~24 hours after the penultimate intake). This result was expected, because of the short plasma half-lives of EGCG, and the once-daily schedule. In our study, the presence of metabolic conjugates of EGCG was shown indirectly. Metabolites were calculated as EGCG after enzymatic hydrolysis. Raised levels of the parent molecule after enzymatic hydrolysis suggested the presence of metabolites. The accumulated EGCG was predominant in its conjugated form, but only trace amounts of EGCG were found in postsupplementation urine. As previously hypothesized (23), conjugated EGCG might be excreted through bile, meaning that it is not simply conjugation that determines renal loss. When we examined the target tissue distribution, despite the very low dose of EGCG administered, all the breast tumor tissues available at surgery (8/12 patients) had measurable levels of EGCG. In the prostate presurgical study (13), only one patient has detectable concentration of EGCG (36 pmol/g ~16 ng/g). Authors explained that the time between the last dose of Poly E and surgical excision of the prostate was more than 24 hours because of restrictions on oral intake on the day of surgery. In our trial, the median time interval between the last administration of GSP and surgery was 5 hours. The long elapsed time of the prostate trial, as suggested by the authors, may explain the undetectable tissue levels at surgery. However, serum levels were obtained while subjects were still on Poly E intervention,

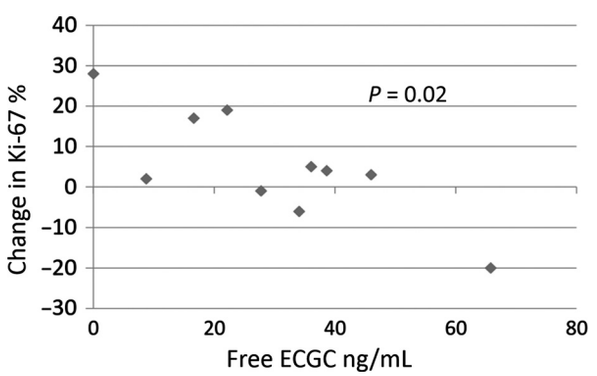

Figure 1.
Free EGCG plasma concentrations by change in Ki-67 between biopsy and surgery, adjusting for Ki-67 at biopsy and diameter of cancer.

suggesting a possible higher bioavailability of GSP compared with Poly E in plasma. Moreover, although a certain commonality between breast and prostate cancer exists (24), a direct comparison between the two organs in terms of biodistribution could be inappropriate. Although our analysis of toxicity is limited by the low number of examined patients, the treatment with GSP in our 12 patients for 28 days was well tolerated. However, considering a relatively high plasmatic concentration obtained with GSP, a future dose escalation trial with GSP, maybe with a "*bis-in-die*" schedule, could be foreseen to both determine the upper safety limit for future long-term intervention trials and to better understand the GSP preventive potential.

Finally, we evaluated the effects of GSP on cell proliferation in breast cancer tissue and on several serum biomarkers. We observed a positive correlation between free EGCG plasma levels and the decrease of tumor Ki-67 between biopsy and surgery. Patients with higher plasma concentration of free EGCG at surgery had a greater decrease of Ki-67 ($P = 0.02$, Fig. 1). Notably, the only case with a 30% increase of Ki-67 occurred in a patient with no detectable plasma free EGCG and the triple-negative histology, in line with its highly proliferating curve 4 weeks apart (25). However, our results on Ki-67 should be interpreted with caution, as the lack of a control arm limits the interpretations of our findings. Very recently, a randomized double-blind placebo-controlled trial examining the effects of 1-year intervention of an oral green tea extract (containing 843 mg EGCG) in 800 postmenopausal women did not show any overall reductions in mammographic density, nor in circulating biomarkers of breast cancer risk (26, 27). In our pilot study, most circulating biomarkers were not modulated. We noted a slight increase of testosterone concentrations after GSP supplementation, although within normal ranges. A potential explanation is the inhibitory effect of EGCG on testosterone glucuronidation and, consequently, a decreased renal excretion (28). Prediagnostic testosterone is associated with increased breast cancer risk both in pre- and postmenopausal women (29, 30), but testosterone's direct effect at the androgen receptor of breast tissue is antiproliferative, proapoptotic, and inhibits ER activity (31, 32). On the other hand, bicalutamide, a nonsteroidal antiandrogen, has been associated with clinical responses in triple-negative breast cancer with androgen receptor expression (33).

Several authors have addressed the hypothesis of adding green tea to breast cancer therapy (34, 35). In particular, the coadministration of green tea and tamoxifen seems to improve the outcomes in breast cancer cell lines (36, 37) and animal models (38, 39). Furthermore, observational studies have evaluated green tea in relation to gynecologic cancers and a meta-analysis reported that green tea intake was associated with a 23% decrease in endometrial cancer risk (40, 41), which is the biggest concern of patients taking tamoxifen for adjuvant treatment and prevention (42). With all the caution necessary when extrapolating data from preclinical studies or retrospective trials, a future step might be the evaluation of the combination of GSP with low-dose tamoxifen (43), which has shown a potential preventive effect on high-risk DCIS in postmenopausal women (44).

In conclusion, we have shown for the first time that GSP is able to reach human breast tissue. Further clinical investigation is needed to confirm the potential of GSP in cancer prevention.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

Authors' Contributions

Conception and design: M. Lazzeroni, D. Serrano, M. Cazzaniga, G. Pruneri, A. Riva, G. Petrangolini, P. Morazzoni, A. DeCensi, B. Bonanni
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