

Green Tea Catechins -Pharmacokinetic Properties and Health Beneficial Effects

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Abstract

In this review, we provide information on the pharmacokinetic properties of green tea catechins and their beneficial health effects. The major catechins in green tea are (-)-epicatechin (EC), its hydroxyl derivative (-)-epigallocatechin (EGC), and their gallic acid esters, (-)-epicatechin-3-gallate (ECg) and (-)-epigallocatechin-3-gallate (EGCg). We developed an analytical method for determination of the presence of green tea catechins in human serum using ion-pair HPLC with electrochemical detection to estimate the pharmacokinetic parameters of target catechins. The C_{max} values indicated that catechin absorption was relatively low. One of the galled catechins, EGCg, had a longer half-life than the non-galled catechins. Green tea catechins, in particular, have attracted attention as cancer preventive agents in terms of their low toxicity and being readily available to the general population. Several epidemiological studies revealed that green tea consumption reduces cancer incidence. Numerous *in vitro* cell culture studies have shown that EGCg, which is defined as a major green tea catechin contributing to green tea's anticancer effects, inhibits cell growth concomitant with induction of apoptosis. We have previously found that the cell death-inhibiting gene, *Bcl-xL*, was decreased by EGCg. These results support the hypothesis that EGCg regulates cytoplasmic NF- κ B and subsequently induction of apoptosis. Green tea consumption may also play a role in preventing other lifestyle diseases, such as cardiovascular diseases and stroke, due to its hypocholesterolemic and hypotensive activities. In conclusion, habitual green tea drinking may promote human health by preventing lifestyle-related diseases.

Introduction

The tea plant (*Camellia sinensis*), a native of southern China, has been known for more than two thousand years in Chinese botany and medicine. Lu Yu, who lived from 733 to 804, is respected as the Sage of Tea for his contributions to Chinese tea culture. He is best known for his seminal book, *Ch'aChing*, or The Classic of Tea, the first definitive work on the cultivation, preparation, and drinking of tea. Eisai (1141-1215) was a Japanese Zen Buddhist monk who, after studying in China, brought back new tea seeds and introduced the tea ceremony. He also wrote the first book about the health benefits of drinking green tea, '*Kissa- Yohjoh-Ki*' (Maintaining Health by Drinking Tea). Sen Rikyu (1522-1591) established the foundation of *Chanoyu* (The Japanese style of tea ceremony) which has been said to offer a comprehensive model for life. Currently in Japan, green tea is regarded as a healthy beverage by the public. The term 'green tea' refers to the product manufactured from fresh tea leaves by careful steaming or roasting to avoid oxidation of the polyphenolic components known as catechins. The major catechins in green tea are (-)-epicatechin (EC), its hydroxyl derivative (-)-epigallocatechin (EGC), and their respective gallic acid esters, (-)-epicatechin-3-gallate (ECg) and (-)-epigallocatechin-3-gallate (EGCg) (Figure 1). Among green tea catechins, EGCg is abundant in green tea leaves, and has been shown to exhibit strong health-promoting activity [1,2], according to structure activity relationship assessment on EGCg, two close parallel aromatic rings and a third aromatic ring vertical to the two parallel rings may play a key role in the pharmacophore activity. This activity may be associated with the number of -OH groups in the catechin [3].

An understanding of the pharmacokinetic properties of green tea catechins is vital for promoting the human health benefits of its consumption. To assess the pharmacokinetics of green tea catechins after ingestion, we developed a new analytical method for detecting green tea catechins in serum. Pharmacokinetic study using this method showed individual variation of catechin serum concentration after ingestion of same amount of green tea catechins [4]. This

observation indicated the existence of unknown factors affecting the pharmacokinetics of green tea catechins.

Numerous studies have shown that catechins have anti-cancer, hypotensive, and hypocholesterolemic activities [5-10]. Consistently, inverse relationships between green tea consumption and the reduction of cancer incidence, the risk of developing hypertension, and cholesterol level have also been demonstrated in epidemiological studies.

Previously, the *in vitro* anti-cancer effects of a green tea extract on Adult T-cell Leukemia (ATL), which is an endemic disease caused by a latent infection of human retrovirus HTLV-1, have been demonstrated [11]. Furthermore, to investigate an intervention study, the *in vivo* effects of drinking green tea on HTLV-1 provirus load in asymptomatic HTLV-1 carriers have been conducted [12]. By integrating the results of these studies, we proposed a possible mechanism of the anti-cancer effect of EGCg was proposed in this review.

Habitual green tea drinking has been shown to reduce other lifestyle-related diseases. We review experimental and epidemiological evidence to demonstrate that green tea consumption is good for human health.

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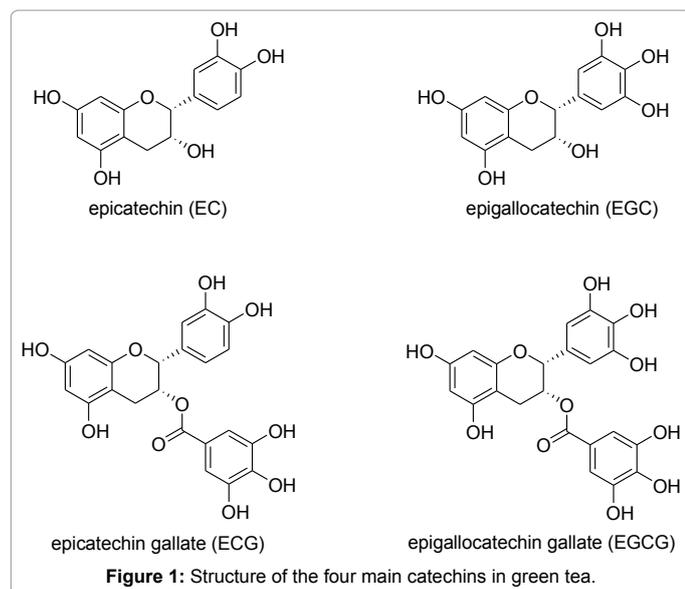


Figure 1: Structure of the four main catechins in green tea.

Method of analysis for green tea catechins

Although one regular cup of green tea contains several tens of milligrams of catechins, the serum concentration of catechins after ingestion reaches a maximum between several tens and several hundreds of ng/mL [13]. In order to assess the pharmacokinetics and beneficial health effects of catechins, a sensitive, reproducible, and straight-forward method is needed to quantify serum catechin concentration.

Generally, tea catechins are analyzed using high-performance liquid chromatography (HPLC) with ultraviolet (UV), chemiluminescence (CL), or electrochemical detection (ECD) also commonly used are capillary electrophoresis (CE) and gas chromatography with mass spectrometry (GC-MS) [14-18]. These methods have been used for determining catechin concentrations in tea, rat plasma, human plasma, and urine. Although GC-MS can be used for the determination of catechin concentration with high sensitivity, the required sample pretreatment is too demanding for routine analysis. HPLC is easier to use and more widely available. Previous work has shown that HPLC-ECD is more sensitive for the determination of catechin concentrations than HPLC-UV. Moreover, ECD is well suited to the detection of polyphenolic compounds like catechins because these analytes can be selectively oxidized by the appropriate electrical potential and flow current, the magnitude of this current is associated with the analyte concentration. Therefore, HPLC-ECD is an excellent candidate technique for the selective and highly sensitive detection of catechins in serum.

After ingestion of green tea, a large portion of catechins are present as glucuronate or sulfate conjugates in the serum. For the quantification of total catechins in serum, samples were treated with β -glucuronidase and sulfatase for hydrolysis to the free form of catechins. It has been reported that type H-2 β -glucuronidase, which also contains sulfatase activity, effectively hydrolyzes conjugates in plasma samples but concomitantly cleaves the ester bonds of EGCg and ECg. Therefore, these enzymes convert gallated catechins (EGCg and ECg) to their non-gallated forms (EGC and EC) [5,19]. Total catechins are expressed as EGCg + EGC and ECg + EC, which unfortunately makes it difficult to determine the exact concentrations of each

individual catechin, due to some EGC and EC being derived from EGCg and ECg via treatment with the enzyme. Therefore, gallic acid (GA), derived from the gallated catechins, must be measured simultaneously to elucidate whether or not cleavage has occurred.

We developed a new simultaneous detection system of green tea catechins and GA in human serum using ion-pair HPLC with an ECD detection system. All the analytes (4 catechins and GA, and Ethyl gallate as an internal standard) were separated and obtained as single peaks, and the accuracy, reproducibility, and sensitivity of this method were sufficient to measure the target catechins and GA in serum [4].

Pharmacokinetic properties of green tea catechins

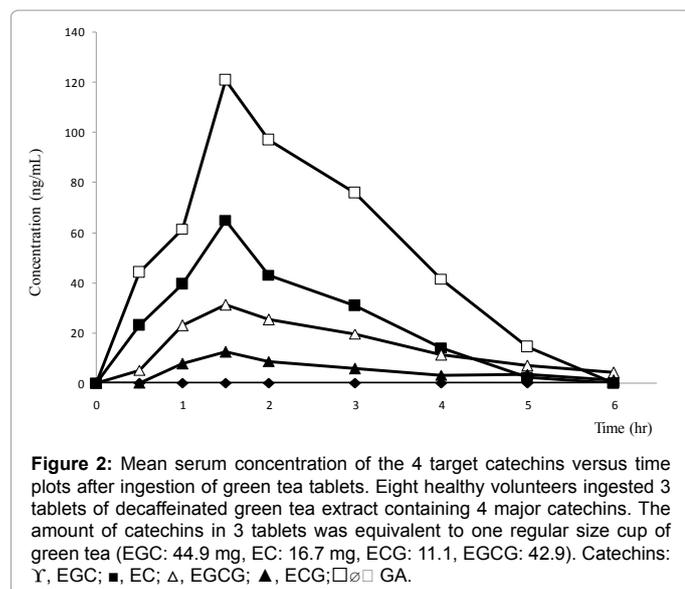
A pharmacokinetic study with 8 healthy volunteers was conducted using our method, and changes in the levels of the four catechins and GA were monitored over time (Figure 2). No GA was detected in any of the samples. Other samples spiked with a known amount of standard catechins in phosphate buffer saline and treated with the deconjugation enzymes yielded a small amount of GA. The enzymatic decomposition of the gallated catechins may have been prevented by an unknown component in the serum. It has been reported that gallated catechins, specifically EGCg, preferentially bind with albumin in blood and can avoid metabolic conjugation [20,21]. The green tea extract tablets taken by subjects contained 16.7 mg of EC, 44.9 mg of EGC, 11.1 mg of ECG, and 42.9 mg of EGCg, and mean C_{max} (n=8) reached 34.7, 60.6, 20.9, and 42.8 ng/mL, respectively. These C_{max} values indicated that the amount of catechin absorption was relatively low. Gallated catechins such as EGCg and ECg had longer half-lives than the non-gallated catechins, EGC and EC, probably due to the majority of gallated catechins preferentially binding to serum proteins and existing in the non-conjugated free form, delaying excretion. Moreover, efflux transporters, such as multidrug resistance-associated protein 2 (MRP2), a real so likely factors affecting half-life times; catechin metabolites are substrate of MRPs [22]. Li et al. demonstrated that non-gallated catechins were preferentially effluxed during their absorption across the small intestines using MRPs expressed in a Caco-2 monolayer model, consistent with our observation [23]. Catechins may also be substrates of another transporter, p-glycoprotein (P-gp) [24]. Functional polymorphisms of P-gp have been shown both in human studies and in *in vitro* studies using cells stably transfected with various mutants of human P-gp [25]. Individual variation in C_{max} and AUC of catechins after ingestion of green tea extract was observed in our pharmacokinetic study, which may be associated with the variation of P-gp activity among the subjects [26]. Recently, Misaka et al. reported a significant catechin-drug interaction; green tea ingestion greatly reduced plasma concentration of β -blocker nadolol mediated by organic anion-transporter OATP1A2 [27]. The catechin-drug interaction competitive antagonism or direct inhibition for transporters should be kept in mind in the clinical field.

Fewer studies have reported the interaction between catechins and efflux transporters, possibly due to the instability of polyphenolic structures in cell culture medium or saline buffers caused by antioxidant level, pH of culture condition, concentration of proteins or the presence of metal ions [28]. To improve the stability of catechins in saline buffers, addition of a small amount of ascorbic acid (around 100 μ M) as an antioxidant is recommended [22,24].

Anti-cancer effects of green tea drinking

Human studies

General cancer: Imai et al. showed an association between green



tea consumption and incidence of cancer in a prospective cohort study of a Japanese population with 8,552 individuals over the age of 40 residing in a town in Saitama Prefecture surveyed regarding their lifestyle habits, including their daily consumption of green tea [29]. An association between green tea consumption and cancer incidence was uncovered, with an increase in green tea consumption significantly decreasing the incidence of cancer among females. Although not statistically significant, males also showed a reduction in the relative risk of cancer with increased consumption of green tea, this was found among both smokers and nonsmokers. In a comparison of age-specific cancer incidence rates among females, an obvious reduction in age-related increases in cancer incidence with increased green tea consumption was observed, especially for individuals in their 60s and 70s.

Prostate cancer: The association between green tea consumption and prostate cancer risk has been shown by Kurahasi et al. and the Japan Public Health Center-based Prospective Study Group. A total of 49,920 men were prospectively followed up (from 1990 to 2004); 404 men were newly diagnosed with prostate cancer, and of these, 114 were advanced cases, 271 were localized, and 19 were at an undetermined stage. Green tea consumption was associated with a dose-dependent decrease in the risk of developing advanced prostate cancer. The multivariate relative risk was 0.52 (95% confidence interval: 0.28, 0.96) for men drinking 5 or more cups/d compared with less than 1 cup/d ($P_{\text{trend}}=0.01$). Thus, green tea consumption may be associated with a decreased risk of advanced prostate cancer [30]. Consistent results were shown in a one-year proof-of-principle study of 60 volunteers with high-grade prostate intra-epithelial neoplasia (HG-PIN) [31]. Compared to the placebo group, the control group receiving 600 mg/d of green tea extract had reduced development of prostate cancer from HG-PIN. Notably, no significant side effects or adverse effects were found in the 1-year study period. These results indicate that long term ingestion of green tea extract is safe and effective for treating premalignant lesions before development of prostate cancer.

Breast cancer: Zhang et al. conducted a case-controlled study in southeast China to identify the protective effects of green tea consumption against breast cancer; 1009 women newly diagnosed with breast cancer were recruited to the study and an equal number of healthy women were selected as the control group. The dose-response

relationships observed for the duration of green tea consumption and the number of cups consumed per day was similar. The author concluded that the regular consumption of green tea is associated with a reduced risk of breast cancer [32]. On the other hand, Suzuki et al. reported in a pooled analysis of two prospective studies with 35,000 Japanese women that there was no association between consumption of green tea and risk of breast cancer [33]. Because catechin absorption from the intestines to the bloodstream is relatively low [4], a high dose of about 10 cups/d in addition to long duration of green tea consumption may be necessary to achieve an inverse association between green tea consumption and cancer incidence. Although inconsistent results have been observed in a case-controlled study and a prospective study, Li et al. showed that there was a statistically significant 19% reduction of breast cancer incidence among women with high green tea intake in the most recent meta-analysis of two cohort studies and five case-controlled studies [34-41].

Colorectal cancer: Yuan et al. have jointly conducted the Shanghai Cohort Study involving 18,244 men to investigate the association between urinary levels of EGC and its methyl metabolite (Me-EGC) as validated biomarkers of specific tea polyphenols and the risk of colorectal cancer. After a 16 year follow-up period, 162 subjects developed colorectal cancer. Among subjects with at least 5 years of follow-up, higher levels of Me-EGC were associated with lower risk of colon cancer. Compared with undetectable Me-EGC, odds ratios (95% confidence interval) for colon cancer in the lowest, intermediate and highest tertiles of detectable Me-EGC were 0.49 (0.25-0.96), 0.32 (0.16-0.67) and 0.41 (0.20-0.84), respectively ($P_{\text{trend}}=0.006$); polyphenol biomarkers were not associated with rectal cancer risk. This study strongly supports long term green tea consumption for prevention of colon cancer in humans [42].

Lung cancer: A case-controlled study conducted in Okinawa, in the south of Japan, showed that daily tea consumption significantly decreased the risk of lung cancer, especially for squamous cell carcinoma [43]. Contrarily, Li et al. demonstrated in a population-based cohort study in Miyagi, in northeastern Japan, that there was no association between green tea consumption and the risk of lung cancer [44]. To address these conflicting views, Wang et al. performed a recent meta-analysis of 38 lung cancer studies (26 case-controlled studies and 12 cohort studies) with 59,041 cases and 396,664 controls. The results showed that overall tea consumption was significantly associated with a decreased risk of lung cancer (RR, 0.78; 95%CI, 0.70-0.87) [45].

Leukemia cancer: Based on epidemiological evidence and our preliminary experimental study [11], authors attempted to apply green tea for Adult T-cell Leukemia (ATL). The causative agent of ATL is the human retrovirus, HTLV-1. HTLV-1-infected individuals are prevalent world-wide but clustered the endemic area including southwestern Japan. Therefore, we investigated green tea as a chemopreventive agent in ATL development. We conducted an intervention study to investigate the *in vivo* effect of green tea on HTLV-1 provirus load in peripheral blood lymphocytes (PBLs) of HTLV-1 carriers. The subjects enrolled in this study were asymptomatic HTLV-1 carriers. They were randomly assigned to two groups. GT (-): no intake of any green tea capsules ($n = 46$), and GT (+): received 9 green tea capsules per day ($n = 37$). Among the subgroup with a higher provirus load, a decreasing trend in HTLV-1 provirus load values was observed in the GT (+) group (regression coefficient (RC) = -0.072, SE=0.430), but not in the GT (-) group (RC= +0.012, SE=0.043). A significant difference in HTLV-1 provirus load from baseline to each follow-up month between the GT (-) and GT (+) groups observed at the 5 month [12].

Summary for human studies: Green tea consumption may preferentially offer some protection against hormone-associated cancer cells, including prostate and breast cancer cells [46]. Although green tea catechins, especially EGCG, strongly inhibit cellular proliferation or cell viability in other cancer cell lines, including oral, esophageal, bladder and skin cells, less information from cohort, case-controlled, or interventional studies on humans have been performed [5]. Recently, Hou et al. reviewed 17 epidemiological studies for stomach cancer, and found that all studies that analyzed men and women separately suggested a reduced risk in women compared to men, albeit the difference was not significant [47]. The inconsistent results between the epidemiologic studies may be due to variables such as differences in tea preparation and consumption, the methods of tea production, the bioavailability of tea compounds, and genetic variation in how the human body responds to tea consumption.

Proposed Mechanism of the Anti-cancer Effects (Experimental Studies)

We have shown that EGCG markedly inhibits cell proliferation. Furthermore, under identical conditions, EGCG suppressed *Bcl-xL* mRNA expression in non-small-cell lung cancer A549 cells [48]. Bcl-xL, a member of the Bcl-2 family, inhibits apoptosis by blocking the mitochondrial cytochrome *c* release [49, 50]. A decrease in *Bcl-xL* gene expression may lead to the promotion of cell death. We predict that NF- κ B inactivation by catechins, which is an upper stream event, may be essential for the induction of apoptosis. Several cell culture studies have focused on one of the hallmarks of apoptosis induction by green tea catechins, namely NF- κ B [51, 52]. NF- κ B is a nuclear transcription factor that regulates expression of genes that are critical of the regulation of apoptosis. NF- κ B is bound in a complex in cytosol with inhibitor protein I κ B. When I κ B is degraded by proteasomes, NF- κ B enters the nucleus and transcript target genes such as apoptosis-inhibiting genes. The suppression of NF- κ B activation may lead to induce apoptosis. Gupta et al. revealed that EGCG reversed the degradation of I κ B α , inhibitor protein of NF- κ B, in a cytoplasmic extract. Subsequently NF- κ B activation is down-regulated and apoptosis is induced in A431 human epidermoid carcinoma cells [53]. On the other hand, the vascular endothelial growth factor (VEGF) is the most critical regulator in development of solid tumors. Indeed, human monoclonal antibody against VEGF, Bevacizumab, is utilized as the molecular target in the clinical use. Recently, Gu et al. reported that EGCG suppresses breast tumor growth by inhibiting the activation of NF- κ B and VEGF expression. Oral administration of EGCG significantly reduced tumor VEGF expression in a mouse breast cancer model, in which mammary glands were inoculated with breast cancer cells. It was also shown that EGCG significantly inhibited the activation of NF- κ B in cultured cells [54]. Integrating these evidences, we hypothesized possible mechanism of apoptotic cell death and VEGF suppression by EGCG. That is, EGCG stabilize I κ B and abrogates NF- κ B activation. Then, suppression of the apoptosis-inhibiting protein Bcl-xL causes the release of cytochrome *c* from mitochondria. The activation of caspase signaling induces apoptosis, which is accompanied by a concomitant reduction in VEGF expression might lead to the suppression of angiogenesis (Figure 3).

Green Tea Effect on Lifestyle-related diseases

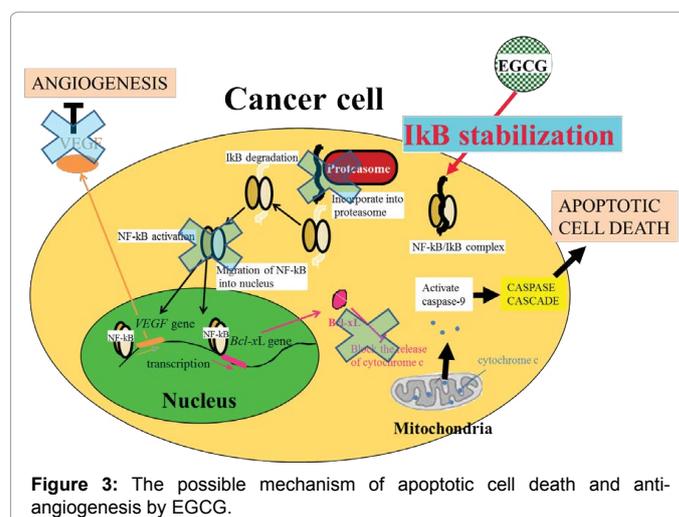
Recently, Kokubo et al. reported on the impact of green tea consumption on the incidence of cardiovascular disease and stroke in the Japanese population [55]. In this study, high green tea consumption was found to be inversely associated with the incidence of cardiovascular disease, stroke and stroke subtypes. The result verified

by 'the Japan Public Health Center-Based Study Cohort was consistent with distinct study of 'The Tokamachi-Nakasato cohort study in Japan' [56].

Hypertension is one of the major risk factors of cardiovascular disease or stroke. Yang et al. reported that habitual tea consumption of 120 mL/d or more for 1 year significantly reduced the risk of developing hypertension in the Chinese population. This demonstrates the negative correlation between the amount and duration of tea consumption on the systolic and diastolic blood pressures. Multiple logistic regression models of the effect of the amount and duration of tea consumption on hypertension have been generated. Compared with non-habitual tea drinkers, the risk of developing hypertension was decreased by 45% for those who drank 120 to 599 mL/d, and was further reduced by 65% for those who drank 600 mL/d [6]. One possible mechanism of the effect of green tea on hypertension is the activation of the Akt and endothelial NO synthase (eNOS) pathways. Akt is a serine/threonine protein kinase that is activated by a phosphatidylinositol-3 kinase, which regulates phosphorylation eNOS. eNOS is involved in NO release and vasorelaxation. Ihm et al. assessed the level of phosphorylated Akt and eNOS in aortic rings in a rat model of metabolic syndrome (MS). Although MS rats showed low levels of phosphorylated eNOS and Akt, treatment with a green tea extract (namely, oral administration of 25 mg/kg/d for 12 weeks) increased eNOS and Akt phosphorylation [7].

The effect of green tea administration on serum cholesterol levels is shown. Wu et al. conducted a 2-month controlled green tea intervention study. Postmenopausal women were randomized into three arms: placebo, 400 mg of EGCG as a green tea extract capsule (PPE), or 800 mg of EGCG as a PPE. Total cholesterol and LDL cholesterol decreased significantly in both PPE groups. The LDL-cholesterol level differed significantly between the placebo and PPE groups [8]. While the mechanisms by which green tea influences LDL-cholesterol levels remain unknown, animal studies showed that green tea extracts inhibit intestinal absorption of lipids and up regulate lower-density lipoprotein receptors in liver may lead to increase the efflux of cholesterol from liver cells [57].

Most recently, the effect of green tea on blood pressure and lipid profile was assessed by meta-analysis of randomized clinical trial. Based on the results of meta-analysis which included 20 RCTs and 1536 participants, green tea intake results in significant reductions in systolic blood pressure, total cholesterol, and LDL-cholesterol. The



effects appear greater with longer duration of intervention [58].

Conclusion

Since green tea catechins absorption after one regular cup of green tea intake is relatively low (estimated in 10s to 100s nM range), it is difficult to obtain a similar result of anti-cancer effects observed in *in vitro* cell culture study (in 10s to 100s μ M range). However, epidemiological studies shown in this review suggesting that larger portion of green tea consumption for a long term might reduce not only the risk of cancer, but also other lifestyle-related disease incidence. We concluded habitual green tea drinking is good for health.

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