Theaflavin-3,3′-digallate and penta-O-galloyl-β-D-glucose inhibit rat liver microsomal 5α-reductase activity and the expression of androgen receptor in LNCaP prostate cancer cells

Hung-Hsiao Lee1, Chi-Tang Ho2 and Jen-Kun Lin1,3

1Institute of Biochemistry, College of Medicine, National Taiwan University, Taipei, Taiwan and 2Department of Food Science, Rutgers University, Brunswick, New Jersey, USA
3To whom correspondence should be addressed
Email: jklin@ha.mc.ntu.edu.tw

Androgens play a critical role in regulating the growth, differentiation and survival of epithelial cells in many androgen-responsive organs, such as prostate and skin. The enzyme steroid 5α-reductase (EC 1.3.99.5) catalyzes the conversion of testosterone (T) to a more active androgen, dihydrotestosterone (DHT). DHT then binds to androgen receptors (AR) and functions in the nucleus to regulate specific gene expression. Androgens via their cognate receptor may be involved in the development and progression of benign prostate hyperplasia, prostate cancer, hirsutism, male pattern alopecia and acne. The aim of this study was to determine whether theaflavin-3,3′-digallate (TF3) and penta-O-galloyl-β-D-glucose (5GG) have inhibitory effects on androgen production and action. We found that TF3 and 5GG inhibit rat liver microsomal 5α-reductase activity. Furthermore, TF3 and 5GG significantly reduced androgen-responsive LNCaP prostate cancer cell growth, suppressed expression of the AR and lowered androgen-induced prostate-specific antigen secretion and fatty acid synthase protein level. In conclusion, our result suggests that TF3 and 5GG might be useful chemoprevention agents for prostate cancer through suppressing the function of androgen and its receptor.

Introduction

Prostate carcinoma is the most frequently diagnosed malignancy and the second leading cause of death as a result of cancer in men in the western world (1). In the human prostate, androgens mediate critical processes involved in the normal development, organizational structure and mature function of the gland (2). Evidence shows that androgens can also be risk factors for prostate cancer development (3). The androgenic hormones testosterone (T) and dihydrotestosterone (DHT) exert their cellular effects by means of interaction with the androgen receptor (AR), a ligand-dependent transcriptional factor. AR belongs to the steroid/nuclear receptor superfamily, and possesses higher affinity for DHT than for T. In the prostatic cell, T is transformed into DHT by the enzyme 5α-reductases. After T or DHT binding to the AR, ligand-activated AR complexed with co-activator proteins and general transcription factors, bind to androgen-response elements (AREs) located in the promoter regions of androgen-regulated genes and serves to activate or to repress transcription (4–7).

Steroid 5α-reductase is a membrane-bound NADPH-dependent enzyme that catalyzes the reduction of Δ4,5 double bond of a variety of 3-oxo-Δ4 steroids including the conversion of T to the more potent androgen DHT, the first step of androgen actions in the prostate (8,9). 5α-Reductase has been proposed to have a role in the development of prostate cancer and possibly may be responsible for differences in prostate cancer mortality among different racial groups (10). There are two different isoforms of 5α-reductase (5α-R1 and 5α-R2), which have been characterized in humans, monkeys, rats and mice, each encoded by different genes. The average sequence identity between isoforms within a given species is ~47%, while the sequence identity between the same isozyme across species is 60% for 5α-R1 and 77% for 5α-R2 (11). Generally, 5α-R2 is thought to play a major role in prostate cancer because it is predominantly expressed in this tissue. However, some evidence shows that, in the human prostate, the 5α-R1 isoform is expressed mainly in the epithelial cells, whereas the 5α-R2 isoform is localized mainly in the stromal compartment (12,13). As a consequence, in advanced prostate cancer, characterized by an abnormal proliferation of epithelial cells, the 5α-R1 might become to be the predominant isoform probably responsible for the androgen metabolism. Inconsistent with the above findings, human prostate cancer cell lines, including androgen-dependent LNCaP or androgen-independent PC3 and DU145 cells, expressed mainly 5α-R1 (14–16). Moreover, it has been shown that 5α-R1 activity is three to four times greater in malignant than in benign prostate tissues, but 5α-R2 activity is similar in these two diseases (17). Therefore, 5α-reductase inhibitors have been synthesized as potential drugs for treatment of human benign prostate hyperplasia and prostate cancer. In fact, synthetic 5α-reductase inhibitors have been shown to decrease prostate size in vivo in prostatic carcinoma rat model (18,19) and decrease primary prostatic cellular proliferation (20).

Black tea is consumed at high levels in the world, and thought to exert a possible inhibitory effect against tumorigenesis and tumor growth because of the biological activities of its polyphenols. The major black tea polyphenols, theaflavins, include theaflavin (TF1), theaflavin-3-gallate (TF2A), theaflavin-3′-gallate (TF2B) and theaflavin-3,3′-digallate (TF3) (Figure 1). Among black tea theaflavins, TF3 is considered generally to be the more effective components for the inhibition of carcinogenesis. In our previous studies, TF3 has been found to possess anti-proliferative activity on several tumor cell lines including A431 and NIH3T3, through blocking the growth factor binding to its receptor (21). TF3 may also exert its anti-inflammatory and cancer chemopreventive actions by suppressing the activation of nuclear factor κB (NFκB) through inhibition of inhibitor κB kinase (IKK) activity (22). In addition, TF3 may inhibit TPA-induced protein kinase C (PKC) and transcription activator protein-1 (AP-1) binding activities (23).

Abbreviations: AR, androgen receptor; DHT, dihydrotestosterone; FAS, fatty acid synthase; 5GG, 1,2,3,4,6-penta-O-galloyl-β-D-glucose; TF1, theaflavin; TF2A, theaflavin-3-gallate; TF2B, theaflavin-3′-gallate; TF3, theaflavin-3,3′-digallate.

Carcinogenesis vol.25 no.7 pp.1109–1118, 2004
DOI: 10.1093/carcin/bgh106

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1,2,3,4,6-Penta-O-galloyl-β-D-glucose (pentagalloylgucose, 5GG), one kind of galloylgucose, contains one glucose that esterified with five gallic acids on its five hydroxyl groups of the glucose. 5GG is structurally similar to (--)epigallocatechin gallate (EGCG) in containing galloyl groups (24) and an important component in traditional Chinese crude drugs. It has been found that 5GG is also an effective biological reagent in inhibiting succinate dehydrogenase, ubiquinol-1 oxidase for mitochondrial respiration in rat liver (25). 5GG also inhibits both NADH dehydrogenases I/II, α-galactosidase, xanthine oxidase and H⁺, K⁺-ATPase (26,27). In our previous reports, we demonstrated that 5GG might be an effective antitumor reagent because of its ability to induce apoptosis through activation of caspase-3 in HL-60 (28) and inhibit the invasion of mouse melanoma by suppressing matrix metalloproteinase-9 expression (29).

In this study, we examined whether 5GG and theaflavins have inhibitory effects on androgen production and action of prostate cancer. We used rat liver microsomes as 5α-reductase enzyme source. Unlike human, only 5α-R1 is detected in rat liver. We found that 5GG and theaflavins inhibit 5α-R1 activities. Furthermore, they significantly reduced androgen-responsive LNCaP prostate cancer cell growth, suppressed expression of the AR and lowered the secretion of prostate-specific antigen (PSA). In conclusion, our result suggests that 5GG and theaflavins can attenuate the function of androgen and AR, which may be useful for the prevention or treatment of prostate cancer.

Materials and methods

Materials

TF1, TF2A, TF2B and TF3 were isolated from black tea (30). 5GG was isolated from the leaves of Macaranga tanarins (L.) as described previously (24). EGCG, gallic acid and n-propyl gallate were purchased from Sigma (St Louis, MO). All compounds were dissolved in 100% DMSO and adjusted the concentration to 100 mM as stock solution. T, DHT and androst-4-ene-3,17-dione were obtained from Sigma and dissolved in 100% ethanol. 14C-T (57 mCi/mmol) was purchased from Amersham (Arlington Heights, IL).

Preparation of rat liver microsomes

Rat liver microsomes were prepared by the method of Liang et al. (31). One female Sprague-Dawley rat (body wt, 400 g) was fasted overnight to decrease the concentration of liver glycogen and was then killed by carbon dioxide asphyxiation. The liver was removed and washed in ice-cold homogenizing buffer (0.32 M sucrose, 1 mM dithiothreitol and 20 mM potassium phosphate, pH 6.5). The following procedures were all carried out at 4°C. The tissue was minced in a beaker with a pair of scissors. The minced tissue was then homogenized in homogenizing buffer. The homogenate was then centrifuged at 10 000 g for 10 min. The resulting pellet was washed twice with 2 Vol homogenizing buffer. The combined supernatant from the two centrifugations was further centrifuged at 105 000 g for 1 h. The resulting pellet (microsomes) was suspended in homogenizing buffer. The microsome suspension was divided into small aliquots and stored at −70°C. The microsomes were diluted with 40 mM potassium phosphate, pH 6.5, immediately before use.

5α-Reductase assay

Briefly, the reaction solution contains 1 mM dithiothreitol, 40 mM potassium phosphate, pH 6.5, 100 μM NADPH, [14C]testosterone (3.5 μM) and liver microsome (20 μg protein) in a total volume of 0.5 ml, with or without designated concentrations of polyphenols. The reaction was started with the addition of the enzyme preparation. After incubation at 37°C for 20 min, the reaction solution was extracted with 2 ml ethyl acetate. The ethyl acetate phase (upper phase) was transferred to a tube and evaporated to dryness with nitrogen gas. The steroids were taken up in 50 μl ethyl acetate and chromatographed on a TLC plate (Merck silica gel 60F254 type), using chloroform–methanol (96:4) as the developing solvent system at 4°C. For comparison, standards of the unlabeled steroids (10 μg each of testosterone, DHT and androst-4-ene-3,17-dione) were also submitted to TLC. The plate was viewed under a UV lamp (254 nm) to locate testosterone and androst-4-ene-3,17-dione. Since DHT cannot be visualized under UV, it was located with iodine vapors. Detection of the radio-labeled steroids was performed by autoradiography. 14CJH and 14CJDT were measured by densitometer (IS-100 Digital Imaging System). The conversion from T to DHT was calculated from the ratio of the radioactivity of DHT to the sum of the radioactivities of T and DHT (31,32).

Cell cultures and treatments

The human prostate cancer cell line LNCaP was obtained from The American Type Culture Collection (Rockville, MD) and propagated in 24-well, 60 or 100 mm culture dishes at the desired density in RPMI 1640 medium supplemented with 10% fetal calf serum (FCS) (Gibco, Grand Island, NY), 1% penicillin/streptomycin (P-S) in a 5% CO2 atmosphere at 37°C. LNCaP cells were also cultured in 10% charcoal-stripped FCS (cFCS) and 1% (P-S). The cells were treated with polyphenols at designated concentrations with or without T at physiological concentrations (25 nM) in phenol red-free RPMI 1640 medium.

Cell viability and PSA protein expression

LNCaP cells were seeded at 2 × 10^4 cells/well in 24 well plates. After 48 h the medium was changed to serum-free RPMI 1640 medium, and the cells were incubated for an additional 24 h to deplete endogenous steroid hormones prior to experiments. Cells were then treated with different concentrations of polyphenols with or without 25 nM T. After 4 days incubation, spent media were harvested, and levels of PSA in spent media were quantified by an enzyme linked immunosorbent assay (ELISA). The human PSA ELISA kit was obtained from (Cytimmune, MA). Cell density was quantified by MTT assay. The protein levels of PSA was normalized by cell density measurements and expressed as a percentage over control (T alone).

Western blot analysis

For immunoblotting of the AR, LNCaP cells were grown under the same conditions described above and treated with or without polyphenols in the presence of T. Whole cell lysates were prepared using Gold lysis buffer (10% (v/v) glycerol, 1% (v/v) Triton X-100, 1 mM sodium orthovanadate; 20 mM Tris-base, pH 7.9; 1 mM sodium pyrophosphate; 100 mM β-glycerophosphate; 10 mM NaF; 137 mM NaCl; 5 mM EGTA; 1 mM PMSF; 10 μg/ml aprotinin; 10 μg/ml leupeptin). Protein content was quantified by the Bio-Rad DC protein assay (Bio-Rad, Hercules, CA). Eight percent polyacrylamide reducing SDS gels were run and electrotransferred onto an immobile membrane (PVDF; Millipore, Bedford, MA). The PVDF membranes were immediately blocked with blocking solution containing 1% bovine serum albumin dissolved in phosphate buffered-saline (PBS). The membranes were then immunoblotted with a primary monoclonal antibody against AR (1:2000 dilution; Pharmingen, San Diego, CA) or fatty acid synthase (FAS) (1:2000 dilution; Transduction Laboratories, Lexington, KY) for 1 h at room temperature. Membranes were incubated with an anti-mouse IgG secondary antibody conjugated to horseradish peroxidase (1:3000 dilution; Amersham, Arlington Heights, IL) at room temperature for 1 h. Blots were washed between each step with PBS and visualized by enhanced chemiluminescence substrate (ECL, Amersham, Arlington Heights, IL) then exposure to X-ray film.

Results

5α-Reductase activity in rat liver microsome

In mammalian cells, 5α-reductase is very tightly associated with microsomal membranes. The assay of 5α-reductase activity, therefore, has been characterized by the rate of conversion of testosterone to DHT by microsomal preparations of rat liver in the presence of NADPH (33). Previous study shows that green tea catechins including ECG and EGC are potential 5α-reductase inhibitors (34). For this reason, we further examined the in vitro effect of 5GG and theaflavins whose structures were related to EGCG on 5α-reductase activity. The data from these experiments are shown in Figure 2. 5GG, TF2A, TF2B and TF3 can inhibit 5α-reductase activity. The data in Figure 2 revealed inhibition of 5α-reductase activity in a dose-dependent manner by 5GG and theaflavins at concentrations up to 20 μM. TF3 was found to be the most effective inhibitor of 5α-reductase among the polyphenols tested. The maximum
inhibition found in TF3 was 89% at 20 μM. The IC₅₀ values of these compounds were listed in Table I. The inhibitory effect was in the order: TF3 > 5GG > TF2B > TF2A > EGCG > TF1. Among these compounds, TF1, the only one that does not present any gallate group, has no inhibitory activity up to 100 μM. Since the presence of the gallate group in the catechin molecule may be important for the inhibition activity of 5α-reductase, we next examined the inhibition effect of two other gallate group-containing compounds, gallic acid and n-propyl gallate. However, both of them have no inhibitory effect for 5α-reductase up to 100 μM (Table I) consistent with the previous report (34).

Kinetic study of 5α-reductase

Kₘ and Vₚₙₐₓ values for 5α-reductase were determined in rat liver microsome from Lineweaver-Burk plots using different T concentrations as shown in Figure 3. In an enzyme reaction, Vₚₙₐₓ means the maximum velocity when the enzyme molecules were saturated with high substrate
Kinetic studies of 5GG and theaflavins inhibition of 5α-reductase

The inhibition of 5α-reductase activity by 5GG and theaflavins were examined at varying concentrations of T or NADPH. The Lineweaver-Burk plots (Figure 4) of these data indicate that 5GG, TF3 and TF2B are non-competitive inhibitors for T but they are competitive inhibitors for NADPH. This mode of inhibition is similar to EGCG for inhibition of 5α-R1 (35). Taken together, these results suggest that 5GG, TF3 and TF2B may inhibit 5α-reductase by interacting with the NADPH binding site, but not the testosterone binding site. The \( K_i \) values of the three compounds for inhibiting T or NADPH binding are listed in Table II. \( K_i \) is a dissociation constant for inhibitor and enzyme binding, which means the affinity of enzyme for inhibitor. For the substrate testosterone binding to 5α-reductase, 5GG is the most effective non-competitive inhibitor. On the other hand, TF3 is the best competitive inhibitor for NADPH binding. Therefore, the results shown in Figures 2 and 4 clearly indicated that 5GG and theaflavins, especially TF3, inhibited 5α-reductase activity, which is the first step of the androgen action in prostate.

5GG and theaflavins inhibit testosterone-induced cell growth in LNCaP cells

The LNCaP (lymph node carcinoma of the prostate) human prostate cancer cell line is a well-established and androgen-dependent cell line (36). LNCaP cells retain most of the characteristics of human prostatic carcinoma, like the dependence on androgens, the presence of ARs, the production of acid phosphatase and PSA. More importantly, LNCaP cells express only 5α-R1 but not 5α-R2 (15). For this reason, the LNCaP cell line becomes an attractive model for the in vitro studies on the biology of human prostate cancer (37). In this study, we first examined the effects of 5GG and theaflavins on androgen-stimulated growth of LNCaP cells. LNCaP cells were incubated with varying concentrations of 5GG or theaflavins with or without T for 4 days. The MTT assay was performed to measure cell viability. In the absence of 5GG or theaflavins, T alone apparently stimulates LNCaP cell number 50% on average above untreated control in Figure 5. 5GG, TF3, TF2B or EGCG (10-40 \( \mu \)M) treatment resulted in a dose-dependent inhibition of LNCaP cell growth to the degree similar to that in no androgen stimulated controls (Figure 5. –T) at higher concentrations of these compounds (20 and 40 \( \mu \)M) in the presence of T. Compared with the six polyphenols tested in this experiment, 5GG and TF3 seem to exhibit higher inhibitory activity on LNCaP cell growth than TF2B and EGCG. In contrast, TF1 and TF2A showed little inhibitory activity. Therefore, we next examined the effect of 5GG and TF3 on AR expression, which is important for androgen action.

5GG and TF3 inhibit the expression of AR protein

After T is transformed into DHT by the enzyme 5α-reductases, DHT will bind to AR and result in a series of androgen actions. Since AR is the essential mediator for androgen action, we need to determine the effect of 5GG, theaflavins and EGCG on the AR protein expression. Therefore, a western blot analysis was performed to detect whether AR protein levels were changed by treatment with these compounds. Among the six polyphenols assessed, 5GG and TF3 significantly reduced the amount of AR protein at a dose of 40 \( \mu \)M concentrations. \( K_m \) measures the substrate concentration at which the reaction rate is \( V_{\text{max}}/2 \) and it is often associated with the affinity of enzyme for substrate. The mean value for \( K_m \) in our data was 2.45 ± 0.39 \( \mu \)M and that for \( V_{\text{max}} \) 3.11 ± 0.33 nmol of DHT/min/mg protein.

![Fig. 2. Inhibition of rat liver microsomal 5α-R1 by 5GG (filled square), EGCG (filled diamond) and theaflavins: TF2A (triangle), TF2B (square), TF3 (diamond). Values represent mean ± standard error of three separate experiments.](https://academic.oup.com/carcin/article-abstract/25/7/1109/2390706)

![Fig. 3. Determination of \( K_m \) and \( V_{\text{max}} \) values for rat liver microsomal 5α-R1 by the Lineweaver-Burk plots.](https://academic.oup.com/carcin/article-abstract/25/7/1109/2390706)
TF3 and 5GG inhibit rat liver 5α-reductase activity

(Figure 6a). TF2A, TF2B, TF1 and EGCG had minor effects at the concentration tested. We further examined the dose-dependent effect of 5GG and TF3 on AR protein expression. In Figure 6b and c, the inhibitory effect of 5GG and TF3 occurred in a dose-dependent fashion. Although 5GG and TF3 decreased AR protein levels, they did not affect the expression of β-actin or heat shock protein 70 (Hsp70), a chaperone protein for AR, suggesting that 5GG and TF3 probably do not have a broad inhibitory effect on gene expression.

![Fig. 4. Determination of $K_i$ values for rat liver microsomal 5α-R1 in the presence of (a) 5GG, (b) TF3 and (c) TF2B by the Lineweaver-Burk plots. Initial reaction velocities ($V$) were determined for different substrate (T or NADPH) concentrations and as a function of inhibitor concentration: 0 μM (filled square), 2.5 μM (filled triangle), 4 μM (open triangle) 5 μM (filled diamond), 6 μM (open diamond), 10 μM (filled circle).]

<table>
<thead>
<tr>
<th>Compounds</th>
<th>$K_i$ (μM)</th>
<th>NADPH</th>
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</thead>
<tbody>
<tr>
<td>Testosterone</td>
<td></td>
<td></td>
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<tr>
<td>5GG</td>
<td>2.68 ± 0.44</td>
<td>0.48 ± 0.02</td>
</tr>
<tr>
<td>TF3</td>
<td>5.59 ± 0.77</td>
<td>0.36 ± 0.18</td>
</tr>
<tr>
<td>TF2B</td>
<td>6.51 ± 0.57</td>
<td>2.60 ± 1.38</td>
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The $K_i$ values were determined by Lineweaver-Burk plots.
Evidence shows that alteration of 5α-reductase or AR leads to modulate androgen-responsive genes. PSA is one of the androgen-responsive genes because the promoter of PSA gene contains functional ARE (38). PSA is specifically produced by both prostate epithelial cells and prostate cancer and is the most commonly used serum marker for diagnosing cancers. In patients with prostate carcinomas, an increase of the serum PSA level is observed usually (39). In Figure 7, we further examined if 5GG and TF3 can actually block androgen action by measuring the secretion level of PSA in LNCaP cells.

PSA in spent media from cell growth experiments were quantified by immunometric assay and normalized by cell density. LNCaP cells were grown in serum-free RPMI media with or without T. As shown in Figure 7, T strongly up-regulates PSA production in LNCaP cells. In the presence of T, treatment of cells with varying concentrations of 5GG or TF3 significantly decreases the secretion levels of PSA in a dose-dependent manner. On the other hand, previous reports suggest that FAS, a key lipogenesis enzyme, could be also regulated by androgens. Androgen treatment of LNCaP cells results in a 3–4-fold stimulation of the steady-state mRNA levels of FAS and leads to an up to 10-fold stimulation of FAS activity.
The over-expression of FAS in prostate may simply reflect the mitogenic or secretory stimulus produced by androgen exposure. Rapid cell division requires the biosynthesis of cell membranes, and the specialized secretory function of prostate epithelial cells requires the synthesis of storage vesicles and secretory components (42). Evidence shows that FAS inhibitor might be a selective target to prostate cancer cells and have been proposed as therapeutic agents (43). Here we report that 5GG inhibit the expression of FAS protein in LNCaP cells. As shown in Figure 8, western blot analysis showed that treatment of 5GG greatly reduced the expression of FAS in a dose-dependent manner. Taken together, 5GG and TF3 actually inhibit androgen actions in LNCaP cells by down-regulating PSA secretion and FAS protein expression.

Discussion

Environmental factors, such as diet, have an important role in modulating cancer incidence and mortality, and differences in diet may explain geographical differences in prostate cancer mortality. Since androgens regulate the growth and function of the normal prostate and prostate cancer, dietary components capable of altering this growth signaling pathway in the prostate may affect prostate cancer development and progression. Flavonoids are naturally occurring polyphenolic compounds widely distributed in fruits, vegetables and beverages. In recent years, many flavonoids have been shown to possess cancer chemopreventive effects. Several studies have demonstrated that certain flavonoids have potential chemopreventive effects on prostate cancer through different mechanisms. Green tea polyphenols have been found to inhibit prostate carcinogenesis in vitro and in vivo. EGCG, the major polyphenolic constituent of green tea, inhibit growth of several types of human prostate cancer cell by inducing cell cycle arrest and apoptosis (44,45). EGCG also down-regulate the expression of the AR (46) and inhibit 5α-reductase activity (34,35). In addition, some evidence shows that EGCG inhibit the growth and rapidly reduced the size of human prostate tumors in nude mice (47). Moreover, green tea polyphenols were effective in inhibiting prostate cancer development and abolished distant site metastases in TRAMP mice by oral infusion (48). It has been demonstrated that green tea polyphenols possess prostate cancer...
chemoprevention effects on testosterone-mediated induction of ornithine decarboxylase, which is an important contributor of prostate cancer development (49). Relatively, black tea polyphenols can inhibit insulin-like growth factor-1 (IGF-1)-induced signaling in DU145 prostate cancer cells (50). In this study, we discovered that 5GG and TF3, which contain several galloyl groups inhibit prostate cancer by attenuating the function of androgen and AR.

The early development and growth of prostate cancer is highly dependent on androgens. In the prostatic cell, T is transformed into a more active form, DHT, by the enzyme 5α-reductases. DHT then binds to AR to exert its biological functions. Since excessive 5α-reductase activity has been proposed to be a possible contributing factor in prostate cancer development or progression (10), the development and progression of prostate cancer may be affected by diets containing inhibitors of 5α-reductase. In our study, we found that 5GG and certain theaflavins including TF3 and TF2B reduce androgen function by inhibiting 5α-R1 activity in rat liver microsomes (Figures 2 and 4, Tables I and II). Meanwhile, they can also inhibit testosterone-induced cell growth, PSA secretion and FAS protein expression in LNCaP cells, which express only 5αR1 (Figures 5, 7 and 8). Based on these findings, we suggest that 5GG and TF3 may inhibit prostate cancer cell growth due partly to their inhibition effects on 5α-R1. It has been shown that dual 5α-reductase inhibitor is more effective, physiologically and clinically, than only the 5α-R2 inhibitor finasteride, the first drug approved in the USA for BPH treatment (51,52). Perhaps the use of combined 5α-R1/5α-R2 inhibitors may halt or ameliorate the progression of BPH or prostate cancer more efficiently.

In Table I, we elucidated the effects of 5GG, theaflavins and EGCG on 5α-reductase activity. Interestingly, we found that the IC_{50} value of TF3 was 18.9-fold less than EGCG and these data suggested that black tea polyphenols are stronger 5α-R inhibitors than green tea polyphenols. Moreover, the inhibitory effect of six polyphenols on 5α-reductase was in the order: TF3 > 5GG > TF2B > TF2A > EGCC > TF1. The similar result was also shown in previous studies that the inhibition of iNOS protein is in the following order: TF3 > EGCC > TF2 > TR > TF1 (53). Compared with the structures of these compounds, we found that gallate group containing polyphenols are better inhibitors of 5α-reductase. Nevertheless, gallic acid and n-propyl gallate, the two compounds having simple gallal or gallaloyl groups, did not have an inhibitory effect on 5α-reductase. This suggests that the galloyl group alone is not sufficient for the enzyme inhibition. A previous study observed that the catechol group might be necessary for potent inhibition of 5α-R1 (35). In comparing the structures of testosterone and tea polyphenols including catechins and theaflavins, we have noted the following features. First, the A, B and C rings of catechins and theaflavins are in a plane while the four fused rings of testosterone are also in a plane. The hydrophobic interactions among these compounds may occur. Secondary, the galloyl groups in EGCG, TF2A, TF2B and TF3 are rotatable, which may be affected in the binding of these compounds to the 5α-R1. Finally, the B ring in EGCG is freely rotatable, while the B rings of theaflavins are fused and non-rotatable. This may be one of the reasons that theaflavins show stronger inhibitory effect than EGCG. Therefore, we need to examine further what structures in the polyphenol compounds are important for the inhibition of 5α-reductase.

For many years, androgen ablation or endocrine therapy has remained the mainstay of treatment for prostate cancer. Unfortunately, cancer cells eventually escape the steroid requirement and progress into the androgen-independent phenotype during androgen ablation therapy. In many recurrent or advanced prostate cancers, AR is still expressed, either mutated or amplified. The mutated but functionally intact AR can be activated by lower concentrations of androgens or other non-androgenic ligands such as growth factors. Therefore, AR is not only important in androgen-dependent prostate cancer cells but also in androgen-independent cancers. In this study, we next focused on whether 5GG and theaflavins inhibit AR expression. In Figures 6–8, 5GG and TF3 significantly decrease the expression and function of AR. The abilities of 5GG and TF3 on reducing AR protein levels might make them a good chemopreventive or chemotherapeutic agent for prostate cancer.

In Figures 7 and 8, we found that 5GG and TF3 inhibit androgen action by reducing PSA secretion and FAS protein expression. PSA is an androgen-regulated serine protease and may be involved in the pathogenesis of prostate cancer (54). The proteolytic capacity of PSA in tumor microenvironments has the potential to cleave a number of proteins that may influence prostate cancer development or progression. One such protein that can be cleaved by PSA is insulin-like growth factor binding protein-3, which functions as the major serum binding protein for IGF-1, a growth factor for prostate cancer cells (55). In addition, androgen treatment results in FAS gene over-expression in prostate cancer cells may simply reflect the rapid cell division requires high fatty acid synthesis. Taken together, 5GG and TF3 can inhibit the expression of androgen-regulated genes, which are important for prostate cancer progression.

Another important observation of our study was that 5GG and TF3 have better inhibitory effects than EGCG on 5α-reductase activity, testosterone-induced LNCaP cell growth and the expression of AR protein. Since green tea polyphenols have been found to inhibit prostate carcinogenesis in vitro and in vivo, we could expect that 5GG and TF3 may exhibit powerful inhibitory effects on prostate cancer development.

In summary, we hypothesize that the natural polyphenolic 5GG and TF3 exhibit their inhibitory effects on androgen action in prostate cancer cells by two aspects including inhibition of (i) 5α-reductase activity and (ii) AR protein levels. Therefore, 5GG and TF3 may have the potential to become chemopreventive or chemotherapeutic agents for prostate cancer.

Acknowledgements

This study was supported by the National Science Council NSC 91-2320-B-002-006 and NSC 91-2311-B-002-037, by the National Health Research Institute NHSI-EX91-8913BL and by the Ministry of Education, 89-B-FA01-1-4.

References


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Received September 16, 2003; revised January 3, 2004; accepted January 26, 2004