Chemoprevention of Human Prostate Cancer by Oral Administration of Green Tea Catechins in Volunteers with High-Grade Prostate Intraepithelial Neoplasia: A Preliminary Report from a One-Year Proof-of-Principle Study

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Abstract

Green tea catechins (GTCs) proved to be effective in inhibiting cancer growth in several experimental models. Recent studies showed that 30% of men with high-grade prostate intraepithelial neoplasia (HG-PIN) would develop prostate cancer (CaP) within 1 year after repeated biopsy. This prompted us to do a proof-of-principle clinical trial to assess the safety and efficacy of GTCs for the chemoprevention of CaP in HG-PIN volunteers. The purity and content of GTCs preparations were assessed by high-performance liquid chromatography [(−)-epigallocathechin, 5.5%; (−)-epicatechin, 12.24%; (−)-epigallocatechin-3-gallate, 51.88%; (−)-epicatechin-3-gallate, 6.12%; total GTCs, 75.7%; caffeine, <1%]. Sixty volunteers with HG-PIN, who were made aware of the study details, agreed to sign an informed consent form and were enrolled in this double-blind, placebo-controlled study. Daily treatment consisted of three GTCs capsules, 200 mg each (total 600 mg/d). After 1 year, only one tumor was diagnosed among the 30 GTCs-treated men (incidence, ~3%), whereas nine cancers were found among the 30 placebo-treated men (incidence, 30%). Total prostate-specific antigen did not change significantly between the two arms, but GTCs-treated men showed values constantly lower with respect to placebo-treated ones. International Prostate Symptom Score and quality of life scores of GTCs-treated men with coexistent benign prostate hyperplasia improved, reaching statistical significance in the case of International Prostate Symptom Scores. No significant side effects or adverse effects were documented. To our knowledge, this is the first study showing that GTCs are safe and very effective for treating premalignant lesions before CaP develops. As a secondary observation, administration of GTCs also reduced lower urinary tract symptoms, suggesting that these compounds might also be of help for treating the symptoms of benign prostate hyperplasia. (Cancer Res 2006; 66(2): 1234-40)

Introduction

The incidence of prostate cancer (CaP) is steadily increasing in the U.S. and Europe. Actually, it has become the second leading cause of cancer-related deaths among men in western countries, thus representing a major (and growing) health and social problem. When truly organ-confined, radical prostatectomy or radiation therapy are the therapeutic approaches of choice, but after it has spread to local and distant sites, hormonal therapy remains the most generally used chemotherapy for this disease. However, in nearly all men, advanced CaP eventually becomes refractory to hormonal therapy, which results in cancer recurrence. Because of the unfavorable prognosis of high-grade organ-confined and extraprostatic CaP, early detection at potentially curable stages makes sense, however, screening has never been shown to decrease CaP mortality. On the other hand, the fact that CaP onset and progression takes considerable time to occur can be considered as an important opportunity for treating premalignant lesions. Thus, at present, prevention may be the best approach to fight this frequent disease.

Lifestyle-related factors, particularly the diet, are considered to be the major contributors to CaP promotion. Although clinical evidences are still rather sparse and not definitive, several epidemiologic studies have focused on the lower incidence of CaP in Asian countries where green tea is consumed regularly (1), as compared with western populations. Moreover, the risk for CaP returns in Asian immigrants to the U.S. if original dietary habits are abandoned (1). Recently, a case-control study conducted in China showed that green tea consumption is etiologically associated with CaP, suggesting the protective effect of green tea against this disease (2). This body of evidence has suggested that administration of biologically active compounds from green tea might be effective in lowering the incidence of CaP.

It is well known that the active compounds released and found in highest amounts in the dry matter of green tea infusion are catechins, the most common of which are (−)-epigallocatechin-3-gallate (EGCG), (−)-epigallocathechin (EGC), (−)-epicatechin-3-gallate (ECG), and (−)-epicatechin (EC). Green tea catechins (GTCs), and especially EGCG, have been shown to be potent chemopreventive agents *in vitro* and in many *in vivo* animal models of induced carcinogenesis (3, 4). The systematic study of the biological and biochemical properties of GTCs only started quite recently, searching for possible molecular explanations for their effect on cancer cells. Endocrine changes occurring upon GTCs or EGCG administration (5), and/or inhibition of 5-α-reductase (the prostatic enzyme transforming testosterone into the more active androgen, 5-α-dihydrotestosterone) have been often suggested as key events capable of inhibiting CaP burden (6, 7). We previously showed that apoptotic cell death was specifically induced by EGCG in both SV40-immortalized (PNT1A) and tumorigenic (PC-3) human prostate cells by the activation of caspase-cascade without

Note: S. Bettuzzi and M. Brausi contributed equally to this work.

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Table 1. Complete list of individual variables

Case no.	Age (a)	PSA at the time of enrollment (b)	Prostate volume at the time of enrollment, mL (c)	Prostate volume at the end of the study, mL (d)	No. of HG-PIN cores/total cores taken at the time of enrollment (e)	No. of total cores taken at the end of the study (f)
(A) Placebo arm						
1	56	7.00	45	50	4 of 8	10
2	71	8.20	90	90	1 of 8	10
3	61	8.00	38	32	2 of 8	10
4	56	1.15	25	28	1 of 8	10
5	78	7.00	35	35	1 of 10	12
6	61	4.22	43	40	4 of 8	12
7	74	19.19	52	50	2 of 10	12
8	59	21.70	75	75	2 of 10	10
9	68	5.60	60	65	3 of 8	8
10	70	7.12	70	73	6 of 10	10
11	61	4.33	36	36	1 of 12	10
12	68	5.61	35	38	1 of 12	12
13	73	3.30	45	45	2 of 14	12
14	60	4.92	85	85	1 of 12	12
15	74	2.82	28	30	1 of 10	12
16	58	5.40	35	32	1 of 8	8
17	71	5.25	38	38	6 of 10	12
18	55	9.90	70	70	1 of 8	10
19	70	6.77	70	70	1 of 12	12
20	59	5.32	22	22	1 of 10	10
21	64	8.53	80	80	1 of 8	12
22	63	5.80	60	60	3 of 12	12
23	74	15.22	33	33	4 of 10	10
24	72	2.64	25	23	1 of 10	10
25	68	9.40	100	100	3 of 12	14
26	68	35.70	35	35	1 of 10	12
27	57	6.11	60	60	1 of 10	12
28	68	4.76	45	45	1 of 10	10
29	56	2.07	25	26	1 of 10	12
30	60	6.07	38	35	1 of 8	12
Mean ± SD	65.1 ± 6.8	7.9 ± 6.9	49.9 ± 21.5	50.0 ± 21.8	N/A	N/A
				Total cores	296	330
				No. of monofocal HG-PIN	18	
				No. of plurifocal HG-PIN	12	
(B) GTC arm						
31	62	15.61	56	50	1 of 10	12
32	57	5.50	45	48	1 of 10	10
33	54	8.33	55	48	1 of 12	12
34	61	8.00	52	58	1 of 8	12
35	63	5.13	68	70	2 of 10	12
36	75	7.93	43	45	3 of 10	12
37	48	4.50	38	35	1 of 10	10
38	68	7.40	55	60	1 of 8	10
39	65	5.50	35	30	2 of 12	12
40	63	4.22	35	33	2 of 12	12
41	70	17.00	42	45	1 of 10	10
42	70	9.88	26	25	6 of 10	12
43	70	6.40	32	31	4 of 10	12
44	64	6.41	35	37	1 of 10	12
45	69	10.43	90	95	5 of 10	14
46	65	6.78	38	42	2 of 8	10

(*Continued on the following page*)

Table 1. Complete list of individual variables (Cont'd)

Case no.	Age (a)	PSA at the time of enrollment (b)	Prostate volume at the time of enrollment, mL (c)	Prostate volume at the end of the study, mL (d)	No. of HG-PIN cores/total cores taken at the time of enrollment (e)	No. of total cores taken at the end of the study (f)
(B) GTC arm						
47	60	4.65	27	25	1 of 8	10
48	61	6.02	42	44	6 of 12	12
49	69	3.30	50	48	2 of 10	12
50	61	6.26	82	75	2 of 14	14
51	58	5.36	40	38	1 of 8	10
52	64	7.00	85	87	2 of 10	12
53	65	7.60	110	100	1 of 8	14
54	72	13.60	46	44	1 of 10	12
55	67	2.37	30	30	2 of 12	12
56	65	12.00	45	40	1 of 10	10
57	65	15.85	50	55	1 of 14	12
58	66	3.40	38	42	1 of 8	10
59	75	6.42	45	38	1 of 10	12
60	60	6.30	35	33	1 of 10	10
Mean ± SD	64.4 ± 5.9	7.6 ± 3.8	49.0 ± 19.8	48.3 ± 19.5	N/A	N/A
				Total cores	304	346
				No. of monofocal HG-PIN	17	
				No. of plurifocal HG-PIN	13	
(C) Summary and statistical analysis						
Placebo arm						
Mean ± SD	65.1 ± 6.8	7.9 ± 6.9	49.9 ± 21.5	50.0 ± 21.8		
P values (95% confidence, end vs. enrollment)				0.813		
GTC arm						
Mean ± SD	64.4 ± 5.9	7.6 ± 3.8	49.0 ± 19.8	48.3 ± 19.5		
P values (95% confidence, end vs. enrollment)				0.427		
P values (95% confidence, placebo vs. GTC)	0.670	0.819	0.862	0.756		

NOTE: (a) Age of subject at the time of diagnosis, (b) total serum PSA at the time of enrollment, (c) prostate volume at the time of enrollment, (d) prostate volume at the end of study, (e) total number of HG-PIN cores / total number of HG-PIN cores versus total cores taken at the time of enrollment, (f) total number of HG-PIN cores taken at the end of study of volunteers of placebo arm (A) and GTC arm (B). At the end of the study, only 1 of 24 patients in the placebo arm and 2 of 29 patients of the GTC arm were diagnosed for HG-PIN. The summary of such data (mean values ± SD) are also provided (C). Statistical analysis (*t* test analysis) shows that none of the variables considered were significantly different in the two arms of the study with 95% confidence (age, $P = 0.670$; PSA, $P = 0.819$; prostate volume at the enrollment, $P = 0.862$; prostate volume at the end of study, $P = 0.756$). Also prostate volumes at the enrollment were not significantly different from those at the end of the study in placebo arm ($P = 0.813$) or GTC arm ($P = 0.427$).

any significant effect on benign controls (normal human prostate epithelial cells in primary culture from cystectomy; ref. 8).

The transgenic adenocarcinoma of the mouse prostate (TRAMP) mouse model is a well known *in vivo* animal model for CaP displaying *in situ* and invasive carcinoma of the prostate that mimics the whole spectrum of human CaP progression from prostate intraepithelial neoplasia (PIN) to androgen-independent disease (9, 10). Recent studies have shown that GTCs administration can actually prevent CaP development in this model (11). We confirmed this finding, showing that p.o. administration of GTCs to TRAMP mice reduced CaP incidence at 24 weeks from 100% to 20% without any side effects (8), and suggested that GTC's action might be mediated by the induction of the expression of Clusterin (CLU). The *CLU* gene is potently up-regulated during prostate gland involution (12), but is down-regulated in human CaP specimens (13, 14) and, as previously reported by us, in PNT1a and PC-3 cells, it exerts antiproliferative (15) and proapoptotic activities (16–19). In the prostate glands of TRAMP mice responding to GTCs treatment, CLU expression was maintained at high levels, and, shortly before the induction of casp-9 expression/activation, and concomitantly to decreased expression of histone H3 mRNA (a specific marker of cell proliferation; ref. 8), localized to the nuclei, in which it is known to

exert a proapoptotic role (17–19). These data, altogether, confirmed that GTCs exert potent and selective *in vitro* and *in vivo* proapoptotic activity on prostate cancer cells.

Nevertheless, no definitive clinical data demonstrating the efficacy of GTCs as chemopreventive agents in humans have thus far been produced. Thus, clinical studies for the evaluation of safety and effectiveness of these agents in cancer chemoprevention, both individually and in combination, are needed (20). Our preclinical results prompted us to do a proof-of-principle clinical trial to assess the possible efficacy of GTCs for the chemoprevention of CaP.

In order to obtain this information as quickly as possible, the study was done in a selected population of 60 human volunteers with high-grade PIN (HG-PIN), the main premalignant lesion of CaP (21), known to result in a substantial number of cancers in a 1-year period (22, 23). Because, at present, no treatment is given to these patients until CaP is diagnosed, our study can be envisaged as an attempt to fill this therapeutic void. The primary end point of this study was to determine a possible difference in the prevalence of CaP in the GTCs-treated arm in comparison to placebo. Possible changes in total serum prostate-specific antigen (PSA) values, as well as possible variations in lower urinary tract symptoms (LUTS), as assessed by International Prostate Symptom Score (IPSS; ref. 24) and quality of life score (QoL; ref. 25) in men with coexistent benign prostate hyperplasia (BPH) were also pursued as secondary observations.

Materials and Methods

Study design. The study was conducted on volunteers with HG-PIN. At present, in Italy, this condition is clinically managed simply by performing saturation biopsies of the prostate every 3 to 6 months, in search of possible coexisting CaP (21, 26). Recent studies have quantified the risk for invasive prostate cancer in men with HG-PIN, and it was suggested that the prevalence of CaP was as high as 30% within 1 year after repeated biopsy (22, 23). These data suggest that in the near future, therapy of HG-PIN may become a useful approach for inhibiting the development of CaP. Clinical benefits in case of effective treatment would include reduced morbidity, enhanced quality of life, delayed surgery or radiation, and increase in the interval for surveillance requiring invasive procedures (26).

To investigate whether the administration of GTCs could prevent malignancy in men at high-risk, 60 volunteers (Caucasian men) bearing HG-PIN lesions, to whom no therapy is commonly given, were enrolled in this double-blind, placebo-controlled study. Because pure HG-PIN is a rather rare finding, recruitment time extended up to 18 months to reach the appropriate number of subjects for each arm. Volunteers were randomly assessed to a placebo- or GTCs-arm by simple randomization. More precisely, subjects were called for an informative interview within 2 weeks from the time of diagnosis and asked to join the study by signing the informed consent. That same day, they were alternatively assigned to the placebo- or GTCs-arm and given the appropriate treatment. To all subjects, capsules were given by the urologist according to the double blind method. Compliance with study medication was assessed by pill count/returned blister packs. Due to the enthusiastic adherence to the study by highly motivated subjects at high risk for CaP, all volunteers took the complete medication assigned in all cases, thus the compliance was very good in both arms and did not differ between the two groups. The GTCs-arm volunteers received three capsules per day containing 200 mg each of GTCs, for a total of 600 mg of GTCs per day. GTCs were given as a high-quality preparation whose content was determined by high-performance liquid chromatography as previously published (8). Content was as follows: EGC, 5.5%; EC, 12.24%; EGCG, 51.8%; ECG, 6.12%; total GTCs, 75.7%; virtually caffeine-free (caffeine <1%). In the second arm, men received placebo (three identical capsules per day). The primary end point was the prevalence of CaP during the 1-year study in the two arms. Although the study was specifically designed for assessing the possible chemopreventive action of GTCs, we also recorded possible changes in total serum PSA values during the whole study, and possible variations in LUTS, as assessed by IPSS and QoL scores before GTCs administration, and after 3 months of treatment in a subset of men with coexistent BPH, not receiving any other therapy. In case of cancer diagnosis, subjects were excluded from the chemoprevention trial (failure of chemoprevention) and recommended to clinical management.

Eligibility criteria. Men ages 45 to 75 years old with HG-PIN found after the collection of 8 to 18 needle biopsies according to prostate volume. All subjects were volunteers, who were made aware of the study details, and agreed to sign an informed consent form.

Exclusion criteria. Men aged >75 years, consuming green tea or derived products, vegetarians, taking antioxidants, and following antiandrogenic therapy. Patients diagnosed with cancer were excluded from the chemoprevention trial (failure of chemoprevention) and recommended to clinical management.

Clinical trial results and follow-up studies. Medical history, physical examination, and evaluation of total PSA were carried out every 3 months following the recruitment date. Six and 12 months after the beginning of the study, each subject had prostate mapping by 8 to 14 core needle biopsy examination, the number chosen according to prostate volume. Subjects exhibiting a sudden increase of total PSA levels, alterations of other clinical variables or symptoms of prostate disease (referred during physical examination) underwent needle biopsy earlier, at the physician's discretion. During the whole study, the urologists were continuously in contact with volunteers to detect any possible adverse or side effects.

Total serum PSA determination. All total serum PSA determinations were carried out in the same central laboratory of the hospital by using a two-site immunoluminometric *in vitro* commercially available assay kit for quantitative determination of human prostate–specific antigen in human serum and plasma.

Statistical analysis. For PSA, data are expressed as mean values ± SD. For IPSS and QoL, data are expressed as mean values. Statistical significance was calculated by the Student's t test and P values are indicated with 95% confidence. Multivariate analyses of variables in Table 1 were done with the one-way MANOVA test.

Results

Primary end-point: prevalence of prostate cancer. Table 1A and B shows the complete list of individual variables (a, age of subject at the time of diagnosis; b, total serum PSA at the time of enrollment; c, prostate volume at the time of enrollment; d, prostate volume at the end of study; e, total number of HG-PIN cores versus total cores taken at the time of enrollment; f, total number of HG-PIN cores taken at the end of study; total number of monofocal or plurifocal HG-PIN lesions) of 30 volunteers of placebo-arm (A) and 30 volunteers of GTCs-arm (B). The summary of such data (mean values ± SD), are also provided (C). Statistical analysis (t test analysis) showed that none of the variables considered were significantly different in the two arms of the study with 95% confidence (age, $P = 0.670$; PSA, $P = 0.819$; prostate volume at the time of enrollment, $P = 0.862$; prostate volume at the end of study, $P = 0.756$). Also, prostate volumes at the enrollment were not significantly different from those at the end of the study in the placebo-arm ($P = 0.813$) or the GTCs-arm ($P = 0.427$). In addition, multivariate analysis using all the above variables at the time of initial diagnosis showed no significant differences in the two arms with a 99.99% confidence. Thus, none of these variables may account for the difference in prevalence of CaP in the two arms of the study, which are reported in Table 2. After 1 year of treatment, only one cancer was diagnosed among the 30 men that received GTCs daily, with a final incidence of about 3%; instead, nine cancers were found among the 30 men treated with placebo, with a final incidence of 30%, a figure close to that expected

(21, 22). In particular, in the placebo-arm, six cancers were found 6 months after the recruitment (at the 6-month biopsy check), and three more cancers were found at the 12-month biopsy check (end of the study). The only cancer found in the GTCs-arm was detected at the 12-month biopsy check. Altogether, this suggests a 90% chemoprevention efficacy of GTCs in men subjected to high risk for developing CaP (Table 2). Statistical analysis showed that this result was highly significant ($P < 0.01$; Table 2).

Secondary observation: total serum PSA values. It is known that PIN lesions do not significantly elevate serum PSA per se. In the cohort of 60 men enrolled in the study, total serum PSA ranged from 0.70 to 35.70 ng/mL (mean, 7.7 ng/mL) at the recruitment time. In the 30 men of the placebo-arm, PSA varied from 1.15 to 35.70 ng/mL (mean, 7.97 ng/mL), whereas in those of the GTCs-arm, it ranged from 0.70 to 15.85 ng/mL (mean, 7.57 ng/mL). Although total serum PSA value was not taken into consideration for assigning volunteers to the placebo- or GTCs-arm, the mean PSA values was very similar in the two arms at the beginning of the study (Table 1C). GTCs treatment did not significantly affect PSA values throughout the study, probably because of high individual differences reflected by different total range between placebo- and GTCs-arm (Fig. 1). In any case, it may be worth noticing that the mean value of total PSA was always lower in the GTCs-arm at any time point with respect to control, and a trend toward a more stable total PSA value was clearly evident in GTCs-treated men.

Secondary observation: changes in LUTS as assessed by IPSS and QoL scores. In the cohort of volunteers, 18 out of 30 in the placebo-arm and 17 out of 30 in the GTCs-arm had BPH and were suffering LUTS. We quantified LUTS at the beginning of the study and after 3 months of treatment by assessing both IPSS (27) and QoL (25) scores. Volunteers agreed not to undergo any therapy for LUTS during the 3-month subtrial. Changes in LUTS are shown in Table 3. A decrease, small but significant ($P < 0.05$), in IPSS score was found in GTCs-treated arm for 3 months as compared with placebo. Improvement of IPSS was found in 65% of GTCs-treated men. Also, QoL score decreased in 35% of the men in the study following GTCs treatment, reaching close to statistical significance ($P = 0.08$), whereas no changes were found in placebo-treated men.

Medical events and side effects. No significant side or adverse effects were documented throughout the whole study as reported during physical examinations that were done every 3 months. In the study, only two cases of diarrhea on each arm were reported and rated as very mild disorders. These rare events have been considered to be unrelated to GTCs administration because the incidence reported in the GTCs-treated arm was identical to that reported in the placebo arm (two versus two), and they disappeared spontaneously within 3 days, thus allowing study completion for these subjects.

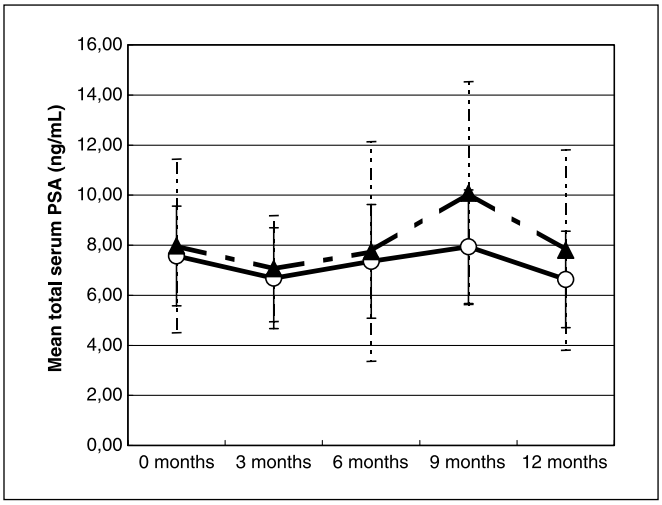

Figure 1. Trend of serum total PSA (mean ± SD) in volunteers of the placebo-arm (▲) and the GTC-arm (○) during the 1-year study.

Discussion

To our knowledge, this is the first study showing that GTCs have potent *in vivo* chemoprevention activity for human CaP. Altogether, our data suggest that up to 90% of chemoprevention efficacy can be obtained by GTCs administration in men prone to develop CaP. Thus, administration of GTCs could be an effective therapy for treating premalignant lesions of high-risk subjects, thus filling a therapeutic void (21) by taking advantage of an important window of opportunity for treatment before CaP develops. If confirmed, our finding suggests a new scenario in which the incidence of this disease could be greatly reduced by simply making GTCs available to the elderly or men at high-risk, resulting in a tremendous social and clinical impact, especially in the Western countries. The fact that no side or adverse effects have been reported confirm that GTCs, at least at the dosage used here, are safe in humans. This was also shown previously (28). In fact, in preparation for future trials, a study was conducted to determine the safety and pharmacokinetics of green tea polyphenol administration by using pure EGCG

Table 2. Prevalence of prostate cancer in placebo arm and GTC arm at the 6-month biopsy checkpoint and at the end of the 1-year study (12 months biopsy checkpoint)

Study arm	6-months biopsy check	12-months biopsy check	Total no. of CaPs diagnosed/ total no. of recruited subjects	Prevalence of prostate cancer
Placebo	6 of 30	3 of 24*	9 of 30	30.0%
GTCs	0 of 30	1 of 30	1 of 30	3.3%
P value (95% confidence, placebo vs. GTC)				<0.01

*Six patients, diagnosed with prostate cancer at the 6 months biopsy check, left the study and were not subjected to the subsequent 12 months biopsy check. Statistical analysis (*t* test analysis) shows that the difference in prostate cancer prevalence between the two arms was highly significant ($P < 0.01$).

Table 3. Changes in LUTS as assessed by IPSS and QoL scores the in placebo arm and GTC arm after 3 months of treatment

	Initial IPSS (mean)	3 months IPSS (mean)	*P*	Percentage of men with reduced IPSS (%)	Initial QoL (mean)	3 months QoL (mean)	*P*	Percentage of men with reduced QoL (%)
Placebo	8.27	7.00	0.14	46	1.30	1.47	0.27	7
GTCs	11.12	9.12	0.04	65	2.06	1.76	0.08	35

NOTE: Statistical analysis (*t* test analysis) and *P* values (95% confidence) are indicated, showing that the decrease in IPSS score was statistically significant ($P < 0.05$).

or Polyphenon E, a defined, decaffeinated green tea polyphenols mixture with a composition very similar to our GTCs preparation (28). Preliminary clinical trials showed that Polyphenon E is effective in the prevention of human papilloma virus–infected cervical lesions (29).

It is worth noticing that, as a secondary observation, we found a small but statistically significant improvement in LUTS in 65% of GTCs-treated volunteers with coexistent BPH as assessed by IPSS. Improvements were also found with regard to QoL score in the same men. This suggests that GTCs administration might also be of help to relieve the symptoms of BPH, although the possible mechanisms of action of GTCs on benign prostate diseases is still unknown. Nevertheless, because this result was not accompanied by a significant reduction of prostate volume (Table 1C), it seems unlikely to be related to a possible antiandrogenic action. Unfortunately, due to the fact that our study was not specifically designed to investigate the possible effects of GTCs on BPH, no further data are available on this cohort of men. Thus, the positive effect on LUTS recorded here should just be considered a rather promising observation for future research suggesting that other potential benefits may accompany GTCs administration.

It seems rather obvious that the chemopreventive effect exerted by GTCs on CaP development must be quickly confirmed by a larger study. Considering that all volunteers enrolled in this study were Caucasians, it would be particularly important to check whether GTCs treatment is also effective in high-risk men with different genetic backgrounds. Although follow-ups will continue for up to 5 years in the cohort of subjects studied here, a larger confirmatory study extending GTCs administration for up to 5 years would allow us to understand whether CaP onset could be definitively prevented or simply delayed by the treatment, and to exclude possible negative effects caused by long-term GTCs treatment. In addition, comparison of the histologic features of the tumors diagnosed in the two arms will also permit us to verify the possible effects of GTCs on cell differentiation, clinical stage and aggressiveness of CaPs, goals that could not be achieved in the present contribution. The importance of this issue is supported by a recent report showing that Finasteride was effective in reducing the prevalence of CaP by 28.8% over a 7-year period, but apparently, cancers with Gleason grade from 7 to 10 were more common in the Finasteride arm than in the placebo arm (30). The number of cancers found at the end of the trial were too small to evaluate the effect of GTCs on cancer grade. We still believe that knowing whether or not long-term GTCs administration affects the grading and staging of CaPs is crucial information that is needed and could possibly be obtained with a second confirmatory study employing a much larger number of volunteers.

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References

1. Nelson WG, De-Marzo AM, Isaacs WB. Prostate cancer. N Engl J Med 2003;349:366–81.
2. Jian L, Xie LP, Lee AH, Binns CW. Protective effect of green tea against prostate cancer: a case-control study in southeast China. Int J Cancer 2004;108:130–5.
3. Liao S, Kao YH, Hiipakka RA. Green tea: biochemical and biological basis for health benefits. Vitam Horm 2001;62:61–94.
4. Liao S, Umekita Y, Guo J, Kokontis JM, Hiipakka RA. Growth inhibition and regression of human prostate and breast tumors in athymic mice by tea epigallocatechin gallate. Cancer Lett 1995;96:239–43.
5. Kao YH, Hiipakka RA, Liao S. Modulation of endocrine systems and food intake by green tea epigallocatechin gallate. Endocrinology 2000;141:980–7.
6. Liao S. Hiipakka RA. Selective inhibition of steroid 5α-reductase isozymes by tea epicatechin-3-gallate and epigallocatechin-3-gallate. Biochem Biophys Res Commun 1995;214:833–8.
7. Hiipakka RA, Zhang HZ, Dai W, Dai Q, Liao S. Structure-activity relationships for inhibition of human 5α-reductases by polyphenols. Biochem Pharmacol 2002; 63:1165–76.
8. Caporali A, Davalli P, Astancolle S, et al. The chemopreventive action of catechins in the TRAMP mouse model of prostate carcinogenesis is accompanied by clusterin over-expression. Carcinogenesis 2004;25:2217–24.
9. Gingrich JR, Barrios RJ, Morton RA, et al. Metastatic prostate cancer in a transgenic mouse. Cancer Res 1996; 56:4096–102.
10. Kaplan-Lefko PJ, Chen TM, Ittmann MM, et al. Pathobiology of autochthonous prostate cancer in a pre-clinical transgenic mouse model. Prostate 2003;55: 219–37.
11. Gupta S, Hastak K, Ahmad N, Lewin JS, Mukhtar H. Inhibition of prostate carcinogenesis in TRAMP mice by oral infusion of green tea polyphenols. Proc Natl Acad Sci U S A 2001;98:10350–5.
12. Bettuzzi S, Hiipakka RA, Gilna P, Liao ST. Identification of an androgen-repressed mRNA in rat ventral prostate as coding for sulphated glycoprotein 2 by cDNA cloning and sequence analysis. Biochem J 1989;257:293–6.
13. Bettuzzi S, Davalli P, Astancolle S, et al. Tumor progression is accompanied by significant changes in the levels of expression of polyamine metabolism regulatory genes and clusterin (sulfated glycoprotein 2) in human prostate cancer specimens. Cancer Res 2000;60:28–34.
14. Scaltriti M, Brausi M, Amorosi A, et al. Clusterin (SGP-2, ApoJ) expression is downregulated in low- and high-grade human prostate cancer. Int J Cancer 2004; 108:123–30.
15. Bettuzzi S, Scorcioni F, Astancolle S, Davalli P, Scaltriti M, Corti A. Clusterin (SGP-2) transient overexpression decreases proliferation rate of SV40-immortalized human prostate epithelial cells by slowing down cell cycle progression. Oncogene 2002;21:4328–34.
16. Scaltriti M, Bettuzzi S, Sharrard RM, Caporali A, Caccamo AE, Maitland NJ. Clusterin overexpression in both malignant and nonmalignant prostate epithelial cells induces cell cycle arrest and apoptosis. Br J Cancer 2004;91:1842–50.

17. Caccamo AE, Scaltriti M, Caporali A, et al. Cell detachment and apoptosis induction of immortalized human prostate epithelial cells are associated with early accumulation of a 45 kDa nuclear isoform of clusterin. Biochem J 2004;382:157–68.
18. Scaltriti M, Santamaria A, Paciucci R, Bettuzzi S. Intracellular clusterin induces G2-M phase arrest and cell death in PC-3 prostate cancer cells. Cancer Res 2004; 64:6174–82.
19. Caccamo AE, Scaltriti M, Caporali A, et al. Ca(2+) depletion induces nuclear clusterin, a novel effector of apoptosis in immortalized human prostate cells. Cell Death Differ 2005;12:101–4.
20. Moyers SB, Kumar NB. Green tea polyphenols and cancer chemoprevention: multiple mechanisms and endpoints for phase II trials. Nutr Rev 2004;62: 204–11.
21. Bostwick DG, Qian J. High-grade prostatic intraepithelial neoplasia. Mod Pathol 2004;17:360–79.
22. Bishara T, Ramnani DM, Epstein JI. High-grade prostatic intraepithelial neoplasia on needle biopsy: risk of cancer on repeat biopsy related to number of involved cores and morphologic pattern. Am J Surg Pathol 2004;28:629–33.
23. Kronz JD, Allan CH, Shaikh AA, Epstein JI. Predicting cancer following a diagnosis of high-grade prostatic intraepithelial neoplasia on needle biopsy: data on men with more than one follow-up biopsy. Am J Surg Pathol 2001;25:1079–85.
24. O'Leary MP. Quality of life and sexuality: methodological aspects. Eur Urol 2001;40 Suppl 43:13–48.
25. Grumann M, Schlag PM. Assessment of quality of life in cancer patients: complexity, criticism, challenges. Onkologie 2001;24:10–5.
26. Steiner MS. High-grade prostatic intraepithelial neoplasia and prostate cancer risk reduction. World J Urol 2003;21:15–20.
27. Denis LJ. Future implications for the management of benign prostatic hyperplasia. Eur Urol 1994;25 Suppl 21: 29–34.
28. Chow HH, Cai Y, Hakim IA, et al. Pharmacokinetics and safety of green tea polyphenols after multiple-dose administration of epigallocatechin gallate and polyphenon E in healthy individuals. Clin Cancer Res 2003;9: 3312–9.
29. Ahn WS, Yoo J, Huh SW, et al. Protective effects of green tea extracts (polyphenon E and EGCG) on human cervical lesions. Eur J Cancer Prev 2003;12:383–90.
30. Thompson IM, Goodman PJ, Tangen CM, et al. The influence of finasteride on the development of prostate cancer. N Engl J Med 2003;349:215–24.

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