



Review

Cancer chemopreventive activity and bioavailability of tea and tea polyphenols

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Abstract

Consumption of tea (*Camellia sinensis*) has been associated with many health benefits including the prevention of cancer. Based on in vitro experiments, many mechanisms have been proposed to account for the cancer chemopreventive activity. The importance of some of these mechanisms in vivo remains in question due to an incomplete understanding of the bioavailability of the polyphenolic compounds in tea. In this article, the literature on the cancer chemopreventive activity of tea and the tea polyphenols is discussed as well as some of the possible mechanisms for this activity. Whereas studies in animal models and with cell lines have demonstrated cancer preventive activity, the epidemiological data remain mixed. This discrepancy may arise from several factors including lifestyle, correlation between animal models and humans, and differences in metabolism among individuals. Results on the bioavailability and biotransformation of the tea polyphenols help explain some of the differences. We hope this article will spark research efforts on some of the important questions regarding tea polyphenol bioavailability and cancer chemoprevention.

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1. Introduction

Tea (*Camellia sinensis*, family Theaceae) is consumed worldwide and is second only to water in popularity as a beverage. Many health benefits have been ascribed to consumption of this beverage including prevention of cancer, heart disease, and cataracts.

The three major forms of tea—green tea, black tea, and oolong tea—differ in how they are produced and in their chemistry. A typical brewed green tea beverage contains 30–42% catechins by dry weight. These include (–)-epicatechin (EC), (–)-epicatechin-3-gallate

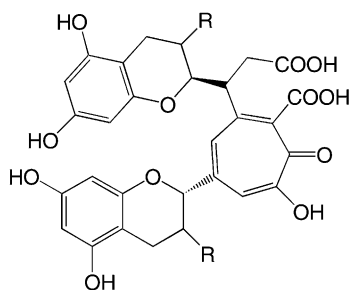
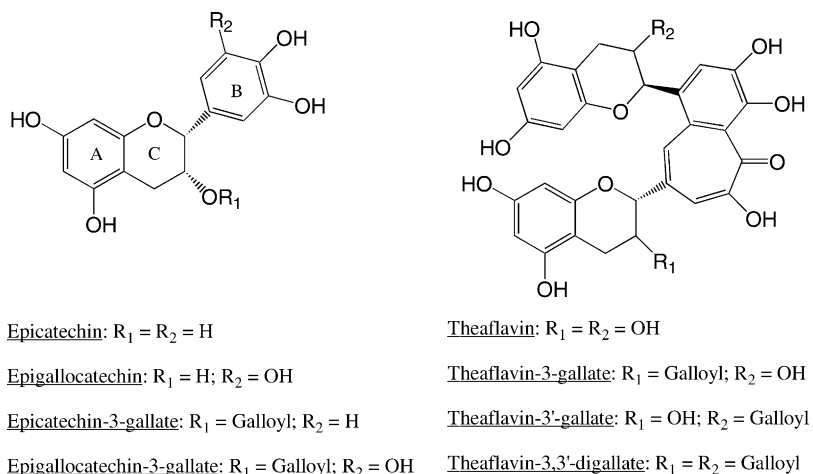
(ECG), (–)-epigallocatechin (EGC) and (–)-epigallocatechin-3-gallate (EGCG) with EGCG being the major component (Fig. 1). In black tea, catechins, theaflavins (TF) and thearubigins (TR) (Fig. 1) account for 3–10, 2–6, and >20%, respectively, of the water-extractable material by dry weight. Tea leaves also contain flavonols, such as quercetin and myricetin as well as the nitrogenous compounds caffeine and theobromine [1].

Whereas many of the beneficial effects of tea have been attributed to the strong antioxidative activity of the tea polyphenolic compounds, other biological mechanisms may also be important. A comprehensive understanding of these potential mechanisms is hindered by the lack of understanding of the bioavailability of the tea polyphenols. Here, we discuss the

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Thearubigins (R = Galloyl or other groups)

Fig. 1. Major tea polyphenols.

cancer chemopreventive activities of the tea and tea polyphenols; potential mechanisms by which these activities are realized; and the current knowledge of the bioavailability and biotransformation of these compounds. The purpose of this review is to discuss what is currently known about the potential cancer preventive activity of the tea compounds and which areas of research require further study.

2. Inhibition of carcinogenesis

2.1. Animal studies

Numerous studies have indicated that green tea, black tea, and pure tea polyphenols inhibit both ultraviolet light and chemically-induced tumorigenesis

in animals. These studies have been previously reviewed [2]. Tea and its constituents have been shown to inhibit carcinogenesis in the skin, lung, esophagus, stomach, liver, small intestine, pancreas, colon and mammary gland [3–11]. Tea and tea polyphenols have demonstrated inhibitory activity during the initiation, promotion, and progression stages of carcinogenesis.

Many studies have attempted to determine the cancer chemopreventive constituents of tea. Whereas inhibition of tumorigenesis by EGCG and theaflavins have been demonstrated in some models [4,12], decaffeinated green or black tea have been shown to be ineffective in the inhibition of UVB-induced complete carcinogenesis [5,13]. Pure caffeine, however, displayed inhibitory activity in this and other models [5,13].

2.2. Human epidemiological studies

The effectiveness of tea as a cancer chemopreventive agent in humans remains inconclusive. Most of the studies showing an inverse relationship between tea consumption and development of cancer were conducted on gastrointestinal cancers in Japan and China where green tea is the main form of tea consumed [2]. Of seven case control studies on the relationship between gastric cancer and tea consumption conducted in China and Japan, four studies found a significant inverse relationship and another two found an insignificant inverse relationship [2]. However, a cohort study in Japan found no association between green tea consumption and gastric cancer risk [14]. A recent case-control study of esophageal cancer patients in Shanghai showed that consumption of green tea was associated with a lower risk of esophageal cancer [15]. Conversely, earlier studies have shown a positive correlation between tea consumption and esophageal cancer. In six of seven studies, the correlation was due to the high temperature of the tea [16]. Indeed, recent studies have confirmed drinking very hot liquids including tea and soup as a risk factor for esophageal cancer [15].

Studies of black tea have also been mixed. For example, a population-based study in The Netherlands found no effect of black tea consumption on the risk of breast, colorectal and stomach cancers [17]. However, a recent study in the US suggests that consumption of black tea reduces colon cancer risk in both men and women [18].

Such inconsistent results may be due to a number of confounding factors including diet, smoking status, age, and alcohol consumption. Additionally, the bioavailability of the tea polyphenols is not clearly understood. It is possible that individual variation in metabolism and gut microflora may significantly alter the uptake and availability of biologically active molecules.

2.3. Mechanistic studies

The cancer chemopreventive effects of EGCG and the other tea polyphenols may be the result of decreased cell transformation and proliferation or increased apoptosis. In vitro, tea polyphenols, especially EGCG, have been shown to cause growth

inhibition and apoptosis in a number of human tumor cell lines including melanoma, breast cancer, lung cancer, leukemia, and colon cancer [19–22]. These effects have been extensively studied in vitro to try to elucidate the potential mechanism(s) of action of EGCG and the other tea polyphenols. While these effects have been observed in vivo in certain animal models, no definitive mechanism(s) has been reported for the tea polyphenols [23].

2.3.1. Antioxidative effects

The antioxidative activity of polyphenols has been well-studied in vitro, but the role of antioxidative activity in chemoprevention in vivo has been much harder to establish. Tea administration has been shown to inhibit oxidative stress induced by carcinogens and tumor promoters [24,25]. Nevertheless, the relationship between such inhibition and reduced tumor formation needs to be established.

2.3.2. Cell cycle effects

EGCG induces cell cycle arrest in the G₀/G₁ phase. In MCF7 breast cancer cells, this arrest is characterized by hypophosphorylation of Rb, loss of cyclin dependent kinase (cdk)2 and cdk4 activity, and overexpression of the cdk inhibitors WAF1/p21 and KIP1/p27 [26,27]. Similar molecular events are observed following treatment of EGF-stimulated breast epithelial cells with EGCG [28]. G₀/G₁ arrest in prostate carcinoma cell lines is accompanied by an increase in p53 expression and an induction of WAF1/p21 [29].

2.3.3. Signal transduction effects

Inhibition of MAP kinases and suppression of AP-1 transcription factors can lead to growth inhibition, cell cycle arrest and apoptosis. Recently, it was shown that EGCG and theaflavin-3,3'-digallate (TFdiG) inhibited the growth of and induced apoptosis in Ras-transformed human bronchial cell lines. Both compounds produced H₂O₂, but only EGCG-induced apoptosis was partially inhibited by addition of catalase. Catalase had no effect on TFdiG-induced cell death. EGCG and TFdiG inhibited the phosphorylation of c-jun and ERK1/2 as well as the phosphorylation of EIK-1 and MEK1/2 which are downstream and upstream in the MAP kinase cascade, respectively [30,31].

NF κ B activation by both lipopolysaccharide and TNF α is inhibited by EGCG through the inhibition of I κ B phosphorylation and degradation. Ahmad et al. observed that A431 human epidermoid carcinoma cells were more sensitive to this effect than were normal human epidermal keratinocytes [32]. Since NF κ B is known to be anti-apoptotic, inhibition by tea polyphenols is expected to be pro-apoptotic.

EGCG has been shown to effectively compete for the ligand-binding site of a number of pro-growth receptors. In A431 cells, EGCG blocked binding of epidermal growth factor (EGF) to its receptor and prevented the autophosphorylation of the receptor [33]. In vitro, EGCG inhibits the kinase activity of EGFR, platelet-derived growth factor receptor (PDGFR), and fibroblast-growth factor receptor (FGFR) [2]. The inhibitory effect of EGCG against protein kinase A (PKA) and protein kinase C (PKC) was found to be relatively weak, whereas TFdiG was shown to be more potent [33,34].

Another recently proposed mechanism for EGCG is direct binding to FAS. Using affinity chromatography with immobilized EGCG, the authors were able to detect FAS-EGCG binding [35]. On the basis of this observation and the induction of caspase-8 mediated apoptosis by EGCG, the authors suggest that EGCG binds to FAS to elicit an apoptotic response in U937 monocytic leukemia cells. The concentrations of EGCG necessary for binding were as high as 300–500 μ M.

2.3.4. Other potential mechanisms

Most recently, EGCG was found to inhibit the activity of topoisomerase I in several human colon carcinoma cell lines. The concentrations necessary to inhibit topoisomerase I activity (3–17 μ M) correlated well with concentrations necessary to significantly inhibit cell growth [36]. By comparison, EGCG at concentrations up to 550 μ M did not inhibit topoisomerase II activity [36]. This is an interesting mechanism of action for EGCG given the relatively low concentrations necessary for inhibition and the correlation of topoisomerase I inhibition with phenomena, such as cell cycle arrest, DNA damage, and induction of apoptosis.

The relative importance of each of these potential mechanisms in vivo depends on whether effective tissue concentrations of the tea polyphenols can

be achieved. In many cases, this remains to be determined.

3. Biotransformation and bioavailability

Many of the mechanistic studies of the tea catechins have been conducted on cell lines using concentrations of 10–1000 μ M. As described below, it is unlikely that such high concentrations can be obtained in target tissues other than the skin and GI tract where high amounts of tea polyphenols can be directly applied. Correlating mechanistic data in vitro with effects in vivo must be done with careful consideration of the poor bioavailability of the tea polyphenols.

3.1. Biotransformation

Glucuronidation, sulfation, and methylation represent the major metabolic pathways for tea catechins (Fig. 2). There are species and tissue-specific differences in EGCG and EGC glucuronidation, with humans and mice being more similar than humans and rats [37,38]. Methylated catechins have been observed in the rat including 3' and 4'-O-methyl EC, 4'-O-methyl EGC, and 4''-O-methyl EGC and EGCG [39,40]. 4', 4''-di-O-methyl-EGCG was the major metabolite detected in the bile of the rat following oral EGCG administration [41].

In humans, EGC is detected mainly as the glucuronidated form (57–71%) or sulfated form (23–36%) with only a small amount present as the free form (3–13%) [42]. Methylation of EGC also occurs in humans leading to the formation of 4'-O-methyl EGC, which is present mainly as the glucuronide or sulfate conjugate [2]. In contrast, the sulfated form of EC is more abundant (66%) than the glucuronidated form (33%) [42] while EGCG is present mainly in the free form in the plasma [43].

In addition to these conjugation reactions, the tea catechins undergo metabolism in the gut to form the ring fission products 5-(3',4',5'-trihydroxyphenyl)- γ -valerolactone (M4), 5-(3',4'-dihydroxyphenyl)- γ -valerolactone (M6) and 5-(3',5'-dihydroxyphenyl)- γ -valerolactone (M6') [44]. These metabolic intermediates are further broken down by gut flora to phenylacetic and phenylpropionic acids. M6 was previously shown to form during anaerobic incubation of ECG

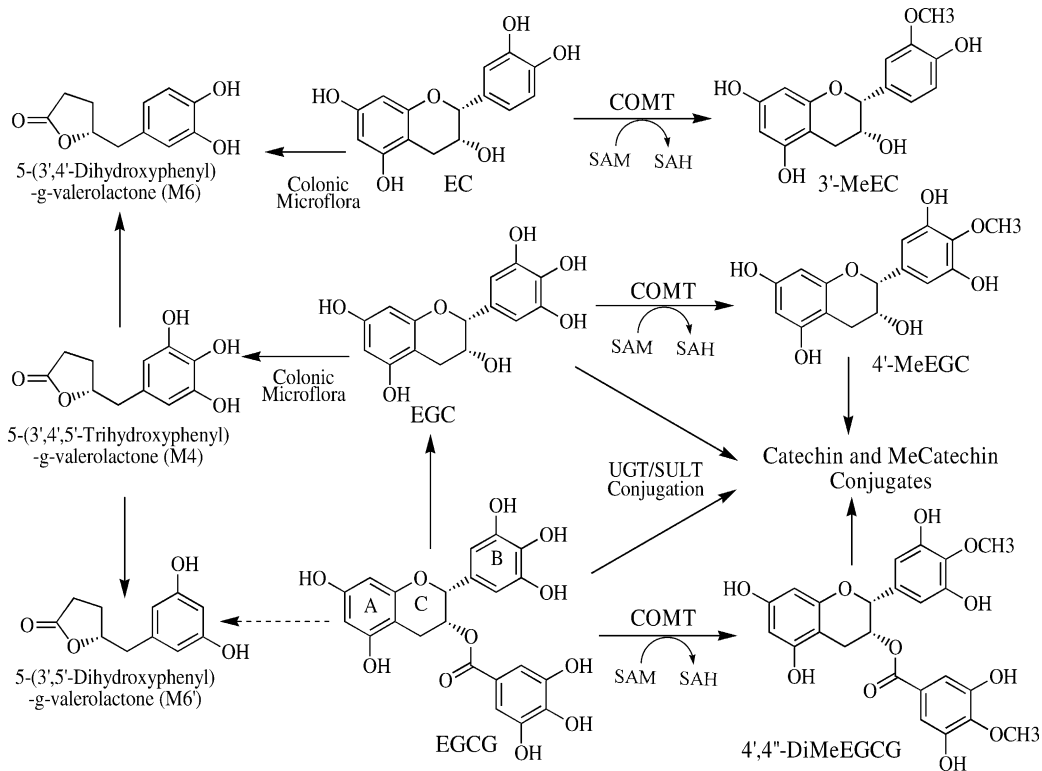


Fig. 2. Metabolic fate of tea catechins (abbreviations: COMT, catechol-*O*-methyltransferase; SAM, *s*-adenosylmethionine; SAH, homocysteine; SULT, sulfotransferase; UGT, uridine 5'-diphosphoglucuronosyltransferase).

and EC with human intestinal bacteria [45]. The metabolic fate of the theaflavins and thearubigins is unknown.

3.2. Pharmacokinetics

The pharmacokinetic parameters of the tea catechins have been thoroughly determined in the rat by both *i.v.* and *i.g.* routes of administration. EGCG, EGC, and EC were fit to a two-compartment model with elimination half-lives of 212, 45, and 41 min, respectively. The absolute bioavailability of EGCG, EGC, and EC following *i.g.* administration of decaffeinated green tea were 0.1, 14, and 31%, respectively [46]. Similarly, another study showed that EGCG levels in the tissues and blood corresponded to 0.0003–0.45% of the ingested dose, further demonstrating the poor bioavailability of EGCG in the rat [47].

Studies of [^3H]-EGCG have been performed in both the rat and the mouse. Following a single *i.g.* dose of

[^3H]-EGCG, radioactivity can be detected throughout the body with 10% of the initial dose present in the blood after 24 h and approximately 1% in the brain, lung, heart, liver, kidney and other tissues [48]. Excretion in the feces is the major route of elimination with 25–30% of the total radioactivity excreted after 24 h. In the rat, *i.v.* administration of [^3H]-EGCG resulted in 77% of the dose being excreted in the bile and only 2% excreted in the urine [49].

Chronic consumption of green tea polyphenols (0.6% w/v) in the drinking water by rats and mice increased plasma catechin levels over 14 day with EGCG levels being lower than EC or EGC levels. High levels of EC and EGC were detected in the rat bladder (800 ng/g), kidney (450 ng/g), large intestine (300–900 ng/g), esophagus (190 ng/g), lung (190–230 ng/g), and prostate (245 ng/g). EGCG levels were highest in the esophagus, intestine and colon. Consumption of green tea polyphenols for 14 more days lead to a decrease in plasma levels to baseline

levels by day 28 [50]. This could represent an induction of conjugating enzymes or excretion mechanisms as an adaptive response.

Many studies have been conducted on the pharmacokinetics of the tea catechins in humans. Yang et al. [51] demonstrated a T_{\max} in the plasma of 1.5 to 2.5 h after consumption of decaffeinated green tea solids (1.5, 3.0, 4.5 g). These levels decreased and were not detectable by 24 h. While EGCG was not detected in the urine, 90% of the total EC and EGC were excreted in the urine by 8 h. The bioavailability of EGCG was found to be less than that of EGC. Chow et al. [43] compared the pharmacokinetic parameters of pure EGCG (200–800 mg, p.o.) and Polyphenon E (decaffeinated green tea catechin mixture). The authors found that the EGCG C_{\max} ranged from 73.7 to 438 ng/ml depending on the dose and was not significantly different when EGCG was given as a pure compound or in combination. AUC (22.5–161.4 min $\mu\text{g/ml}$), $t_{1/2}$ (118–113.5 min) and T_{\max} (127–249 min) were also similar between pure EGCG and Polyphenon E. Interestingly, plasma levels of EGCG increased significantly when the dose increased from 400 to 600 mg. This may be due to a saturable first pass elimination mechanism.

The pharmacokinetics of the TF and the TR are less well characterized. Using high performance liquid chromatography–electrospray mass spectrometry, Mulder et al. [52] detected TF in the plasma of human volunteers at 1 ng/ml following consumption of 700 mg of pure theaflavins. The authors report a peak urine concentration of 4.2 ng/ml at 2 h. No other reports have been made on the pharmacokinetics of TF or TR.

4. Concluding remarks

Despite the demonstration of cancer prevention by tea in many animal studies, epidemiological studies have yielded mixed results concerning the effectiveness of tea as a cancer chemopreventive agent in humans. This may be due to several factors. (1) The dose of the chemopreventive agent is generally higher in animal studies than is typically consumed by humans. (2) The model of carcinogenesis, especially certain chemical carcinogens, may not be

relevant to human carcinogenesis. (3) Interindividual variation in metabolism of tea constituents as well as other confounding factors may mask the effects of tea consumption on cancer. Some of these discrepancies may be explained once we have a better understanding of the bioavailability of tea polyphenols. Knowledge of the bioavailability and activities of tea constituents would allow a clearer interpretation of the mechanisms by which EGCG and other tea polyphenols exert their cancer preventive effects.

The poor bioavailability of EGCG, theaflavins, and thearubigins maybe in part explained by Lipinski's Rule of 5 [53]. This rule is based on the ability of a molecule to pass through transient pores formed in the plasma membrane by the movement of the phospholipid acyl tails and also a molecule's ability to form hydrogen bonds. It states that if a compound has a molecular weight of >500, contains 5 or more hydrogen-bond donors and/or 10 or more hydrogen-bond acceptors, it is likely to be poorly bioavailable. Likewise, it is expected that the tea polyphenols will have a large polar surface area due to the numerous hydrogen bond donors and acceptors. These groups probably result in the formation of a large hydration shell due to interaction with water molecules [54]. Such an increase the apparent size of the molecules would make them less likely to pass through transient pores form in the lipid bilayer by rotation of the acyl chains of phospholipids [54]. Therefore, EGCG could be predicted to be less bioavailable than EGC, EC, and the metabolites M4, M6 and M6', which are much smaller and form fewer hydrogen-bonds and are thus likely to be more bioavailable. Theaflavins and thearubigins are predicted to have extremely low bioavailability.

Careful studies on the bioavailability of tea constituents as well as the mechanism(s) of action of these compounds are needed. Definitive conclusions on the effectiveness of tea as a cancer chemopreventive agent will have to come from well-designed intervention and observational epidemiological studies.

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References

- [1] D. Balentine, Manufacture and chemistry of tea, in: M. Huang, C.T. Ho, C. Lee (Eds.), *Phenolic Compounds in Food and their Effects on Health*, ACS, Washington, DC, 1992, pp. 103–117.
- [2] C.S. Yang, P. Maliakal, X. Meng, Inhibition of carcinogenesis by tea, *Ann. Rev. Pharmacol. Toxicol.* 42 (2001) 25–54.
- [3] G. Caderni, C. De Filippo, C. Luceri, M. Salvadori, A. Giannini, A. Biggeri, S. Remy, V. Cheyner, P. Dolara, Effects of black tea, green tea, and wine extracts on intestinal carcinogenesis induced by azoxymethane in F344 rats, *Carcinogenesis* 21 (2000) 1965–1969.
- [4] J. Cao, Y. Xu, J. Chen, J. Klauing, Chemopreventive effects of green and black tea on pulmonary and hepatic carcinogenesis, *Fundam. Appl. Toxicol.* 29 (1996) 244–250.
- [5] F.-L. Chung, M. Yang, A. Rivenson, M. Iatropoulos, J. Reinhardt, B. Pittman, C.-T. Ho, S. Amin, Inhibition of lung carcinogenesis by black tea in Fischer rats treated with a tobacco-specific carcinogen: caffeine as an important constituent, *Cancer Res.* 58 (1998) 4096–4101.
- [6] J. Landau, Z.-Y. Wang, G.-Y. Yang, W. Ding, C.S. Yang, Inhibition of spontaneous formation of lung tumors and rhabdomyosarcomas in A/J mice by black and green tea, *Carcinogenesis* 19 (1998) 501–507.
- [7] Y.-R. Lou, Y.-P. Lu, J.-G. Xie, M.-T. Huang, A.H. Conney, Effects of oral administration of tea, decaffeinated tea, and caffeine on the formation and growth of tumors in high-risk SKH1 mice previously treated with ultraviolet B light, *Nutr. Cancer* 33 (1999) 146–153.
- [8] T. Majima, M. Tsutsumi, H. Nishino, T. Tsunoda, Y. Konishi, Inhibitory effects of beta-carotene, palm carotene, and green tea polyphenol on pancreatic carcinogenesis initiated by by *N*-nitrosobis(2-oxopropyl)amine in Syrian golden hamsters, *Pancreas* 16 (1998) 13–18.
- [9] M. Suganuma, Y. Ohkura, S. Okabe, H. Fujiki, Combination cancer chemoprevention with green tea extract and sulindac shown in intestinal tumor formation in min mice, *Jpn. Cancer Res. Clin. Oncol.* 127 (2001) 69–72.
- [10] J. Weisburger, A. Rivenson, K. Garr, C. Aliaga, Tea, or tea and milk, inhibit mammary gland and colon carcinogenesis in rats, *Cancer Lett.* 114 (1997) 323–327.
- [11] C.S. Yang, Z.-Y. Wang, Tea and cancer, *J. Natl. Cancer Inst.* 85 (1997) 1038–1049.
- [12] G.-Y. Yang, Z. Liu, D.N. Seril, J. Liao, W. Ding, S. Lim, F. Bondoc, C.S. Yang, Black tea constituents, theaflavins, inhibit 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone (NNK)-induced lung tumorigenesis in A/J mice, *Carcinogenesis* 18 (1997) 2361–2365.
- [13] M.T. Huang, J. Xie, Z. Wang, C.T. Ho, Y. Lou, C. Wang, G. Hard, A.H. Conney, Effects of tea, decaffeinated tea, and caffeine on UVB-induced complete carcinogenesis in SKH-1 mice: demonstration of caffeine as a biologically important constituent of tea, *Cancer Res.* 57 (1997) 2623–2629.
- [14] Y. Tsubono, Y. Nishino, S. Komatsu, C.-C. Hsieh, S. Kanemura, I. Tsuji, H. Nakatsuka, A. Fukao, H. Satoh, S. Hisamichi, Green tea and the risk of gastric cancer in Japan, *New Engl. J. Med.* 344 (2001) 632–636.
- [15] Y. Gao, J. McLaughlin, W. Blot, B. Ji, Q. Dai, J. Fraumeni, Reduced risk of esophageal cancer associated with green tea consumption, *J. Natl. Cancer Inst.* 86 (1994) 855–858.
- [16] Z.Y. Wang, L. Chen, M.-J. Lee, C.S. Yang, Tea and cancer prevention, in: J. Finley, D. Armstrong, S. Nagy, S. Robinson (Eds.), *Hypernutritious Foods*, AGSCIENCE Inc., Auburndale, FL, 1996, pp. 239–259.
- [17] R. Goldbohm, M. Hertog, H. Brants, G. van Poppel, P. van den Brandt, Consumption of black tea and cancer risk: a prospective cohort study, *J. Natl. Cancer Inst.* 88 (1996) 93–100.
- [18] L. Su, L. Arab, Tea consumption and the reduced risk of colon cancer; results from a national prospective cohort study, *Proc. Am. Assoc. Cancer Res.* 41 (2000) 5141.
- [19] L. Chung, T. Cheung, S. Kong, K. Fung, Y. Choy, Z. Chan, T. Kwok, Induction of apoptosis by green tea catechins in human prostate cancer DU145 cells, *Life Sci.* 68 (2001) 1207–1214.
- [20] M. Lea, Q. Xiao, A. Sadhukhan, S. Cottle, Z.-Y. Wang, C.S. Yang, Inhibitory effects of tea extracts and (–)-epigallocatechin gallate on DNA synthesis and proliferation of hepatoma and erythroleukemia cells, *Cancer Lett.* 68 (1993) 231–236.
- [21] S. Uesato, Y. Kitagawa, M. Kamishimoto, A. Kumagai, H. Hori, H. Nagasawa, Inhibition of green tea catechins against the growth of cancerous human colon and hepatic epithelial cells, *Cancer Lett.* 170 (2001) 41–44.
- [22] G.-Y. Yang, J. Liao, K. Kim, E. Yurkow, C.S. Yang, Inhibition of growth and induction of apoptosis in human cancer cell lines by tea polyphenols, *Carcinogenesis* 19 (1998) 611–616.
- [23] Y.-P. Lu, Y.-R. Lou, J.-G. Xie, P. Yen, M.-T. Huang, A.H. Conney, Inhibitory effect of black tea on the growth of established skin tumors in mice: effects on tumor size, apoptosis, mitosis and bromodeoxyuridine incorporation into DNA, *Carcinogenesis* 18 (1997) 2163–2169.
- [24] M.-T. Huang, C.-T. Ho, Z.-Y. Wang, T. Ferraro, T. Finnegan-Olive, Y.R. Lou, J.M. Mitchel, J.D. Laskin, H. Newmark, C.S. Yang, Inhibitory effect of topical application of green tea polyphenol fraction on tumor initiation and promotion in mouse skin, *Carcinogenesis* 13 (1992) 947–954.
- [25] Y. Xu, C.-T. Ho, S. Amin, C. Han, F.-L. Chung, Inhibition of tobacco-specific nitrosamine-induced lung tumorigenesis in A/J mice by green tea and its major polyphenol as antioxidants, *Cancer Res.* 52 (1992) 3875–3879.
- [26] N. Ahmad, P. Cheng, H. Mukhtar, Cell cycle dysregulation by green tea polyphenol epigallocatechin-3-gallate, *Biochem. Biophys. Res. Commun.* 275 (2000) 328–334.
- [27] Y. Liang, S. Lin-Shiau, C. Chen, J. Lin, Inhibition of cyclin-dependent kinases 2 and 4 as well as induction of Cdk inhibitors p21 and p27 during growth arrest of human breast carcinoma cells by (–)-epigallocatechin gallate, *J. Cell. Biochem.* 75 (1999) 1–12.
- [28] M. Liberto, D. Cobrinik, Growth factor-dependent induction of p21(CIP1) by the green tea polyphenol, epigallocatechin gallate, *Cancer Lett.* 154 (2000) 151–161.
- [29] S. Gupta, N. Ahmad, A.-L. Nieminen, H. Mukhtar, Growth inhibition, cell-cycle dysregulation, and induction of apoptosis by green tea constituent (–)-epigallocatechin-3-gallate in

- androgen-sensitive and androgen-insensitive human prostate carcinoma cells, *Toxicol. Appl. Pharmacol.* 164 (2000) 82–90.
- [30] J. Chung, J. Park, H. Phyu, Z. Dong, C.S. Yang, Mechanisms of inhibition of the ras-MAP kinase pathway in 30.7b ras 12 cells by tea polyphenols (–)-epigallocatechin-3-gallate and theaflavin-3,3'-digallate, *FASEB J.* 15 (2001) 2022–2024.
- [31] G.-Y. Yang, J. Liao, C. Li, J. Chung, E. Yurkow, C.-T. Ho, C.S. Yang, Effect of black tea and green tea polyphenols on c-jun phosphorylation and H₂O₂ production in transformed and non-transformed human bronchial cell lines: possible mechanisms of cell growth inhibition and apoptosis induction, *Carcinogenesis* 21 (2000) 2035–2039.
- [32] N. Ahmad, S. Gupta, H. Mukhtar, Green tea polyphenol epigallocatechin-3-gallate differentially modulates nuclear factor κB in cancer cells versus normal cells, *Arch. Biochem. Biophys.* 376 (2000) 338–346.
- [33] Y.-C. Liang, S.-Y. Lin-Shiau, D.-F. Chen, J.-K. Lin, Suppression of extracellular signals and cell proliferation through EGF receptor binding by (–)-epigallocatechin gallate in human A431 epidermoid carcinoma cells, *J. Cell. Biochem.* 67 (1997) 55–65.
- [34] W. Chen, Z. Dong, S. Valcic, B.N. Timmermann, G.T. Bowden, Inhibition of ultraviolet-B-induced *c-fos* gene expression and p38 mitogen-activated protein kinase activation by (–)-epigallocatechin gallate in a human keratinocyte cell line, *Mol. Carcinog.* 24 (1999) 79–84.
- [35] S. Hayakawa, K. Saeki, M. Sazuka, Y. Suzuki, Y. Shoji, T. Ohta, K. Kaji, A. Yuo, M. Isemura, Apoptosis induction by epicatechin gallate involves its binding to FAS, *Biochem. Biophys. Res. Commun.* 285 (2001) 1102–1106.
- [36] S. Berger, S. Gupta, C. Belfi, D. Gosky, H. Mukhtar, Green tea constituent (–)-epigallocatechin-3-gallate inhibits topoisomerase I activity in human colon carcinoma cells, *Biochem. Biophys. Res. Commun.* 288 (2001) 101–105.
- [37] M. Piskula, J. Terao, Accumulation of (–)-epicatechin metabolites in rat plasma after oral administration and distribution of conjugating enzymes in rat tissue, *J. Nutr.* 128 (1998) 1172–1178.
- [38] J. Terao, Dietary flavonoids as antioxidants in vivo: conjugated metabolites of (–)-epicatechin and quercetin participate in antioxidant defense in blood plasma, *J. Med. Invest.* 46 (1999) 159–168.
- [39] K. Okushio, M. Suzuki, N. Matsumoto, F. Nanjo, Y. Hara, Identification of (–)-epicatechin metabolites and their metabolic fate in the rat, *Drug Metabol. Disp.* 27 (1999) 309–316.
- [40] K. Okushio, M. Suzuki, N. Matsumoto, F. Nanjo, Y. Hara, Methylation of tea catechins by rat liver homogenates, *Biosci. Biotech. Biochem.* 63 (1999) 430–432.
- [41] K. Kida, M. Suzuki, N. Matsumoto, F. Nanjo, Y. Hara, Identification of biliary metabolites of (–)-epigallocatechin gallate in rats, *J. Agric. Food Chem.* 48 (2000) 4151–4155.
- [42] M.-J. Lee, Z.-Y. Wang, H. Li, L. Chen, Y. Sun, S. Gobbo, D. Balentine, C.S. Yang, Analysis of plasma and urinary tea polyphenols in human subjects, *Cancer Epidemiol. Biomarkers Prev.* 4 (1995) 393–399.
- [43] H.H.S. Chow, Y. Cai, D.S. Alberts, I. Hakim, R.T. Dorr, F. Shahi, J. Crowell, C.S. Yang, Y. Hara, Phase I pharmacokinetic study of tea polyphenols following single-dose administration of epigallocatechin gallate and Polyphenon E, *Cancer Epidemiol. Biomarkers Prev.* 10 (2001) 53–58.
- [44] C. Li, M.-J. Lee, S. Sheng, X. Meng, S. Prabhu, B. Winnik, B. Huang, J. Chung, S. Yan, C.-T. Ho, C.S. Yang, Structural identification of two metabolites of catechins and their kinetics in human urine and blood after tea consumption, *Chem. Res. Toxicol.* 13 (2000) 177–184.
- [45] M. Meselhy, N. Nakamura, M. Hattori, Biotransformation of (–)-epicatechin 3-O-gallate by human intestinal bacteria, *Chem. Pharm. Bull.* 45 (1997) 888–893.
- [46] L. Chen, M.-J. Lee, H. Li, C.S. Yang, Absorption, distribution, and elimination of tea polyphenols in rats, *Drug Metabol. Disp.* 9 (1997) 1045–1050.
- [47] K. Nakagawa, T. Miyazawa, Absorption and distribution of tea catechin, (–)-epigallocatechin-3-gallate, in the rat, *Nutr. Sci. Vitaminol.* 43 (1997) 679–684.
- [48] M. Sukanuma, S. Okabe, M. Oniyama, Y. Tada, H. Ito, H. Fujiki, Wide distribution of [³H](–)-epigallocatechin gallate, a cancer preventive tea polyphenol, in mouse tissue, *Carcinogenesis* 19 (1998) 1771–1776.
- [49] T. Kohri, F. Nanjo, M. Suzuki, R. Seto, N. Matsumoto, M. Yamakawa, H. Hojo, Y. Hara, D. Desai, S. Amin, C. Conaway, F.-L. Chung, Synthesis of (–)-[4-³H]epigallocatechin gallate and its metabolic fate in rats after intravenous administration, *J. Agric. Food Chem.* 49 (2001) 1042–1048.
- [50] S. Kim, M.-J. Lee, J. Hong, C. Li, T. Smith, G.-Y. Yang, D. Seril, C.S. Yang, Plasma and tissue levels of tea catechins in rats and mice during chronic consumption of green tea polyphenols, *Nutr. Cancer.* 37 (2000) 41–48.
- [51] C. Yang, L. Chen, M.-J. Lee, D. Balentine, M. Kuo, S. Schantz, Blood and urine levels of tea catechins after ingestion of different amounts of green tea by human volunteers, *Cancer Epidemiol. Biomarkers Prev.* 7 (1998) 351–354.
- [52] T. Mulder, C. van Platerink, P. Schuyf, J. van Amelsvoort, Analysis of theaflavins in biological fluids using liquid chromatography–electrospray mass spectrometry, *J. Chromatogr. B* 760 (2001) 271–279.
- [53] C. Lipinski, F. Lombardo, B. Dominy, P. Feeney, Experimental and computational approaches to estimate solubility and permeability in drug discovery and development settings, *Adv. Drug Deliv. Rev.* 23 (1997) 3–25.
- [54] D.E. Clark, Rapid calculation of polar molecular surface area and its application to the prediction of transport phenomena. Part 1. Prediction of intestinal absorption, *J. Pharm. Sci.* 88 (1999) 807–814.