GREEN TEA CATECHINS EXTRACT (GTE) FOR PATIENTS WITH PRE-NEOPLASTIC LESIONS

An "early therapy" that is very effective at inhibiting human prostate cancer progression.

A tea plantation

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THE NEW HOPE: NATURAL COMPOUNDS TO CURE EARLY PROSTATE CANCER

Prostate cancer (CaP) is the second leading cause of cancer-related death among men in Western countries. At the moment, CaP represents a major health problem, growing as populations age (1). It has been recently announced that CaP is now the most lethal cancer in Italian male population: this finding is certainly in relation with the fact that Italians are now the oldest population in Europe, as recently published on the Lancet journal.
When CaP is truly organ confined, the standard therapies used are radical prostatectomy or radiation therapy. Both can be curative, but when the disease became aggressive and spreads to local and distant sites, the most generally used systemic chemotherapy is hormonal therapy, i.e. androgen ablation. The rationale for the anti-androgen therapy still derives from a Dr C. Huggins' finding that goes back in the '30s, when he found that surgical castration can delay the progression of disease because CaP is androgen-dependent in the early phases. But unfortunately, probably under the pressure of androgen-ablation, too often CaP eventually becomes refractory to hormonal therapy. Under these conditions further progression is unavoidable and the final outcome is usually the loss of the patient because of metastatic diffusion to bones. In consideration of the unfavourable prognosis of metastatic CaP, prevention strategies and early detection at potentially curable stages of the disease are highly desirable. CaP exhibit a peculiar feature we can take advantage of : progression and diagnosis of clinical disease take decades, i.e. CaP is a very slow progressing disease. This is also the explanation why diagnosis is usually in elderly men. Therefore CaP is an ideal target for chemoprevention strategies (2, 3). We will show that chemoprevention of CaP in humans with safe, natural and very effective compounds is actually possible at early stages of development in a real clinical setting.

Only after the advent of Prostate Specific Antigen (PSA) testing in the early 1990's, diagnosis of CaP became common in younger men. The finding of a raise in PSA, although not a definitive proof of cancer, induced millions of men to receive needle biopsy. As a consequence, patients were diagnosed with CaP at increasingly younger ages. This has led to a huge increase in radical prostatectomy. Surgical treatment of CaP is costly, and important side-effects such as impotency are common among patients. But the most frighting aspect of this story is that early screening and radical prostatectomy have not been shown to decrease CaP mortality or survival time (1, 4-8).

In this scenario, inhibition of progression of sub-clinical CaP toward more aggressive stages would be the most effective therapy. Our work gave an important contribution to pave a new way to address this issue. The new hope now rely on natural compounds showing anti-tumor activity against CaP. In the next part of this work we will show that administration of a Green Tea Extract rich in Catechins (GTE) is now the most promising approach available for patients bearing pre-neoplastic lesion, subject to confirmatory trials.

The tea room in a tipical Japanese house

ANTI-CANCER ACTIVITY OF GREEN TEA EXTRACT IN VITRO AND IN ANIMAL MODELS

Firstly we started in vitro testing of the potential of GTE for chemoprevention working with cell lines. Under these conditions, we showed that GTE specifically triggered self-killing (apoptosis) of SV40-immortalized and prostate cancer cells (SV40-immortalized cells mimic the early stages of cell transformation). Very importantly, benign cells were unaffected. Thus, the anti-cancer effect was very specific to transformed cells and already effective against early stage of disease. The finding that CaP progression can be inhibited in vitro in cell-culture systems was then challenged in vivo working with animal models, such as the TRansgenic Adenocarcinoma of the Mouse Prostate (TRAMP) model, to validate the preliminary results. In this case, we decided to administer 0.3% of GTE in drink-

Picture by R. Longo

Green tea catechins can help against pre-neoplastic lesions

ing water to animals at weaning. Thus, our experimental model was aimed at preventing CaP development and not at curing clinically relevant CaP at late stage.
Composition and purity of the GTE extract was tested routinely in our laboratory by HPLC and it was as follows: total Catechins, 75.7%; EGCG, 51.9%; EC 12.2%; ECG 6.1%; EGC 5.5%. The GTE extract was virtually caffeine-free (<1.0%). For in vitro experiments in cell lines we used pure EGCG, the most active compound, while the GTE extract was used in vivo in animals for confirmation of the in vitro results. The results have been published (9) and a brief comment of this finding will follow below.

But confirmation of anti-tumor activity of GTE was only part of the project. In fact, we not only wanted to confirm the chemopreventative properties of GTE in vivo, but also to gain insight into the mechanism of action, since GTE apparently affects cancer cells but not their normal counterparts (10-11). In our study, we have also attempted to identify candidate biomarkers to monitor patient's response to GTE administration which can be potentially useful in the clinic. One of the most interesting genes popping up from our studies was Clusterin (CLU), also known as Apolipoprotein J (ApoJ). In our hands, intracellular accumulation CLU, and particularly accumulation of its nuclear form nCLU, had a potent anti-proliferative effect, also inducing pro-apoptotic activity in prostate cancer cells and thus committing transformed cells to death. Actually, CLU was found down-regulated in prostate cancer (12; see also www.oncomine.org). We have already proposed that CLU is a tumor-suppressor of CaP. Expression of CLU is lost in TRAMP mice during CaP progression. We found that GTE induced the expression of CLU back to high levels in cancer cells and responsive animals. Therefore, the key of anti-tumor action of GTE seems to be related to specific regulation of gene expression (9). This appear to be very different from the usual explanation which involves the well-known but generic "anti-oxidant" properties typical of catechins and EGCG in particular. Anti-oxidant properties are often used to explain their anti-cancer activity. In the case of CLU, for instance, its expression is up-regulated by many stressors, but do not change or is up-regulated by oxidative stress (ROS): thus, thinking very naively, anti-oxidants would have been expected to show the opposite effect on CLU from the one we have measured. This issue will be further discussed below.

The effects of GTE on CLU expression was very specific. In fact, gene expression was evaluated in SV-40 immortalized prostate cancer epithelial cells (PNT1A) and in tumorigenic, poorly differentiated, androgen-independent prostate cancer cells (PC-3) in close comparison to normal human prostate epithelial cells in primary culture obtained from cystectomies. PNT1A cells are non tumorigenic, so they mimic the early phases of cell transformation. PC-3 cells are metastatic and androgen-independent, thus closely resembling the late stage of aggressive CaP. In this system, we found that GTE, and particularly EGCG (the most abundant catechin in Green Tea), was more effective at killing PNT1A cells than PC-3 cells, i.e. the IC50 for PNT1a cells was lower. However, GTE did not reduce the growth of normal primary prostate cells, even at very high doses.
We interpreted this experimental data as suggesting that there is a selective action of GTE toward the early lesions of CaP. Remarkably, GTE specifically enhanced the expression of CLU in both PNT1A and PC-3 cells when IC50 concentrations were given, in comparison to normal primary cells were CLU expression was unchanged. Enhanced expression of CLU occurred in parallel with caspase activation and apoptosis induction, showing that GTE activated the apoptotic cell death pathway in transformed, but not normal, cells (9).

The TRAMP mice spontaneously develop in situ and invasive carcinoma of the prostate, mimicking human disease progression from Prostatic Intraepithelial Neoplasia (PIN) to androgen-independent and metastatic disease in an age-related fashion (13, 14). In this system, SV-40 T/t early antigens are specifically expressed in the prostate under the control of the Probasin promoter, an androgen-dependent gene. Disruption of cell cycle regulation is a consequence of abrogation of p53 and Rb function and inhibition of PP2A activity. Therefore, the mice develop microscopic lesions by 12 weeks of age, with all male animal harbouring clinical evidence of CaP upon autopsy by 24 weeks of age. Metastatic disease is frequently evident by 30 weeks. It has been proven that castration speeds up metastatic diffusion. We showed that GTE delays tumour development in the TRAMP model. Inhibition of CaP progression occurred in 80% of males. This important results showed that GTE are very effective in the animal model as anti-tumor agents. At the same time, these data made the TRAMP model an ideal experimental system for study of the impact of GTE on gene expression. In addition, none of the mice treated with GTE developed metastatic cancer, suggesting that GTE may also inhibit the spreading of disease at distant sites (9).

GENE EXPRESSION ANALYSIS OF RESPONSE TO GTE

We used the TRAMP model to further investigate gene expression and dis-regulation during development of CaP, comparing wild-type non-transgenic, TRAMP mice fed water and TRAMP mice given 0.3% GTE from weaning as the sole source of drinking water. GTE administration at 0.3% concentration reduced tumor development by 80% at 24 weeks. This dosage corresponds to 8 – 16 cups of green tea per day for a human. Our results confirmed those of Gupta (15) who gave only 40% of the GTE concentration used here showing 65% fewer mice with tumors at the same time-point.
Histological staining of prostate tissue from 24-week-old wild-type and TRAMP mice showed that the tissues obtained from GTE-treated animals had reverted to near normal (9). In these specimens, pro-apoptotic intracellular nuclear CLU was potently induced in epithelial cells. Animals responding to GTE displayed enhanced CLU expression

A flower of Camellia sinensis

followed by activation of caspase 9, while those refractory to GTE did not express either CLU or caspase 9 as measured by Western blot (9). These data from histology, Northern and Western blot studies were confirmed by RT-qPCR (16). We found that expression of CLU mRNA by RT-qPCR fell as mice aged from 17 to 24 weeks in wild-type. CLU expression fell much further in untreated TRAMP mice developing CaP. In TRAMP mice responding to GTE, CLU expression increased at 24 weeks, but in TRAMP mice unresponsive to GTE (i.e. which developed CaP, only 20% of animals) CLU expression fell to its lowest level at 24 weeks (16). Thanks to these experimental data, we infer that induction of pro-apoptotic nuclear CLU may mediate GTE action in transformed cells (9). More importantly, these important pre-clinical results encouraged us to study whether GTE administration was also effective in humans.

ANTI-CANCER EFFECT OF GTC IN HUMANS: THE CLINICAL TRIAL.

All together, results obtained by bench work prompted us to perform a clinical trial with GTE against human CaP. Although much of this preclinical work suggests that GTE may actually exert anti-cancer effects, experimental confirmation in humans had not yet been established. High-Grade Prostatic Intraepithelial Neoplasia (HGPIN) has been considered the most likely pre-invasive stage of CaP, but there are no treatment options for patients diagnosed with HGPIN. Following discovery of HGPIN, patients are tested by repeated biopsies every six months until clinical disease is diagnosed, then standard therapies are suggested.

We conducted a Phase II, placebo-controlled, proof-of-concept clinical trial to assess the efficacy of GTE in the prevention of CaP in patients diagnosed with HGPIN only (17). The primary endpoint of the study was the impact of GTE administration on the prevalence/progression of CaP. The secondary endpoints included the effect on total serum PSA levels and the possible changes in Lower Urinary Tract Symptoms (LUTS) as assessed by the International Prostate Symptom Score (IPSS; ref. 18) and measurement of Quality of Life (QoL; ref. 19) in men with co-existent BPH. As stated before, the most of patients experience LUTS, and actually presence of LUTS and BPH are the leading event to undergo needle biopsy.
Eligible males were 45-75 years of age with HGPIN discovered following saturation biopsy; 8-18 needle cores were collected depending on the volume of the prostate. Exclusion criteria included age >75 years, consumption of green tea or derived products, vegetarian diet, routine use of anti-oxidant products, and prior anti-androgen therapy.
A total of 60 subjects were enrolled in the trial and randomized in a double-blind fashion: 30 were treated with GTE capsules, 30 with placebo capsules of the same appearance. GTE were administered orally at 200 mg TID (total daily dose: 600 mg, equal to 3 capsules) for one year. Saturation prostate biopsy was performed at baseline (diagnosis), 6 months and 12 months; PSA was measured at 3, 6, 9 and 12 months. In the event of a diagnosis of CaP, the subject was withdrawn from the trial and referred for clinical management. At the end of the study, we found a significantly lower prevalence of CaP among the subjects who received GTE ($P<0.01$). CaP was diagnosed in one of the subjects treated with GTE (3%) versus 9 subjects among the placebo-treated subjects (30%). Subjects with BPH who received GTE also showed important improvement in both IPSS score and QoL score, with significant improvement noted in IPSS. This was the first clinical study to show that GTE reduce the incidence of cancer in humans. The incidence of cancer diagnosis was reduced by 90% during the first year. A secondary benefit observed in this trial was symptomatic reduction of LUTS in men with BPH (17).

As recently published (20), we followed the treatment of the participants in the trial. Additional biopsies were performed in 21 subjects in the two years following the conclusion of the study (i.e under condition of suspension of treatment). All together, 9 subjects from the placebo-arm and 13 from the GTE-arm underwent this fourth prostate mapping. The two arms remained balanced despite the low follow-up rate (57% and 55%, respectively). The mean follow-up from the end of GTE administration was 23.3 months for placebo-arm (range: 12-30) and 19.1 months for GTE-arm (range: 12-30). Three further cancer diagnoses appeared during follow-up, two in the placebo arm and one in the GTE-arm. The final result for the entire trial was 11 cancers detected in untreated HGPIN subjects versus 2 in those who took GTE for 1 year. The final difference in cancer prevalence is highly significant ($p < 0.01$) by χ^2 test analysis.

The early emergence of benefit already observed at 6 months (17) and the stable clinical condition found 2 years after suspension of treatment (20) suggests a rapid treatment effect on early lesions as suggested by our work in vitro. The treatment with GTE led to an almost 80% reduction in CaP diagnosis, from 53% to 11%, over three years of follow-up, suggesting that an important decrease of morbidity and mortality related to this disease could be achieved.
At the moment we are actively engaged at measuring the level of expression of CLU (for more information on CLU please see refs 21-23) by immunohistochemistry in tissue specimens obtained by needle biopsy from patients enrolled in the clinical trial. Preliminary data are suggesting that CLU is also up-regulated in humans following administration of GTE.
We do not know whether GTE would exert the same chemopreventative effect in a larger population comprised of men with a different genetic backgrounds (our study was entirely performed in Italy). It is also unknown whether long-term use of GTE affects differentiation, clinical staging, and aggressiveness of CaP, although data in the Japanese population do not suggest positive selection of aggressive CaP. For these reasons, a larger study is needed to confirm the results obtained in our proof-of-concept clinical trial. As a matter of fact, a larger confirmatory trial of these results is currently underway (Kumar N. et al, Moffitt Cancer Centre, Tampa Fl, USA; personal communication).

FINAL REMARKS

CaP is still an elusive disease and the fight against it is far from being over. A critical feature of this disease is that CaP is biologically heterogeneous and polyclonal from its initiation. This is a real challenge for researchers, because the molecular events which are critical for triggering prostate cell transformation have not been clarified yet. Studies and new data about global gene expression will be critical in the near future.
The study of specifically altered gene expression during CaP tumourigenesis is a difficult task, but the scientific information that will ultimately be obtained will be an important reward, leading to a better understanding of the molecular basis of the disease and hopefully to better methods for diagnosis and therapy.
Experts in the field figure that the heterogeneity of CaP may also account for the variability of clinical progression, with some patients presenting with slow growing, indolent tumours and other patients showing a rapidly progressive disease. Therefore, methods for discriminating favourable versus poor prognosis

to better guide clinical managing are urgently needed.

But the real novelty now, brought by our work, is that effective chemoprevention of CaP is actually feasible. At this point, prevention instead of therapy seems to be the key word. Now we have the view on a new scenario, in which the incidence of this disease could be greatly reduced by simply making GTE available to "high-risk" men (elderly, African-American, those with family history of CaP or bad life-style, etc…), with a potential tremendous social and clinical impact. In the Western countries, this novel strategy also suggest that an important decrease of sanitary costs related to this disease is potentially achievable.

In conclusion, a projection of our data suggests that up to 90% of chemoprevention efficacy could be obtained by GTE administration in men prone to develop CaP. Safety of a standardized extract called Polyphenon E, specifically made for human trials, has been already tested (24, 25). Thus, administration of GTE could be an effective therapy for treating premalignant lesions of high risk men by filling a therapeutic void before CaP develops.

ACKNOWLEDGMENTS

We thanks Polyphenon Pharma, New York, USA for supplying Polyphenon E.
Grant sponsor: FIL 2006-2008, University of Parma, Italy; Abo Project 2006, Venezia, Italy; AICR (UK) Grant No. 06-711; Polyphenon Pharma, NY, USA.

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