

# Synergistic anticancer activity of biologicals from green and black tea on DU 145 human prostate cancer cells

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## Abstract

There is considerable interest in the potential of botanicals in preventing and/or alleviating chronic ailments. Among the most studied botanicals are compounds present in green and black teas. Nontoxic tea polyphenols are potent antioxidants, and they also modulate several signalling pathways and inhibit proteins such as MMP-9 or protein plasminogen activator system, making them very attractive potential therapeutics. One criticism of the prophylactic or therapeutic use of green or black tea polyphenols was presumably the poor bioavailability of these chemicals when ingested. However, studies have shown that epigallocatechin-3-gallate (EGCG) and theaflavin (TF) can be detected in the small and large intestine, liver, and prostate of experimental animals after consumption of tea extracts. In particular, a study was carried out on 20 men scheduled for prostatectomy, who were assigned to consume teas for five days before surgery. Tea polyphenols were detected in the prostate. This fact contradicts the common misconception of poor bioavailability of TF and EGCG and makes feasible the application of green or black tea polyphenols as prophylactic and therapeutic agents. Theaflavins and catechins seem to act on cancer cells largely through different pathways, so utilisation of both could offer synergistic anticancer effects, but so far no work has been done on the cumulative effects of EGCG and TF on prostate cancer. Therefore, in this study we have investigated if EGCG in combination with TF can reduce the rate of prostate cancer growth, and we have observed greater cell death compared to application of either TF or EGCG alone.

**Key words:** cancer, prostate, theaflavin, epigallocatechin-3-gallate, synergistic anticancer activity.

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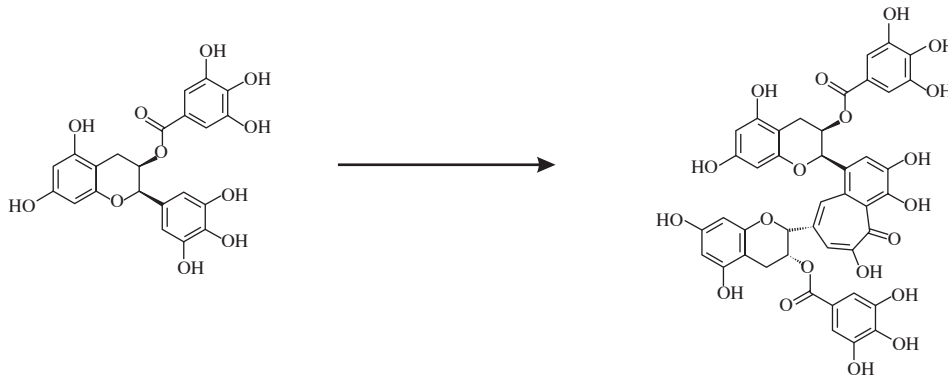
## Introduction

Recently, considerable interest has been observed in the potential of botanicals preventing and/or alleviating chronic ailments [1, 2]. Among the most studied botanicals are compounds present in green and black teas. Consumed by over two-thirds of the world's population, tea produced from the leaves of one plant (*Camellia sinensis*) is the most popular beverage next to water, and the amount of tea consumed is positively correlated to health benefits. Different methods of processing *Camellia sinensis* leads to green, oolong, or black tea [3, 4]. Dried leaves of tea produce green tea, while partially crushed leaves oxidised in a moist environment for short time produce oolong tea, while longer oxidation results in black tea (Fig. 1). The therapeutic properties and prophylactic properties of green and black tea have been credited to catechins of green tea and theaflavins of black tea. Oolong tea is processed in such a way that it can contain both catechins and theaflavins. Tea polyphenols are potent antioxi-

dants, and they also modulate several signalling pathways and inhibit proteins such as metalloproteinase 9 (MMP-9) or protein plasminogen activator system [1, 5].

An increasing body of evidence suggests that the major catechin (epigallocatechin-3-gallate – EGCG) found in green tea has the capacity to impact a variety of human malignancies. In addition to acting as a powerful antioxidant, EGCG also acts as an antiangiogenic factor, promotes apoptosis, and induces cell growth arrest via cell cycle regulatory proteins; it also activates killer caspases, and suppresses oncogenic transcription factors [6]. It has been shown that EGCG reduces PC3 prostate cancer growth via MEK-independent ERK1/2 activation [7], inhibits proliferation via alteration of expression of *CCND1*, *CDK4*, *CDKN1A*, *E2F1*, *MAPK6* and *PCNA* genes [8]. In another androgen-independent prostate cancer cell line (DU 145) EGCG alters genes involved in the functions of transcription, RNA processing, protein folding, phosphorylation, protein degradation, cell motility, and ion transport [9],

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**Fig. 1.** Epigallocatechin-3-gallate converts to theaflavin-3,3'-digallate during black tea production

and induces mRNA expression of interleukin (IL)-6, IL-8, CXCL1, IP-10, CCL5, and transforming growth factor  $\beta$  (TGF- $\beta$ ) [10].

Research on the influence of theaflavin (TF) on prostate cancer cells is less abundant than for EGCG. Nevertheless, it has been found that theaflavin modifies protein kinase B (PKB) pathways in DU145 and LNCaP (androgen-responsive prostate carcinoma cells) pathways [11]. In a different study it was found that theaflavins stimulate G2/M arrest by modifying expression of p21waf1/cip1, cdc25C, and cyclin B proteins in PC-3 cells [12]. However, it was reported that TF, similarly to EGCG, can affect the protein expression of mitogen-activated protein kinases (MAPK) or nuclear factor  $\kappa$ B (NF- $\kappa$ B) pathways [13].

Theaflavins and catechins seem to act on cancer cells largely by different pathways, so treatment of both could offer synergistic anticancer effects. Literature on the synergistic effects of EGCG and TF is limited to the effects of these two polyphenols on suppression of adhesion and invasion of hepatoma cancer cells [14], protection against nitric oxide toxicity [15], and effects of tea polyphenols and ascorbic acid on human lung adenocarcinoma [16]. Prostate cancer is one of most common cancers among men. Therefore, non-toxic natural agents such as EGCG or TF, used either alone or in combination with conventional therapeutics to prevent tumour progression and/or treatment, can be very helpful. For that reason we have

investigated if EGCG in combination with TF can reduce the rate of prostate cancer growth.

## Material and methods

**Cell line.** DU 145 human prostate carcinoma obtained from stock cultures were maintained in Minimum Essential Media (MEM) with L-glutamine, 10% foetal bovine serum (FBS, Life Technologies, Grand Island, NY) with 100 U/ml penicillin and 100  $\mu$ g/ml streptomycin (Sigma-Aldrich®, St. Louis, MO).

**Treatment solutions.** Treatment solutions were prepared by dissolving EGCG (Sigma-Aldrich catalogue # E4143) and theaflavin of black tea (TF, Sigma-Aldrich catalogue # E5550) in DMSO.

**Cell treatment.** Cells were distributed into 24- or 48-well dishes with a density of 50 cells per well and maintained for ~24 hours. Next, cells were treated with EGCG, TF for four hours. Tested doses were: 2.5, 5, 10, 50, 100, and 200  $\mu$ g/ml of EGCG or TF in the media. Other groups included wells with media only or wells with 0.1% DMSO added. Also, in some experiments cells were treated with mixture of 20  $\mu$ g/ml EGCG and 20  $\mu$ g/ml TF to investigate synergist effects. After treatment media was replaced, cells were maintained for seven days followed by fixing with methanol and 0.4% Giemsa stain (Sigma-Aldrich) and colony counts under microscopic (10 $\times$  magnification) examination.

**Table 1.** Colony count of cells treated with epigallocatechin-3-gallate (EGCG) or theaflavin (TF) vs. control

|                      | Media only | Media/<br>DMSO | EGCG<br>20 $\mu$ g/ml | EGCG<br>10 $\mu$ g/ml | EGCG<br>5 $\mu$ g/ml | TF<br>200 $\mu$ g/ml | TF<br>100 $\mu$ g/ml | TF<br>50 $\mu$ g/ml | TF<br>20 $\mu$ g/ml |
|----------------------|------------|----------------|-----------------------|-----------------------|----------------------|----------------------|----------------------|---------------------|---------------------|
| <b>Colony counts</b> | 113.2%     | 100.0%         | 76.9%                 | 98.5%                 | 106.8%               | 55.8%                | 95.6%                | 86.5%               | 74.9%               |
| <b>Std. dev.</b>     | 20.6%      | 20.2%          | 28.9%                 | 22.0%                 | 16.9%                | 36.2%                | 27.1%                | 16.9%               | 38.0%               |
| <b>p value</b>       | NS         | –              | < 0.009               | NS                    | NS                   | < 0.000001           | < 0.000001           | NS                  | NS                  |
| <b>N</b>             | 8          | 8              | 32                    | 32                    | 32                   | 32                   | 32                   | 32                  | 32                  |

Average colonies counted in Media/DMSO wells were considered as 100%, p describes significance of statistical differences between media/DMSO and media only, or EGCG + TF, or EGCG, or TF, Std. dev. – standard deviation, NS – differences not statistically significant, N – number of wells

**Table 2.** Colony count of cells treated with epigallocatechin-3-gallate (EGCG), theaflavin (TF), and EGCG + TF vs. control

|               | Media only | Media/DMSO | EGCG + TF<br>20 µg/ml | EGCG<br>20 µg/ml | TF<br>20 µg/ml |
|---------------|------------|------------|-----------------------|------------------|----------------|
| Colony counts | 99.5%      | 100%       | 50.2%                 | 67.9%            | 82.3%          |
| Std. dev.     | 33.4%      | 35.8%      | 37.2%                 | 35.2%            | 32.7%          |
| p1 value      | NS         | –          | < 0.000001            | < 0.000001       | < 0.002        |
| p2 value      |            |            | < 0.000001            |                  |                |
| p3 value      |            |            | < 0.000001            |                  |                |
| N             | 40         | 40         | 160                   | 160              | 160            |

Average colonies counted in Media/DMSO wells were considered as 100%; p1 describes significance of statistical differences between media/DMSO and media only, or EGCG + TF, or EGCG, or TF; p2 describes differences between EGCG + TF and EGCG; p3 describes significance of statistical differences between EGCG + TF and TF. Std. dev. – standard deviation, NS – differences not statistically significant, N – number of wells

Statistical analysis was carried out using the Origin 8 program (OriginLab Corporation, Northampton, MA 01060). Statistical significance was set at a  $p < 0.05$ , as calculated using ANOVA test.

## Results and discussion

Epigallocatechin-3-gallate and TF inhibit proliferation of many cancer cell lines, as has been reported by numerous papers [17-19]. Our initial experiment showed that cell proliferation was reduced in concentrations similar to those published by other authors (Table 1).

Albrecht *et al.* tested the potency of EGCG for the inhibition of cell proliferation in PC-3 prostate cancer cells and reported similar concentration values [7, 13, 20-22] to ours. It is of great importance that EGCG had no effect on cell proliferation in the RWPE-1 non-tumorigenic prostate epithelial cell line. The authors suggest that ERK1/2 activation via a MEK-independent, PI3-K-dependent signalling pathway is accountable for the inhibition of proliferation in PC-3 cells [7]. Work done on DU 145 showed inhibition of proliferation mediated via inhibition of phosphorylation of ERK1/2 and p38 pathways [23]. They also observed inhibition of two metalloproteinases (MMP-2, MMP-9) critical in cancer cell invasion and metastasis. No work was done with TF on DU145 prostate cancer cells. However, as we stated in the introduction, TFs were used to limit proliferation in different prostate cancer cells in similar concentrations as those used in our experiments [13, 17, 22].

One of the criticisms of prophylactic or therapeutic use of green or black tea polyphenols was presumably poor bioavailability of these chemicals. Work done by Henning *et al.* showed that EGCG and TF can be detected in the small and large intestine, liver, and prostate of C57BL mice after consumption of tea extracts. The prostate bioavailability of TF was 70% higher than that of EGCG. They also performed a study in which 20 men

scheduled for prostatectomy were randomly assigned to consume green or black tea for five days before surgery. Tea polyphenols were detected in prostate samples from men consuming both green and black teas. This pioneering human study showed that polyphenols and theaflavins are bioavailable in the human prostate [21]. This fact supports the feasibility of providing EGCG and TF polyphenols found in green and black tea as prophylactic and therapeutic treatments.

Usually the combination of different drugs used in cancer therapy is justified by high toxicity or resistance of cancer cells to one or the other drugs (for example platinum-based drugs). Utilising the combination of EGCG and TF is different. These two polyphenols are not toxic and have diverse and mostly independent effects on cancer cells. Therefore, EGCG and TF would be perfect candidates for this type of treatment. Despite the enormous number of studies done on cancer therapy using EGCG [20, 24-26] or TF [17, 27, 28], surprisingly there are no reports on the synergistic effects of these polyphenols on cancer cells. In our research we chose the concentration of 20 µg/ml for both polyphenols as this concentration had a weak anticancer effect for both compounds individually. As seen in Table 2, combining EGCG and TF produced greater cancer cell death than EGCG or TF separately.

## Conclusions

Combining lower concentrations of EGCG and TF results in greater cell death compared to either extract alone at the same concentration. Also, increased cell death rates were statistically significant compared to controls.

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