Minireview
Role of epigallocatechin gallate (EGCG) in the treatment of breast and prostate cancer

Emma C. Stuart, Marissa J. Scandlyn, Rhonda J. Rosengren *

Department of Pharmacology and Toxicology, 18 Frederick Street, Adams Building, University of Otago, Dunedin, New Zealand

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Abstract

Green tea and its major constituent epigallocatechin gallate (EGCG) have been extensively studied as a potential treatment for a variety of diseases, including cancer. Epidemiological data have suggested that EGCG may provide protective effects against hormone related cancers, namely breast or prostate cancer. Extensive in vitro investigations using both hormone responsive and non-responsive cell lines have shown that EGCG induces apoptosis and alters the expression of cell cycle regulatory proteins that are critical for cell survival and apoptosis. This review will highlight the important in vitro mechanistic actions elicited by EGCG in various breast and prostate cancer cell lines. Additionally, the actions of green tea/EGCG in in vivo models for these cancers as well as in clinical trials will be discussed.

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Keywords: Epigallocatechin gallate; EGFR; Prostate cancer; Breast cancer; Apoptosis

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Introduction

Flavanoids are low-molecular weight plant-derived compounds found in fruits, vegetables, herbs, tea and wine (Middleton et al., 2000). They are divided into several different classes based on variations of the same basic structure. One such class is the flavan-3-ols, also referred to as the catechins, which are differentiated by di- or tri-hydroxyl group substitutions on the B ring and meta-5,7-dihydroxy substitution on the A ring (Yang et al., 2001). Catechins are especially concentrated in green tea (Camellia sinensis), accounting for 30–40% of its dry weight, while other flavonoids are present only in small quantities.
(Graham, 1992; Mukhtar et al., 1992; Arts et al., 2000a,b). The major catechins contained in green tea are (−)-epigallocatechin-3-gallate (EGCG), (−)-epigallocatechin (EGC), (−)-epicatechin gallate (ECG), (−)-epicatechin (EC) and catechin (Fig. 1) (Graham, 1992; Mukhtar et al., 1992). EGCG, the most abundant catechin in green tea, is credited with the majority of health benefits associated with green tea consumption. EGCG has demonstrated beneficial effects in studies of Parkinson’s disease (Choi et al., 2002), Alzheimer’s disease (Choi et al., 2001; Obregon et al., 2006), stroke (Choi et al., 2004; Koh et al., 2006), obesity (Kao et al., 2000), diabetes (Anderson and Polansky, 2002; Tsuneki et al., 2004), chemoprevention (Xu et al., 1992; Katiyar et al., 1993; Kavanagh et al., 2001; Chung et al., 2003) and also possesses antioxidant activity (Guo et al., 1999). In this review, the potential applications of EGCG in the treatment of breast and prostate cancer and its possible mechanisms of action will be discussed.

**Prostate cancer — in vitro effect of EGCG**

Several studies have demonstrated a reduction in prostate cancer cell number in response to treatment with EGCG (Gupta et al., 2000; Bhatia and Agarwal, 2001; Chung et al., 2001; Hastak et al., 2003). For example, Gupta et al. (2000) demonstrated that EGCG dose-dependently reduced the cell number of both androgen dependent (expressing the androgen receptor (AR)), LNCaP, and independent (not expressing the AR), DU145, cells. This observation was verified by two studies which demonstrated similar effects on DU145 or LNCaP cell viability after treatment with EGCG (Chung et al., 2001; Hastak et al., 2003). It is important to utilize androgen responsive and unresponsive cell lines when testing potential anti-cancer agents as prostate tumors are composed of both androgen sensitive and insensitive cells (Agarwal, 2000; Adhami et al., 2003). Therefore, an effective treatment should induce death in both cell types. The mechanism through which EGCG induces death in prostate cancer cells has been identified as apoptosis (Gupta et al., 2000, 2003; Hastak et al., 2003). Furthermore, Gupta et al. (2000, 2003) demonstrated that the degree of apoptosis induction following EGCG treatment was similar in both DU145 and LNCaP cells. This suggests that the cytotoxic capacity of EGCG is not influenced by the presence or absence of the androgen receptor, which is a desirable attribute for a prostate cancer treatment.

**In vitro mechanisms of action**

It has been reported that EGCG inhibits the activity of the epidermal growth factor receptor (EGFR) in prostate cancer cells (Bhatia and Agarwal, 2001). Activation of the EGFR, via auto-phosphorylation, leads to the activation of intracellular signaling cascades, such as the mitogen activated kinase pathway (MAPK) and phosphoinositols-3-kinase/AKT (PI3K/AKT) pathway (Casalini et al., 2004). Both of these pathways have roles in anti-apoptotic and growth stimulatory signaling (Pearson et al., 2001; Osaki et al., 2004). Several investigations have identified specific targets, modulated by EGCG, such as specific kinases of intracellular cell signaling cascades (Bhatia and Agarwal, 2001; Siddiqui et al., 2004). For example, EGCG treatment increased the proportion of phospho-ERK, an important signal transducing protein in the MAPK pathway (Bhatia and Agarwal, 2001; Siddiqui et al., 2004). Subsequent investigations demonstrated that EGCG treatment decreased the expression of phospho-PI3K and its substrate phospho-AKT to a similar extent in both DU145 and LNCaP cells. This information reaffirms that the effect of EGCG remains independent of the cell’s AR status. Another important protein governing cell survival is the transfactor NF-κB. NF-κB has overlapping roles in many mitogenic signaling pathways as it is capable of promoting and repressing the expression of proteins involved in survival and apoptosis (Lin and Karin, 2003; Aggarwal, 2004; Monks et al., 2004). Therefore, it is vital for tumor growth, and accordingly, is an appropriate target in cancer treatment strategies. EGCG also modulates this trans-factor, as it reduced NF-κB nuclear localization in prostate cancer cells regardless of their AR status (Hastak et al., 2003; Vayalil and Katiyar, 2004).

Cell cycle arrest can lead to the induction of apoptosis, however the mechanisms facilitating this remain largely unidentified (Hipfner and Cohen, 2004). EGCG has been shown to induce cell cycle arrest at the G1 phase in both LNCaP and DU145 cells (Gupta et al., 2000, 2003; Bhatia and Agarwal, 2001). Progression through the G1 phase is regulated through the cyclin dependent kinases (CDKs). The activity of the CDKs is in turn modulated by two sets of proteins; the cyclins (cyclin D1, D2 and E) and the cyclin dependent kinase inhibitors (CDKI: p21, p27, p16, p18). The cyclins are required by the CDKs to perform their catalytic activity, therefore these promote the progression through this cell cycle checkpoint.

Fig. 1. The structure of the major catechins contained in green tea.
Conversely, the CDKIs inhibit the kinase activity of the CDKs, thus inhibiting the progression from the G1 phase through to the S phase (Orlowski and Furlanetto, 1996; Pavleitch, 1999; Sherr and Roberts, 1999). Modulation of these proteins in prostate cancer cells by EGCG has been reported. Specifically, Gupta et al. (2000, 2003) demonstrated that EGCG increased the expression of p21, p27, p18 and p16 and decreased the expression of the cyclins D1, D2 and E as well as CDK 2, 4 and 6 in both LNCaP and DU145 cells. Moreover, the protein–protein interaction between the cyclins and CDKIs was increased. Thus, it appears that EGCG alters the expression of critical cell cycle regulatory proteins resulting in G1 arrest. Again, this effect is independent of AR status.

Tumors require fresh nutrients, oxygen and a method of waste disposal for further growth and development. These requirements are fulfilled by the formation of new blood vessels which infiltrate the tumor: a process termed angiogenesis. In order for angiogenesis to occur, the connective tissue comprising the extracellular matrix around the tumor must be broken down to allow endothelial cells to migrate and form new blood vessels (Adhami et al., 2003; Gasparini et al., 2005; Harlozinska, 2005). Tumor cells achieve this through the release of matrix metalloproteinases (MMP), thus allowing for tumor metastasis. EGCG modulated this process at concentrations as low as 5 μg/ml, which inhibited the production of the zymogen and subsequent proteolytic activity of the MMPs in DU145 cells (Vayali and Katiyar, 2004). Furthermore, EGCG inhibited the production of vascular endothelial growth factor (VEGF) in colon and breast cancer cells (Jung et al., 2001; Sarippour et al., 2002), and the activation of the VEGF receptor in leukemia and colon cancer cells (Jung et al., 2001; Lee et al., 2004). Therefore, EGCG inhibits various processes required for angiogenesis and metastasis in prostate cancer cells. This antiangiogenic effect coupled with the proapoptotic capacity of EGCG demonstrates that the growth of prostate cancer is inhibited via multiple mechanisms. This could be very important because overexpression of VEGF has occurred following resistance to EGFR inhibitors in ovarian and colon cancer murine xenograft models (Viloria-Petit et al., 2001; Ciardiello et al., 2004). Therefore, EGCG treatment may be able to overcome this problem by targeting both EGFR and VEGF.

Prostate cancer — effect of green tea in vivo models

The transgenic adenocarcinoma of the mouse prostate (TRAMP) model is extensively used in chemopreventative studies, as it emulates the progressive form of prostate cancer. Green tea compounds have demonstrated chemopreventative activity in several studies using this model (Gupta et al., 2001; Adhami et al., 2004; Caporali et al., 2004). Gupta et al. (2001) illustrated a significant reduction in tumor incidence, burden and metastasis, prostate weight and cell proliferation in TRAMP mice following the administration of a 0.1% green tea polyphenol mixture (EGCG, ECG, EGC and EC) in the drinking water. This chemoprevention was accompanied by a reduction in serum insulin-like growth factor (IGF)-I levels and a corresponding increase in IGF-binding protein-3 (IGFBP-3) levels (Gupta et al., 2001). As an increase in IGF-I serum levels and a reduction in IGFBP-3 levels are associated with prostate cancer progression and a poor outcome in patients, modulation of IGF-I and IGFBP-3 may represent a mechanism for chemoprevention with green tea (Gupta et al., 2001). This was further investigated by Adhami et al. (2004). Specifically, TRAMP mice that received a 0.1% green tea polyphenol mixture exhibited lower levels of IGF-1 in the dorso-lateral prostate. Additionally, there were increased levels of IGFBP-3 and a reduction in phosphorylated PI3K, AKT and ERK1/2 compared to control mice. Green tea polyphenols also resulted in significantly reduced polypeptide levels of VEGF, urokinase plasminogen activator, MMP-2 and -9, and TIMP-1 and -2. The findings of Adhami et al. (2004) suggest that a green tea polyphenol mixture inhibits IGF-I signaling in TRAMP mice and this may contribute to the inhibition of prostate cancer progression and invasion.

A similar study conducted by Caporali et al. (2004) in TRAMP mice demonstrated a chemopreventative effect, as there was an 80% reduction in tumor development among mice that received a green tea polyphenol mixture (0.3% green tea polyphenol solution). Clusterin, a widely expressed tumor suppressor protein involved in apoptosis, was down-regulated in TRAMP mice that developed tumors, but was maintained in green tea polyphenol-treated mice. Recently, it has been documented that clusterin exerts regulatory control over NF-κB activity, which has been modulated by EGCG in vitro (Santilli et al., 2003). There is limited information regarding the activity of purified EGCG in prostate cancer models. One study by Liao et al. (1995) used purified EGCG in male athymic nude mice inoculated with 2 different human prostate cancer cell lines, PC-3 (androgen independent) or LNCaP 104-R (androgen dependent). The results showed that EGCG (1 mg/mouse/day, i.p., 14 days) significantly halted the growth of both androgen dependent and independent tumors, but was more effective at reducing the growth of androgen dependent tumors (Liao et al., 1995).

Prostate cancer risk associated with tea consumption

Epidemiological studies investigating the link between green tea consumption and prostate cancer risk are relatively few in number and have yielded conflicting results (Table 1). These studies either used few patients from a population at a low risk of prostate cancer (Jian et al., 2004; Chan et al., 2005) or did not discriminate between green and black tea consumption (Slattery and West, 1993; Jian et al., 1998). Therefore, no firm conclusions can be drawn between green tea consumption and the development of prostate cancer in humans.

Clinical trials with green tea extracts

Several clinical trials have been conducted to determine the ability of green tea extracts to prevent the development and progression of prostate cancer. (Bettuzzi et al. 2006) conducted a study using 60 volunteers with the predominant premalignant lesion of prostate cancer, termed high-grade prostate intraepithelial neoplasia. Patients received green tea compounds in capsule form (3, 200 mg capsules/day). Following one year of treatment, only 3% of patients that received the green tea...
polyphenols presented with cancer compared with 30% in the placebo group. Furthermore, patients that received the green tea capsules exhibited a longer latency to tumor detection and exhibited an improved quality of life. Another clinical trial examined prostate-specific antigen (PSA) levels in 19 patients with hormone refractory prostate cancer following treatment with capsules containing green tea extract (250 mg, twice daily, 2 months) (Choan et al., 2005). No alterations in PSA levels were detected, and patients reported at least one side effect from the green tea capsules. The most common symptoms reported were abdominal discomfort and fatigue. Similarly, a Phase II clinical trial conducted by Jatoi et al. (2003) evaluated the ability of a green tea preparation (6 g green tea/day, 4 months) to sustain low levels of PSA in 42 patients with androgen-independent prostate carcinoma. Only one patient experienced a decline in PSA levels from 229 ng/dl to 105 ng/dl. However, this response was not maintained past two months and did not result in a reduction in tumor mass. Furthermore, 69% of patients reported Grade 1 or 2 toxicity, while six episodes of Grade 3 toxicity and one episode of Grade 4 toxicity also occurred. Although this suggests that green tea possesses limited antineoplastic activity, the trial was conducted in patients with androgen-independent prostate carcinoma, a subset of prostate cancers refractory to most treatment options. Additionally, most patients failed to complete the trial. Each of these clinical trials used green tea preparations containing caffeine, which has previously been reported to be responsible for gastrointestinal symptoms in a Phase I clinical trial (Pisters et al., 2001). Therefore, future trials should employ decaffeinated green tea extract preparations, or purified EGCG.

Breast cancer — in vitro effect of EGCG

EGCG is cytotoxic toward breast cancer cells regardless of their estrogen receptor (ER) status. For example, after treatment with EGCG, cell number was significantly decreased from control in the ER positive cell lines, MCF-7 and BT474 (Liang et al., 1999; Morre et al., 2000) as well as ER negative cell lines, Hs578t, MDA-MB-231, MBA-MB-468 and BT-20 (Liang et al., 1999; Morre et al., 2000; Kavanagh et al., 2001; Masuda et al., 2002, 2003; Chisholm et al., 2004; Roy et al., 2005). ER binding studies provided further evidence which demonstrated that the ER status of the cell lines is not important in EGCG-mediated cytotoxicity. Specifically, Goodin et al. (2002) demonstrated that EGCG weakly bound to both ERα and ERβ in vitro. However, EGCG failed to antagonize estradiol-mediated responses in female immature mice, while it weakly inhibited estradiol-mediated responses in ERβ reporter gene assays. These results suggest that EGCG is not a strong ER antagonist.

In vitro mechanisms of action

Very few studies have examined the mechanism by which EGCG is cytotoxic toward breast cancer cell lines. However, EGCG induces apoptosis in ER negative MDA-MB-468 (Roy et al., 2005) and MDA-MB-231 cells (Chisholm et al., 2004). A plethora of literature has detailed that EGCG induces apoptosis in many other human cancer cell lines (Ahmad et al., 1997; Chen et al., 1998, 2003; Masuda et al., 2001; Gupta et al., 2003). Therefore, it is likely that EGCG induces apoptosis in most, if not all, breast cancer cell lines. Mechanisms through which EGCG-induced apoptosis may be mediated include cell cycle arrest and changes in intracellular signaling cascades. Alterations in the CDKIs, p21 and p27, occur following EGCG treatment in breast cancer cells. For example, Liang et al. (1999) demonstrated that following treatment with EGCG, both p21 and p27 proteins were overexpressed in MCF-7 cells. This correlated well with cell cycle studies, which demonstrated that EGCG increased the proportion of cells arrested in G1. Studies in the ER negative cell line, MDA-MB-231, showed a very similar trend, with increased protein expression of p21 and p27 following EGCG treatment (Masuda et al., 2002).

Table 1

<table>
<thead>
<tr>
<th>Population profile</th>
<th>Risk ratio (95% CI)</th>
<th>Outcome</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>White men in Utah, US</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>− 362 cases</td>
<td>Tea consumption (cups/week):</td>
<td>No association</td>
<td>Slattery and West, 1993</td>
</tr>
<tr>
<td>− 685 controls</td>
<td>≤ 67 years</td>
<td>1.00</td>
<td></td>
</tr>
<tr>
<td></td>
<td>0</td>
<td>1.0</td>
<td></td>
</tr>
<tr>
<td></td>
<td>1–5</td>
<td>0.75 (0.47–1.20)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>&gt;5</td>
<td>1.06 (0.72–1.57)</td>
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<tr>
<td></td>
<td>&gt;67 years</td>
<td>1.00</td>
<td></td>
</tr>
<tr>
<td></td>
<td>0</td>
<td>1.0</td>
<td></td>
</tr>
<tr>
<td></td>
<td>1–5</td>
<td>0.90 (0.47–1.75)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>&gt;5</td>
<td>0.90 (0.59–1.36)</td>
<td></td>
</tr>
<tr>
<td>Three different regions in Canada</td>
<td>Tea consumption (g/day):</td>
<td>Tea intake is associated with a reduced risk of prostate cancer</td>
<td>Jian et al., 1998</td>
</tr>
<tr>
<td>− 617 cases</td>
<td>≤ 0–500</td>
<td>0.89 (0.69–1.16)</td>
<td></td>
</tr>
<tr>
<td>− 637 controls</td>
<td>&gt;500</td>
<td>0.70 (0.50–0.99)</td>
<td></td>
</tr>
<tr>
<td>Men in southeast China</td>
<td>Green tea consumption:</td>
<td>Green tea intake is associated with a reduced risk of prostate cancer</td>
<td>Jian et al., 2004</td>
</tr>
<tr>
<td>− 130 cases</td>
<td>&lt;1 cup/day</td>
<td>1.00</td>
<td></td>
</tr>
<tr>
<td>− 274 controls</td>
<td>1–3</td>
<td>0.53 (0.30–0.94)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>≥3 cups/day</td>
<td>0.27 (0.15–0.48)</td>
<td></td>
</tr>
</tbody>
</table>

Significant differences are indicated in bold typeface.
Significant differences are indicated in bold typeface.

Furthermore, Masuda et al. (2003) determined that EGCG inhibited the activity of the erbB2/HER-2 isoform of EGFR. This data correlates well with an EGCG-mediated decrease in c-κα-induced AKT and STAT3 activity in breast cancer cells. Specifically, Masuda et al. (2002) demonstrated that EGCG inhibited both basal and TGF-α-induced EGFR auto-phosphorylation. It was further established that EGCG inhibited AKT and ERK activation. Therefore, EGCG induces apoptosis in breast cancer cells by modulating intracellular signaling pathways that control cell cycle progression and this response is independent of the ER status of the cell line.

Breast cancer — effect of green tea in in vivo models

The majority of in vivo studies investigating the beneficial effects of green tea constituents in breast cancer chemoprevention have focused on green tea polyphenol mixtures rather than purified individual catechins. Studies of chemical-induced mammary carcinogenesis conducted in rats have demonstrated a protective effect of green tea compounds on tumor burden and survival, but it is still unclear whether this protection is greater at the pre- or post-initiation stage (Bhide et al., 1994; Hirose et al., 1994, 1997, 2002; Tanaka et al., 1997; Kavanagh et al., 2001). Various studies using either green tea extracts or purified EGCG have also been conducted using breast cancer cell xenografts in BALB/c mice inoculated with 4T1 mouse mammary carcinoma cells. These effects were associated with an increase in the Bax/
Breast cancer risk associated with tea consumption and genotype

Epidemiological studies investigating the association between green tea consumption and breast cancer risk have yielded conflicting results (Table 2). Several studies have shown no association (Key et al., 1999; Nagano et al., 2001; Suzuki et al., 2004), while others have demonstrated a chemopreventative effect (Inoue et al., 2001; Wu et al., 2003). Given the inconsistency in the literature regarding chemoprevention with green tea, recent studies have genotyped breast cancer patients to determine whether the enzyme isoforms they possess could influence the protection conferred by green tea. A case–control study conducted among Chinese-, Japanese-, and Filipino-American women demonstrated that green tea intake was associated with a reduction in breast cancer risk, but only in women possessing a low-activity catechol- O-methyltransferase (COMT) allele (Wu et al., 2003). COMT is responsible for the rapid methylation of tea polyphenols and, therefore, differences in methylation capacity between individuals may alter the chemopreventative activity of green tea catechins. The findings of Wu et al. (2003) suggest that chemoprevention by green tea in women possessing the low-activity COMT allele may result from an increased bioavailability of catechins. Another recent study illustrated that green tea consumption is associated with a reduced risk of breast cancer in women possessing the high-activity, but not low-activity, angiotensin-converting enzyme (Yuan et al., 2005). This supports the hypothesis that a possible mechanism of chemoprevention by green tea catechins involves their inhibition of reactive oxygen species via angiotensin-converting enzyme inhibition.

A further mechanism of chemoprevention by green tea catechins involves the alternation of circulating hormone levels. A recent study by Wu et al. (2005) conducted in 130 post-menopausal women observed significantly lower plasma levels of estrone in women regularly consuming green tea compared with non- or irregular tea-drinkers (25.8 pg/ml vs 29.5 pg/ml). Plasma estradiol and androstenedione levels were also present in lower levels in women consuming green tea on a regular basis. The findings of Wu et al. (2005) suggest that alteration of estrone levels may contribute to the chemopreventative activity of green tea polyphenols.

Conclusions

EGCG induces apoptosis in both breast and prostate cancer cells in vitro. The cytotoxic effect of EGCG is not influenced by the hormone receptor status of either prostate or breast cancer cell lines. Furthermore, a ubiquitous mechanism may be responsible for the EGCG-mediated induction of apoptosis (Fig. 2). Upon treatment with EGCG, both breast and prostate cancer cells demonstrated cell cycle arrest in G1 phase. This is likely to be the result of a decrease in the auto-phosphorylative capacity of EGFR and a subsequent reduction in the activity of intracellular signaling cascades, which are activated by EGFR. These changes may lead to alterations in the expression of proteins governing the cell cycle (Fig. 2). While these results are promising, this action has yet to be conclusively proven in in vivo models or in cancer patients.

References


