Review

Bioavailability issues in studying the health effects of plant polyphenolic compounds

Chung S. Yang, Shengmin Sang, Joshua D. Lambert, and Mao-Jung Lee

Department of Chemical Biology, Ernest Mario School of Pharmacy, Rutgers, The State University of New Jersey, Piscataway, NJ, USA

Polyphenolic compounds are common in the diet and have been suggested to have a number of beneficial health effects including prevention of cancer, cardiovascular disease, diabetes, and others. For some dietary polyphenols, certain benficial effects are suggested by epidemiological studies, some are supported by studies in animal models, and still others are extrapolated from studies *in vitro*. Because of the relatively poor bioavailability of many of these compounds, the molecular basis of these beneficial effects is not clear. In the present review, we discuss the potential health benefits of dietary polyphenols from the point of view of bioavailability. Tea catechins, curcumin, and proanthocyanidins are used as examples to illustrate some of the problems that need to be resolved. Further research on both the biological activity and bioavailability of dietary polyphenols is needed to properly assess their usefulness for the prevention and treatment of disease.

Keywords: Bioavailability / Chocolate / Dietary polyphenols / Disease prevention / Tea

Received: June 25, 2007; revised: December 7, 2007; accepted: December 27, 2007

1 Introduction

Polyphenolic compounds occur widely in the plant kingdom and are present at high levels in many edible plants. Some well-studied dietary polyphenols include catechins from tea, curcumin from turmeric, and procyanidins (PCs) and anthocyanidines in grapes, berries, and dark chocolate. The structures of examples of these compounds are shown in Fig. 1.

These compounds have strong antioxidative activities *in vitro* and have been suggested to have various beneficial health effects (reviewed in [1–4]). These include: lowering the risk of cardiovascular diseases; preventing cancer and inhibiting tumor growth; decreasing fat absorption and increasing energy expenditure; suppressing inflammation and decreasing the severity of arthritis; reducing the development of cataracts; and even inhibiting the infectivity of influenza. What are the scientific bases for these claims? If these claims are based on extrapolations from *in vitro* studies, was the bioavailability of the compound considered? Even though a compound has strong antioxidative or other biological activities *in vitro*, it would have little biological activity *in vivo* if little or none of the compound gets to the organ site of interest.

This article discusses the bioavailability issues related to dietary polyphenols and their impact on extrapolating results from experiments *in vitro* to the situation *in vivo*. Tea polyphenols, which are being studied extensively in our laboratory, are used to illustrate some of the key issues. The biotransformation and bioavailability of curcumin and PCs are also discussed.

Correspondence: Dr. Chung S. Yang, Department of Chemical Biology, Ernest Mario School of Pharmacy, Rutgers, The State University of New Jersey, 164 Frelinghuysen Road, Piscataway, NJ 08854-8020, USA
E-mail: csyang@rci.rutgers.edu
Fax: +1-732-445-0687

Abbreviations: $\mathbf{C_{max}}$, maximal concentration; **EC**, (–)-epicatechin; **EGC**, (–)-epigallcatechin; **EGCG**, (–)-epigallocatechin-3-gallate; **i.g.**, intragastric; **MRP**, multidrug resistance-related protein; **PC**, procyanidin; $\mathbf{t_{1/2\beta}}$, terminal half-life; **TPA**, 12-O-tetradecanoyl-phorbol-13-acetate

2 Physiochemical properties of dietary polyphenols

In considering the bioavailability of polyphenols, the Lipinski's Rule of 5 is a very useful guide. This Rule was originally derived empirically for pharmaceutical agents, but is applicable to dietary components. According to Lipinski's Rule of 5, compounds that have five or more hydrogen bond donors (OH and NH groups), ten or more hydrogen bond acceptors (notably N and O), a molecular weight

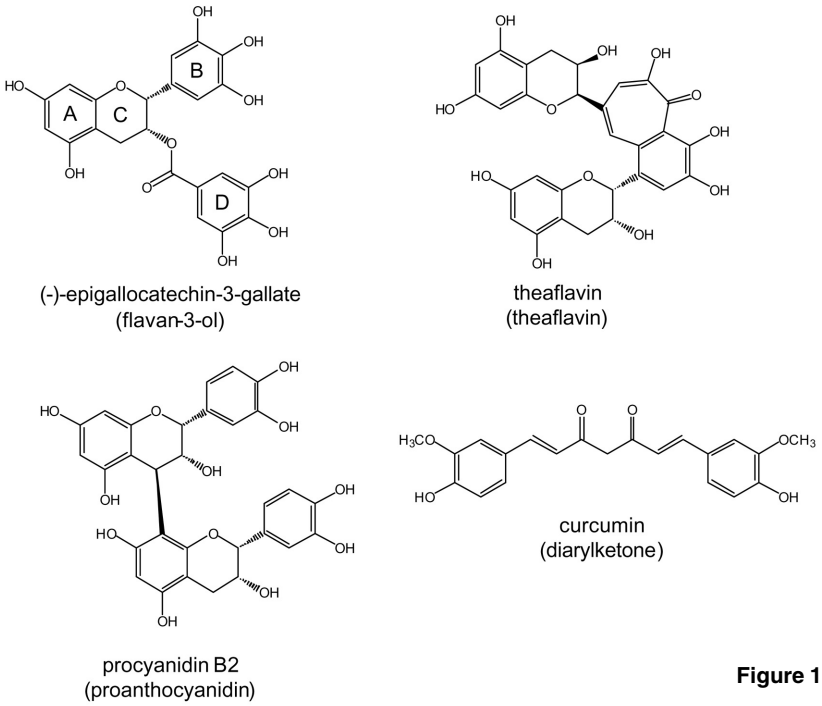

Figure 1. Structures of some major dietary polyphenols.

greater than 500, and Log P greater than 5 are usually poorly absorbed following oral administration. This is due to their large actual size (high molecular weight), high polarity or large apparent size (due to the formation of a large hydration shell) [5, 6].

Dietary polyphenols in general have Log P values of less than 5; however, many violate other rules. For example, (–)-epigallocatechin-3-gallate (EGCG), the most abundant polyphenol in green tea, with a molecular weight of 458 g/mol and 8 phenolic groups, is predicted to be poorly absorbed. (–)-Epicatechin (EC) and catechin, which are also found in tea as well as other foods, have a smaller molecular weight (290 g/mol) and fewer hydrogen bond donors (5 phenolic groups). These compounds are expected to be better absorbed than EGCG. Indeed, both human and animal studies have shown that the bioavailability of EC and catechin are better than EGCG [7–10]. Conversely, theaflavin and theaflavin-3,3′-digallate, which are catechin oligomers found in black tea, have molecular weights of 564 g/mol and 868 g/mol, respectively, and contain and 9 and 14 phenolic groups, respectively. Both of these compounds are predicted to be very poorly absorbed; experimentally, their bioavailability has been shown to be extremely low [11]. Many of the flavonoids in grapes and cranberries are dimers and trimers of catechins. These compounds, as well as PCs from dark chocolate, should be similar to the theaflavins and have very low bioavailability.

Some of the predictions for polyphenol absorbability have been tested experimentally using Caco-2 human colon cancer cells. These cells are a widely accepted *in vitro* system for studying the intestinal permeability of drugs and other compounds [12, 13]. Zhang *et al.* [14] have reported that the apical to basolateral permeability constant (P_{app}) for EGCG and EC in Caco-2 cells was 0.83×10^{-7} and 1.39×10^{-7} cm/s, respectively. These values are low and in good agreement with the predicted relative absorption of these compounds based on Lipinski's Rule of 5. By contrast, the soy isoflavones, genistein and biochanin A, which are predicted by Lipiski's Rule of 5 to have good absorptive characteristics (m.w. less than 300, and number of hydrogen bond donors less than 5), have much higher P_{app} (3.3×10^{-5} and 1.1×10^{-5} cm/s, respectively) in Caco-2 cells [15]. Several studies have examined the permeability of PC oligomers. In experiments with an intestinal epithelium cell monolayer, PC dimers and trimers, but not higher molecular weight polymers, were absorbed [16].

3 Biotransformation and active efflux of dietary polyphenols

The bioavailability of dietary polyphenols is limited not only by the physiochemical properties of the molecule, but also because of enzyme and microbial-mediated biotransformation and active efflux [17–20]. Biotransformation and the efflux of the absorbed material are major factors affecting the biological fate of dietary polyphenols. Most polyphenols are not subjected to phase I metabolisms because the polyphenolic structures make them unfavorable substrates for the cytochrome P450s [17, 21]. The polyphenolic

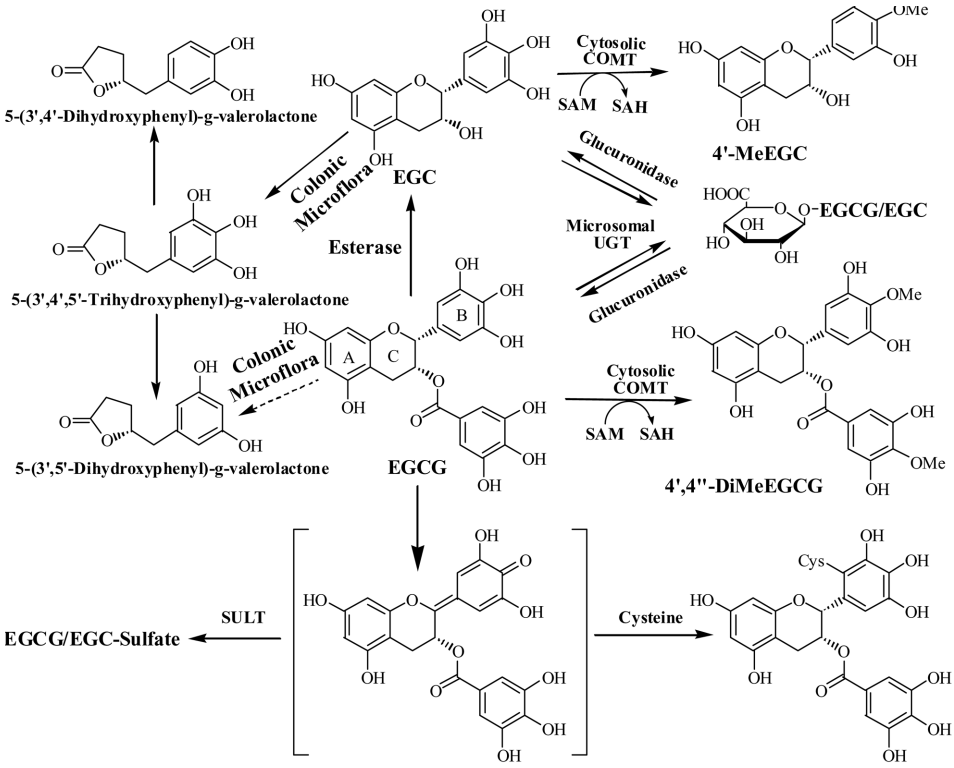

Figure 2. Biotransformation of the green tea catechins. 4′-MeEGC, 4′-*O*-methyl-(–)-epigallocatechin; 4′,4″-di-*O*-methylEGCG, 4′,4″-di-*O*-methyl-(–)-epigallocatechin-3-gallate; COMT, catechol-O-methyltransferase; EGC, (–)-epigallocatechin; EGCG, (–)-epigallocatechin-3-gallate; SAH, *S*-adenosylhomocysteine; SAM, *S*-adenosylmethionine; SULT, sulfotransferase; UGT, UDP-glucuronosyltransferase.

compounds can directly undergo phase II metabolism, predominately methylation, glucuronidation, and sulfation [17, 22–25]. Here, we discuss the biotransformation of tea polyphenols, curcumin, and PCs as examples.

3.1 Biotransformation and pharmacokinetics of tea polyphenols

EGCG and the other tea catechins undergo extensive biotransformation as summarized in Fig. 2. Because of the catechol structure, EGCG and other catechins are readily methylated by catechol-*O*-methyltransferase using *S*-adenosylmethionine as the methyl donor. The favorable methylation sites are the 4′ and 4″ phenolic groups. In the presence of high concentrations of EGCG, 4″-*O*-methyl-EGCG is formed, whereas at low concentrations of EGCG, 4′,4″-di-*O*-methylEGCG is formed. EGCG is also known to be methylated at the 3′ and 3″ positions. (–)-Epigallocatechin (EGC) is also readily methylated to form 4′-*O*-methyl-EGC. This metabolite as well as 4″-*O*-methylEGCG and 4′,4″-dimethylEGCG, have been detected in human and animal plasma and urine after ingestion of tea [10, 24, 26, 27].

In addition to methylation, EGCG and EGC are also known to be glucuronidated by UDP-glucuronosyltransferase (UGT). EGCG-4″-*O*-glucuronide and EGC-3′-*O*-glucuronide are the major metabolites [23]. Human UGT1A1, 1A8, and 1A9 have activity toward EGCG – the intestinal-specific UGT1A8 having the highest catalytic efficiency. EGC-3′-*O*-glucuronide is the major EGC metabolite and the liver microsomes have the highest efficiency. The sulfation of EC has been studied [28]. Studies have revealed that sulfotransferase (SULT)1A1 is largely responsible for this activity in the liver, whereas both SULT1A1 and SULT1A3 are active in the human intestine. The catalytic efficiency for SULT1A1 and SULT1A3-mediated sulfation of EC are 5834 and 55 μL/min/mg [28]. We have observed sulfated metabolites of EGCG in mouse and human urine (Lu, unpublished). Our previous results, and those of others, have shown that more than one phase II reaction can occur on the same polyphenolic molecule [29]. This was confirmed in our recent studies with data-dependent tandem mass spectrometry. We have observed, for example, methyl-EGCG-glucuronide, EGCG-glucuronide-sulfate, dimethyl-EGCG-diglucuronide, and methyl-EGCG-glucuronide-sulfate as urinary metabolites in mice (Sang *et al.*, unpublished).

Active efflux has been shown to limit the bioavailability of many polyphenolic compounds. The multidrug resist-

ance-associated protein 2 (MRP2), which is located on the apical surface of the intestine, kidney, and liver, may facilitate the transport of some polyphenolic compounds in the lumen, kidney, and bile, respectively [30]. Studies with human colon cancer cells have suggested that EGCG and its metabolites are substrates of MRP2 [31–33]. These studies have shown that co-incubation with selective inhibitors of MRP increases the intracellular accumulation of EGCG by greater than tenfold. If so, they are effluxed from the enterocytes into the intestinal lumen, or to be effluxed from the liver to the bile. EGCG and its metabolites are predominantly excreted in the feces with little or none of these compounds in the urine. Indeed, this is observed in both humans and rats [7, 24, 27]. As discussed above, after tea or EGCG treatment of mice, urinary metabolites (in the conjugated forms) can be detected, although at lower levels than are found in the feces [34].

In addition to phase II metabolism, tea catechins can be degraded in the intestinal tract by microorganisms [35]. For example, we have observed the formation of ring fission metabolites 5-(3′,4′,5′-trihydroxyphenyl)-γ-valerolactone (M4), 5-(3′,4′-dihydroxyphenyl)-γ-valerolactone (M6), and 5-(3′,5′-dihydroxyphenyl)-γ-valerolactone (M6′) in human urine and plasma samples several hours after ingestion of tea [36]. Meselhy *et al.* [37] have shown that these compounds are formed under anaerobic conditions by human fecal microflora, and can undergo further degradation to phenylacetic and phenylpropionoic acids. These compounds have certain biological activities [38].

Because of the many factors discussed above, the peak plasma levels of tea catechins are usually in the sub-micromolar range in human subjects or animals following oral administration of the equivalent of two or three cups of green tea. Several investigators have reported the pharmacokinetics of tea polyphenols in human volunteers [9, 10, 39, 40]. For example, we have found that the oral administration of 20 mg green tea solids/kg body weight result in maximal concentration (C_{max}) in the plasma for EGC, EC, and EGCG of 223, 124, and 77.9 ng/mL, respectively [10]. EGCG, EGC, and EC were found to have terminal half-life ($t_{1/2\beta}$) of 3.4, 1.7, and 2 h, respectively. Plasma EC and EGC were present mainly in the conjugated form, whereas 77% of the EGCG was in the free form. Plasma EGC and EC were present as glucuronide and sulfate conjugates with only a small free fraction [9, 41]. EGC was also methylated (4′-*O*-methyl-EGC) in humans [10]. EGCG has also been shown to undergo methylation. The plasma C_{max} of 4′,4″-di-*O*-methylEGCG was 20% of that of EGCG, but the 24-h cumulative excretion of 4′,4″-di-*O*-methylEGCG was tenfold higher than that of EGCG after tea treatment [26]. The ring-fission metabolites, M4, M6, and M6′, have been detected in urine following ingestion of 200 mg EGCG [26, 36].

Chow *et al.* [40] have demonstrated that following 4 weeks of green tea polyphenol treatment (800 mg, once daily), there was an increase in the area under the plasma EGCG concentration-time curve from 95.6 to 145.6 min/(μg min). No such change was observed in the pharmacokinetics of EGCG after repeated treatment at 400 mg, twice daily. Similarly, there was no significant change in the area under the curve of EGC or EC.

Pharmacokinetic studies of the tea catechins have also been conducted in rats and mice [17]. Following intragastric (i.g.) administration of decaffeinated green tea (200 mg/kg) to rats, plasma levels of EGCG, EGC, and EC had elimination half-lives of 165, 66, and 67 min, respectively. The absolute plasma bioavailability of EGCG, EGC, and EC was 0.1, 14, and 31%, respectively. By comparison, the absolute plasma bioavailability of EGCG in mice following i.g. administration of 75 mg/kg, EGCG was 26.5% with greater than 50% present as glucuronide conjugates. Concentrations of EGCG in the small intestine and colon are 20.6 and 3.6 μg/g following i.g. administration. The levels in other tissues are less than 45.8 ng/g [34].

Treatment of rats with a green tea polyphenol preparation (0.6%) in the drinking fluid has been shown to result in increasing plasma levels over a 14-day period with levels of EGC and EC being higher than those of EGCG [8]. Plasma levels then decreased over the subsequent 14 days. When the same polyphenol preparation was given to mice, these levels peaked on day 4 and then decreased to less than 20% of the peak values on days 8–10 [8].

When high pharmacological doses of EGCG are given by oral administration, peak plasma concentrations of 2–9 μM and 7.5 μM have been reported in mice and humans, respectively [42, 43].

3.2 Biotransformation and pharmacokinetics of curcumin

Curcumin, which contains two ferulic acid molecules linked by a methylene bridge and a β-diketone structure in a highly conjugated system, is the major yellow pigment in turmeric (*Curcuma longa*), curry, and mustard. Curcumin has low solubility, and curcumin crystals are not well dispersed in the intestine following oral administration. Most of the ingested curcumin is excreted in the feces and only trace amounts of curcumin (or its metabolites) appear in the blood. The steady-state levels of intact curcumin in the feces (3590 nmol/g) have been reported to be much higher than those of the small intestinal (111 nmol/g) or colonic mucosa (508 nmol/g) when curcumin is administered in the diet (0.2%) [44]. The absorbed curcumin is rapidly metabolized in the intestine and liver to several reduction products (di-, tetra-, and hexahydrocurcumin and hexahydrocurcuminol) [45, 46] and their glucuronide or sulfate conjugates [46–49].

Human studies have shown that doses of up to 180 mg curcumin fail to produce detectable plasma levels [50], and very high doses (up to 8 g) yield curcumin peak levels of

only 0.5–2 μM [51]. Oral consumption of up to 3.6 g curcumin led to curcumin concentrations of 10 nmol/g tissue in human colorectal mucosa [52].

3.3 Biotransformation and pharmacokinetics of PCs

PCs are the major type of proanthocyanidins (known as condensed tannins) in our diet. PCs are mixtures of oligomers and polymers composed of flavan-3-ol units (catechins, epicatechins, and their gallic acid esters) linked mainly through C4→C8 bond and C4→C6 linkage (both are called B-type). The flavan-3-ol units can also be doubly linked by an additional ether bond to C2→O7 (A-type) [53]. Some of the PCs are shown in Fig. 1. PCs are widespread in fruits (grape, cranberry, apple, plum, peach, pear), legume seeds (more particularly broad beans), cereal grains, and different beverages (wine, cocoa, cider) [54, 55]. The estimated intake of PCs varies from tens to hundreds milligrams per day [18].

Radiolabeled PC mixtures fed to animals have been shown to result in urinary and fecal levels of radioactivity (54% of the total radioactivity in urine). Low-molecular-weight products such as hippuric acid, ethylcatechol and various phenolic acids have been identified [56]. Orally administered PC polymers have shown poor absorption in both the chicken and the sheep [57, 58]. Other studies in rats and humans have suggested that cocoa flavanols and PCs are partially absorbed as monomers and dimers [59–63]. After consumption of cocoa (375 mg/kg) as a beverage, PC dimer, EC, and (+)-catechin were detected in the plasma of human subjects with C_{max} of 41 nM, 5.92 μM, and 0.16 μM, respectively [63].

It has been suggested that PCs are cleaved at low pH in the gastric environment, into EC dimers and monomers, which are then absorbed in the small intestine [64, 65]. It has been reported that incubation of PCB2 and PCB5 in simulated gastric juice (pH 1.8) resulted in their degradation to EC as well as isomerization of the dimers. A later study in human volunteers, however, did not support this finding [66]. After consuming 733 mg PCs, samples of the stomach contents were collected from volunteers every 10 min. The composition of PCs and EC monomers and dimers in the stomach did not change during the gastric transit time, indicating that PCs are stable in the gastric milieu and reach the small intestine primarily as polymers.

Once PCs or their fermentation products cross the intestinal barrier, they may be further metabolized by the liver. Three monomethylated and two dimethylated derivatives of PCB3 have been reported following incubation with human liver homogenate and *S*-adenosylmethionine *in vitro* [56, 67]. If absorbed, PCs are extensively metabolized, then methylated, glucuronidated, and sulfated metabolites are expected [56, 68]. Such data, however, are not currently available.

PC dimers and polymers have been shown to be degraded into low-molecular-weight phenolic acids by rat or human colonic microflora grown anaerobically *in vitro*. These compounds are then available for absorption by the colon [67, 69]. The importance of this microbial degradation pathway needs to be studied further.

4 Implications of polyphenol bioavailability for studying bioactivity

A major difficulty in studying the biological activities of dietary polyphenols is correlating biological effects observed *in vivo* with proposed mechanisms based on studies *in vitro*. In the former case, peak plasma and tissue levels of the test compound may reach only sub- to low-micromolar concentrations, whereas the effective concentrations reported in studies *in vitro* are often tens or hundreds of micromolar. Below, we review the literature on the cancer preventive effects of tea polyphenols and discuss the discrepancies between *in vivo* carcinogenesis studies and *in vitro* mechanistic studies. Some hypotheses related to bioavailability issues are presented to explain these discrepancies. These issues are not unique to the tea polyphenols and we briefly discuss the biological activities of curcumin and PCs, which have even lower bioavailability.

4.1 Inhibition of carcinogenesis by tea polyphenols

The inhibition of tumor formation by tea extracts and tea constituents has been demonstrated in different animal models (reviewed in [2, 70–73]). Inhibition of skin, lung, oral cavity, esophagus, stomach, liver, pancreas, small intestine, colon, mammary gland, prostate, and urinary bladder carcinogenesis have been reported. The inhibitory activities have been observed when tea or tea constituents are administered to animals at the initiation, promotion, and progression stages of carcinogenesis. The active constituents for the cancer prevention have been shown to be tea polyphenols and caffeine. Route of administration and organ site affect the activity of these two groups of compounds. For example, oral caffeine was shown to be much more active in the inhibition of skin carcinogenesis than oral tea polyphenols [74]. When applied topically, however, both caffeine and polyphenols demonstrate activity. These results suggest that oral caffeine is more bioavailable to the skin than polyphenols, and that the lack of effect following oral administration is not due to lack of inherent activity on the part of the polyphenols [75]. Both caffeine and tea polyphenols have demonstrated activity against lung tumorigenesis in A/J mice, [76]. Tea polyphenols, but not caffeine, have been shown to inhibit of intestinal carcinogenesis [77]. Some studies have even suggested that caffeine enhances tumorigenesis in the colon [78].

Based on the bioavailability considerations, tea polyphenols are expected to have the greatest activity in the oral cavity and intestinal tract, which have direct contact with orally administered tea polyphenols. For example, holding a tea solution or tea leaves in the mouth has been shown to deliver greater than 100 μM EGCG to the oral epithelial cells [79]. Even after rinsing the mouth vigorously micromolar concentrations of EGCG and other catechins can be measured. These concentrations could inhibit cell growth and induce apoptosis of cancerous and precancerous cells [80, 81]. The colon is also expected to be an optimal site for cancer inhibition by tea polyphenols. As discussed above, EGCG has limited systemic bioavailability, and the unabsorbed EGCG will go through the colon. Even the absorbed EGCG is mostly excreted into the intestine *via* the bile duct. The conjugated EGCG may be hydrolyzed by glucuronidase and sulfatase, and the free form taken up by the colon epithelial cells.

Tea polyphenols have, however, been shown to inhibit tumorigenesis at internal sites including the lung and prostate [82, 83]. The highest concentration of EGCG that we have measured in the mouse lung is approximately 3 μM: the bioavailability of EGCG and other catechins in the prostate and mammary tissues are not known [8]. Inhibition of prostate tumorigenesis in the TRAMP mouse model by tea polyphenols has been reported, whereas the protective effects on mammary tumorigenesis have not been consistent [84]. Inhibition of tumorigenesis at internal sites by tea catechins may be due to indirect effects, or through the activity of catechin metabolites. For example, the effect of EGCG on mammary and prostate carcinogenesis may be the result of effects on hormone metabolism. Such hypotheses need further investigation.

Many mechanisms for the cancer-preventive activity of tea polyphenols have been proposed based on studies with EGCG in human cancer cell lines (reviewed in [2, 70–73]). These include: inhibition of mitogen-activated protein (MAP) kinases and the phosphotidylinositol-3 kinase (PI3K)/Akt pathways; inhibition of activator protein-1 and nuclear factor (NF)-κB-mediated transcription; inhibition of growth factor-mediated signaling; inhibition of cyclin-dependent kinases, matrix metalloproteases, DNA methyltransferase, and dihydrofolate reducatase; binding to and inhibiting activities of insulin-like growth factor-1 receptor and glucose-related protein 78; inhibition of aberrant arachidonic acid metabolism; and other activities. The end results of these effects could be the induction of apoptosis, inhibition of tumor cell growth, or the inhibition of angiogenesis [70, 84]. Most of these effects are observed at concentrations of EGCG greater than 10 μM: concentrations not typically observed *in vivo* following tea consumption.

Several reports have described direct high-affinity targets of EGCG such as 20S proteasome chymotryptic activity, the 67-kDa laminin receptor, vimentin, and Bcl-2 with IC_{50}, K_d, or K_i of 0.003–0.33 μM [85–88]. Such potency has been observed only in cell-free systems and much higher concentrations were required to inhibit cell growth or induce apoptosis in cultured cells [85–88]. This is likely due to the nonspecific binding of EGCG to cellular macromolecules. Although the biological relevance of the effects has been demonstrated in specific cell line systems, the general applicability of these mechanisms for cancer prevention remains unknown.

The lack of agreement between effective concentrations *in vitro* and those *in vivo* raises an important question. Does the use of these high concentrations of EGCG in cell line studies make the results irrelevant for mechanisms of cancer prevention? No clear cut answer is currently available. One may argue that in animals and humans ingesting tea catechins, the cells are consistently exposed to EGCG and other catechins. Even though the concentrations are low, that prolonged exposure can produce significant effects. This seems consistent with the observation that treatment of cancer cells with EGCG for longer periods of time (such as 3 or 4 days) increases the extent of inhibition compared to treatment for 1 or 2 days [89]. There is, however, no quantitative model to provide a clear understanding in correlating the effective concentration *in vitro* to the plasma and tissue concentrations observed in animals or humans. We thus believe that the mechanisms of cancer prevention need to be demonstrated *in vivo*.

One of the approaches that is being used by our laboratory and others is to study cellular and molecular changes in tissues using immunohistochemistry. For example, we observed that the inhibition of 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone (NNK)-induced lung tumorigenesis by green tea extracts is associated with enhanced apoptosis and inhibition of angiogenesis [90]. Inhibition of lung tumor progression from adenoma to adenocarcinoma by green tea polyphenols and caffeine was associated with inhibition of cell proliferation and enhancement of apoptosis in tumor tissues [76]. Increased levels of phosphorylation of extracellular responsive kinase (Erk) 1/2 and c-Jun were observed during the development of adenocarcinoma, and the increased phosphorylation of c-Jun and Erk 1/2 were inhibited by treatment with tea polyphenols and caffeine.

4.2 Inhibition of carcinogenesis by curcumin

The inhibitory effect of curcumin against skin, esophageal, forestomach, duodenal, intestinal, and colon carcinogenesis has been demonstrated in rodent models by many investigators [2, 91–94].

For example, topical application of 10 mmol curcumin to the cheek pouch of Syrian Golden hamsters has been shown to reduce the number of visible DMBA-induced oral tumors by 39.6% and the tumor volume by 61.3% [95]. Administration of 4% curcumin in the diet of azoxymethane (AOM)-treated mice during the initiation period was shown

to reduce the number of colon tumors by 66%. If treatment was started post initiation, tumor number was reduced by 25% [96]. In APC^{min} mice given 0.2 or 0.5% curcumin in the diet, intestinal adenoma multiplicity was shown to be reduced by 39–40% and hematocrit, which is reduced in untreated mice, was partially restored [44]. Topical application of curcumin has also been shown to reduce tumor incidence and multiplicity in both the benzo[a]pyrene/12-*O*-tetradecanoyl-phorbol-13-acetate (TPA) and the DMBA/TPA models of skin carcinogenesis in mice [74, 97].

Effects of curcumin on lung and mammary gland tumorigenesis have not been as dramatic or consistent [1]. As with the tea polyphenols, the poor bioavailability of oral curcumin limits its cancer preventive activity. Curcumin was found to be ineffective at preventing NNK-induced lung tumorigenesis in A/J mice, and liver and kidney tumorigenesis in Long-Evans Cinnamon rats [98, 99]. However, dietary curcumin has been shown to inhibit diethynitrosamine-induced liver cancer and altered hepatic foci in mice and rats, respectively [100, 101]. Effects on mammary tumorigenesis have been mixed. Inano *et al.* [102, 103] have reported that 1% dietary curcumin inhibited the incidence of radiation-induced tumors by 74% and 67% when given during the initiation and post-initiation stages, respectively. By contrast, oral curcumin had no preventive effects against DMBA-induced mammary tumorigenesis [104]. These inconsistent effects may be due to inherent differences in the models used. Overall, it appears that inhibition of carcinogenesis at internal sites depends on the systemic bioavailability curcumin; however, the inhibitory effects observed at some internal sites may be due to curcumin metabolites or indirect effects. Such hypotheses remain to be tested.

Studies with cell lines have shown that curcumin possesses both antioxidant and pro-oxidant activity. Curcumin-induced apoptosis of Caki renal cells was partially inhibited by co-treatment with *N*-acetylcysteine, suggesting the involvement of oxidative stress [105]. Cyclooxygenase 2 and inducible nitric oxide synthase (iNOS) have also been reported as a potential targets for curcumin [93, 106, 107]. Treatment of lipopolysaccharide-injected mice with curcumin (92 μg/kg, i.g.) reduced hepatic iNOS mRNA expression by 50–70% [108].

Curcumin has also been shown to inhibit the activation of the transcription factors, AP-1 and NFκB human leukemia cell lines [109, 110]. Ablation of AP-1 and NFκB activation by curcumin has been demonstrated in TPA-treated mouse skin. Chun *et al.* [111] have further reported that topical curcumin to mouse skin inhibited TPA-mediated activation of Erk and p38 MAP kinases and subsequent activation of NFκB.

Although some of the proposed mechanisms of action for curcumin have been shown *in vivo*, the primary target (or targets) remains unclear from the presently available data. Many, but not all, of the animal models studied involve inflammatory mediators, indicating that curcumin may act primarily as an anti-inflammatory agent, and may thus have cancer preventive effects by an indirect pathway. Further studies of with *in vivo* biomarkers are needed.

4.3 Biological effects of PCs

Many *in vitro* experiments with pure PCs have shown anti-tumor-related activities but few have been confirmed *in vivo* [112–117]. PCs have been shown to inhibit TPA-mediated tumor promotion in the mouse epidermis [118, 119]. Cocoa liquor PCs have also been shown to inhibit rat pancreatic carcinogenesis at the initiation stage as well as lung carcinogenesis in a rat multi-organ carcinogenesis model [117, 120]. A protective role of PCs against cardiovascular diseases has been suggested by many experimental studies, which have shown induction of endothelium-dependent relaxation *via* activation of nitric oxide synthase [121], enhancement of prostacyclin release [122–124], inhibition of oxidative modification of low-density lipoprotein [125–127], and inhibition of platelet aggregation [61, 128].

PC oligomers from grape seeds and skin extracts have been claimed to have diabetes prevention and therapeutic effects. The effects of these PC oligomers on diabetic cataracts, blood sugar, and blood lipid peroxides have been demonstrated in mice [129, 130]. PCs derived from cocoa have also been shown to inhibit diabetes-induced eye lens cataract formation possibly *via* antioxidant effects [130]. A recent study has shown that type-A PC trimers isolated from the aqueous extract of cinnamon have insulin-enhancing activity in *in vitro* assays measuring insulin-dependent glucose metabolism [131].

The aforementioned studies suggest the health beneficial effects of PCs. The poor bioavailability of these compounds raises questions on the active compounds (PCs or their degradation products) and the mechanisms of action. The possibility that large doses of PCs affect the absorption of macronutrients should also be considered. If microbial degradation of PCs to monomeric catechins underlies the biological activities of PCs *in vivo*, then it is unclear why PCs should have superior biological activity to direct treatment with the pure monomers. Further studies are needed to determine the biologica activity of PCs and what the role of PC bioavailability is in influencing these activities.

5 Methods to improve the bioavailability of dietary polyphenols

As discussed, the bioavailability of dietary polyphenols is limited by a number of factors including unfavorable physico-chemical properties, extensive first-pass metabolism, and active efflux. Strategies targeting these issues may be useful in improving the bioavailability of dietary polyphenols and their biological activity. We and other research groups have used several approaches to improve the bioa-

vailability of EGCG including alternative chemical modification to pro-drugs, dosing formulations, and combination with other dietary components.

Acetylation of EGCG to the peracetylated derivative increased the area under curve (AUC) and $t_{1/2}$ of EGCG in the plasma, small intestine and colon [132]. The AUC EGCG in the plasma, small intestine, and colon was increased by 2.4-, 2.8- and 2.4-fold, respectively. Peracetylation similarly increased the intracellular levels of EGCG *in vitro* with an accompanying increase in growth inhibitory activity [132]. Similar increases in *in vitro* biological activities have also been reported by others [133–135]. *In vivo*, Landis-Piwowar *et al.* [136] have demonstrated that peracetylated EGCG had superior growth inhibitory activity to EGCG against MDA-MB-231 human breast cancer xenografts in nude mice. This was associated with a 67% increase in tumor cell apoptosis measured as caspase-3 activity.

We and others have described transdermal formulations of green tea and EGCG [137–139]. These formulations have the advantage of by-passing first pass metabolism and resulting in improved bioavailability. We found that application of EGCG transdermal gel (50 mg/kg) resulted in lower plasma C_{max} than comparable oral doses of EGCG, but dramatically increased plasma AUC (9.1-fold) and $t_{1/2}$ (67.4-fold).

Combination of EGCG and curcumin or genistein, naturally occurring inhibitors of MRP, resulted in a dose-dependent increased cytosolic levels of EGCG in human colon cancer cell lines compared to treatment with EGCG only [33]. These results have been extended to the *in vivo* situation. We have observed that treatment of C57BL/6J mice with dietary curcumin (10 g/kg diet) and EGCG in the drinking fluid (3.2 mg/mL) resulted in increased plasma levels of EGCG compared to mice treated only with EGCG (Lambert *et al.*, unpublished).

Shoba *et al.* [140] have shown that co-treatment of rats with oral curcumin and piperine increased the bioavailability of curcumin by 154% compared to rats treated with curcumin only. We observed similar effects of piperine on the bioavailability of EGCG in mice [141]. Two possible mechanisms have been proposed for the effects of piperine. First, piperine has been shown to increased gastrointestinal transit time [142]. The effect of this would be to increase the residence time of the test compound in the small intestine and increase the likelihood of absorption. Alternatively, piperine has been shown to inhibit glucuronidation activity [140, 141, 143, 144]. This is expected to increase the AUC of the parent compound and decrease elimination.

Further studies are needed to fully develop strategies for improving the bioavailability of dietary polyphenols and to determine whether these methods translate into increased biological activity. A caveat to the effort to increase the bioavailability of dietary polyphenols is the possibility that increased bioavailability could lead to undesirable side effects. Recent studies in humans and animals have shown that high doses of green tea preparations are potentially toxic [145–147]. These data suggest that high doses of EGCG can induce toxicity in the liver, kidneys, and intestine. Toxicity, especially in the liver and kidney, appears to be correlated with the bioavailability of EGCG. In the rat, where bioavailability is low (F = 1.6%), toxicity is confined to the gastrointestinal tract following oral administration [7, 148]. In the dog, where bioavailability is much higher, hepatotoxicity and nephrotoxicity, as well as intestinal toxicity, were observed. Toxicity was greater in fasted, than in pre-fed, dogs [148]. The AUCplasma in the pre-fed dogs was 19.8 μg · h/mL compared to 205 μg.h/mL in fasted dogs following administration of 300 mg/kg, p.o.

6 Conclusions

Many beneficial health effects have been attributed to dietary polyphenols including, prevention and treatment of cancer, heart disease, diabetes, and other conditions. In some of these cases, the claims are supported by studies in animal models of disease using orally administered test compounds. In other cases, however, only *in vitro* experimental systems have been used to demonstrate efficacy. As we have discussed, many dietary polyphenols (especially theaflavins, PCs, and curcumin) have poor bioavailability. It is therefore unclear, in the absence of strong animal model data, whether the results of these *in vitro* studies are meaningful in terms of disease prevention. Four critical lines of research should be explored to gain a clear understanding of the health beneficial effects of dietary polyphenols:

(i) The biotransformation and bioavailability of many dietary polyphenols are not clearly understood. Data on key issues including the tissue distribution and biotransformative pathways for many dietary polyphenols need to be determined.

ii) The potential biological activity of the metabolites of many dietary polyphenols need to be investigated. Such compounds, even if less potent than the parent compound, may still contribute to the overall biological activity of the test compound *in vivo*.

(iii) The claimed health benefits of dietary polyphenols must be demonstrated in appropriate animal models of disease using appropriate routes of administration and in humans at appropriate doses. Whereas *in vitro* studies shed light on the mechanisms of action of individual dietary polyphenols, the importance of these data need to be evaluated in experiments *in vivo*.

(iv) More effort must be made to integrate the available *in vitro* and *in vivo* activity data with the bioavailability data to assess the potential usefulness of various dietary polyphenols. Based on the findings of comprehensive bioavailability studies, it may be necessary to modify *in vitro* model systems to better reflect the situation *in vivo*.

This work was supported by NIH Grants CA88961 Fond CA120915 (to C.S.Y.) and NIH CA125780 and AICR Grant 05A047 (to J.D.L.).

The authors have declared no conflict of interest.

7 References

[1] Yang, C. S., Landau, J. M., Huang, M. T., Newmark, H. L., Inhibition of carcinogenesis by dietary polyphenolic compounds. *Annu. Rev. Nutr.* 2001, *21*, 381 – 406.

[2] Lambert, J. D., Hong, J., Yang, G. Y., Liao, J. *et al.*, Inhibition of carcinogenesis by polyphenols: Evidence from laboratory investigations. *Am. J. Clin. Nutr.* 2005, *81*, 284S – 291S.

[3] Higdon, J. V., Frei, B., Tea catechins and polyphenols: Health effects, metabolism, and antioxidant functions. *Crit. Rev. Food Sci. Nutr.* 2003, *43*, 89 – 143.

[4] Williamson, G., Manach, C., Bioavailability and bioefficacy of polyphenols in humans. II. Review of 93 intervention studies. *Am. J. Clin. Nutr.* 2005, *81*, 243S – 255S.

[5] Lipinski, C. A., Lombardo, F., Dominy, B. W., Feeney, P. J., Experimental and computational approaches to estimate solubility and permeability in drug discovery and development settings. *Adv. Drug Deliv. Rev.* 2001, *46*, 3 – 26.

[6] Clark, D. E., Rapid calculation of polar molecular surface area and its application to the prediction of transport phenomena. 1. Prediction of intestinal absorption. *J. Pharm. Sci.* 1999, *88*, 807 – 814.

[7] Chen, L., Lee, M. J., Li, H., Yang, C. S., Absorption, distribution, elimination of tea polyphenols in rats. *Drug Metab. Dispos.* 1997, *25*, 1045 – 1050.

[8] Kim, S., Lee, M. J., Hong, J., Li, C. *et al.*, Plasma and tissue levels of tea catechins in rats and mice during chronic consumption of green tea polyphenols. *Nutr. Cancer* 2000, *37*, 41 – 48.

[9] Chow, H. H., Cai, Y., Alberts, D. S., Hakim, I. *et al.*, Phase I pharmacokinetic study of tea polyphenols following single-dose administration of epigallocatechin gallate and polyphenon E. *Cancer Epidemiol. Biomarkers Prev.* 2001, *10*, 53 – 58.

[10] Lee, M. J., Maliakal, P., Chen, L., Meng, X. *et al.*, Pharmacokinetics of tea catechins after ingestion of green tea and (−)-epigallocatechin-3-gallate by humans: Formation of different metabolites and individual variability. *Cancer Epidemiol. Biomarkers Prev.* 2002, *11*, 1025 – 1032.

[11] Mulder, T. P., van Platerink, C. J., Wijnand Schuyl, P. J., van Amelsvoort, J. M., Analysis of theaflavins in biological fluids using liquid chromatography-electrospray mass spectrometry. *J. Chromatogr. B Biomed. Sci. Appl.* 2001, *760*, 271 – 279.

[12] Artursson, P., Palm, K., Luthman, K., Caco-2 monolayers in experimental and theoretical predictions of drug transport. *Adv. Drug Deliv. Rev.* 2001, *46*, 27 – 43.

[13] Levy, E., Mehran, M., Seidman, E., Caco-2 cells as a model for intestinal lipoprotein synthesis and secretion. *FASEB J.* 1995, *9*, 626 – 635.

[14] Zhang, L., Chow, M. S., Zuo, Z., Effect of the co-occurring components from green tea on the intestinal absorption and disposition of green tea polyphenols in Caco-2 monolayer model. *J. Pharm. Pharmacol.* 2006, *58*, 37 – 44.

[15] Chen, J., Lin, H., Hu, M., Absorption and metabolism of genistein and its five isoflavone analogs in the human intestinal Caco-2 model. *Cancer Chemother. Pharmacol.* 2005, *55*, 159 – 169.

[16] Deprez, S., Mila, I., Huneau, J. F., Tome, D. *et al.*, Transport of proanthocyanidin dimer, trimer, and polymer across monolayers of human intestinal epithelial Caco-2 cells. *Antioxid. Redox Signal.* 2001, *3*, 957 – 967.

[17] Lambert, J. D., Yang, C. S., Cancer chemopreventive activity and bioavailability of tea and tea polyphenols. *Mutat. Res.* 2003, *523 – 524*, 201 – 208.

[18] Manach, C., Scalbert, A., Morand, C., Remesy, C. *et al.*, Polyphenols: Food sources and bioavailability. *Am. J. Clin. Nutr.* 2004, *79*, 727 – 747.

[19] Scalbert, A., Morand, C., Manach, C., Remesy, C., Absorption and metabolism of polyphenols in the gut and impact on health. *Biomed. Pharmacother.* 2002, *56*, 276 – 282.

[20] Manach, C., Williamson, G., Morand, C., Scalbert, A. *et al.*, Bioavailability and bioefficacy of polyphenols in humans. I. Review of 97 bioavailability studies. *Am. J. Clin. Nutr.* 2005, *81*, 230S – 242S.

[21] Lambert, J. D., Sang, S., Lu, A. Y., Yang, C. S., Metabolism of dietary polyphenols and possible interactions with drugs. *Curr. Drug Metab.* 2007, *8*, 499 – 507.

[22] Lu, H., Meng, X., Yang, C. S., Enzymology of methylation of tea catechins and inhibition of catechol-*O*-methyltransferase by (−)-epigallocatechin gallate. *Drug Metab. Dispos.* 2003, *31*, 572 – 579.

[23] Lu, H., Meng, X., Li, C., Sang, S. *et al.*, Glucuronides of tea catechins: Enzymology of biosynthesis and biological activities. *Drug Metab. Dispos.* 2003, *31*, 452 – 461.

[24] Li, C., Meng, X., Winnik, B., Lee, M. J. *et al.*, Analysis of urinary metabolites of tea catechins by liquid chromatography/electrospray ionization mass spectrometry. *Chem. Res. Toxicol.* 2001, *14*, 702 – 707.

[25] Hu, M., Chen, J., Lin, H., Metabolism of flavonoids *via* enteric recycling: Mechanistic studies of disposition of apigenin in the Caco-2 cell culture model. *J. Pharmacol. Exp. Ther.* 2003, *307*, 314 – 321.

[26] Meng, X., Sang, S., Zhu, N., Lu, H. *et al.*, Identification and characterization of methylated and ring-fission metabolites of tea catechins formed in humans, mice, and rats. *Chem. Res. Toxicol.* 2002, *15*, 1042 – 1050.

[27] Sun, C. L., Yuan, J. M., Lee, M. J., Yang, C. S. *et al.*, Urinary tea polyphenols in relation to gastric and esophageal cancers: A prospective study of men in Shanghai, China. *Carcinogenesis* 2002, *23*, 1497 – 1503.

[28] Vaidyanathan, J. B., Walle, T., Glucuronidation and sulfation of the tea flavonoid (−)-epicatechin by the human and rat enzymes. *Drug Metab. Dispos.* 2002, *30*, 897 – 903.

[29] Lu, H., *Mechanistic studies on the Phase II metabolism and absorption of tea catechins*. Doctoral thesis, Rutgers, The State University of New Jersey, New Brunswick 2002.

[30] Leslie, E. M., Deeley, R. G., Cole, S. P., Toxicological relevance of the multidrug resistance protein 1, MRP1 (ABCC1) and related transporters. *Toxicology* 2001, *167*, 3 – 23.

[31] Vaidyanathan, J. B., Walle, T., Transport and metabolism of the tea flavonoid (−)-epicatechin by the human intestinal cell line Caco-2. *Pharm. Res.* 2001, *18*, 1420 – 1425.

[32] Hong, J., Lu, H., Meng, X., Ryu, J. H. *et al.*, Stability, cellular uptake, biotransformation, and efflux of tea polyphenol (−)-epigallocatechin-3-gallate in HT-29 human colon adenocarcinoma cells. *Cancer Res.* 2002, *62*, 7241 – 7246.

[33] Hong, J., Lambert, J. D., Lee, S. H., Sinko, P. J. *et al.*, Involvement of multidrug resistance-associated proteins in regulating cellular levels of (−)-epigallocatechin-3-gallate and its methyl metabolites. *Biochem. Biophys. Res. Commun.* 2003, *310*, 222 – 227.

[34] Lambert, J. D., Lee, M. J., Lu, H., Meng, X. *et al.*, Epigallocatechin-3-gallate is absorbed but extensively glucuronidated following oral administration to mice. *J. Nutr.* 2003, *133*, 4172 – 4177.

[35] Wang, L. Q., Meselhy, M. R., Li, Y., Nakamura, N. *et al.*, The heterocyclic ring fission and dehydroxylation of catechins and related compounds by *Eubacterium* sp. strain SDG-2, a human intestinal bacterium. *Chem. Pharm. Bull. (Tokyo)* 2001, *49*, 1640 – 1643.

[36] Li, C., Lee, M. J., Sheng, S., Meng, X. *et al.*, Structural identification of two metabolites of catechins and their kinetics in human urine and blood after tea ingestion. *Chem. Res. Toxicol.* 2000, *13*, 177 – 184.

[37] Meselhy, M. R., Nakamura, N., Hattori, M., Biotransformation of (−)-epicatechin 3-*O*-gallate by human intestinal bacteria. *Chem. Pharm. Bull. (Tokyo)* 1997, *45*, 888 – 893.

[38] Lambert, J. D., Rice, J. E., Hong, J., Hou, Z. *et al.*, Synthesis and biological activity of the tea catechin metabolites, M4 and M6 and their methoxy-derivatives. *Bioorg. Med. Chem. Lett.* 2005, *15*, 873 – 876.

[39] Lee, M. J., Wang, Z. Y., Li, H., Chen, L. *et al.*, Analysis of plasma and urinary tea polyphenols in human subjects. *Cancer Epidemiol. Biomarkers Prev.* 1995, *4*, 393 – 399.

[40] Chow, H. H., Cai, Y., Hakim, I. A., Crowell, J. A. *et al.*, Pharmacokinetics and safety of green tea polyphenols after multiple-dose administration of epigallocatechin gallate and polyphenon E in healthy individuals. *Clin. Cancer Res.* 2003, *9*, 3312 – 3319.

[41] Yang, C. S., Chen, L., Lee, M. J., Balentine, D. *et al.*, Blood and urine levels of tea catechins after ingestion of different amounts of green tea by human volunteers. *Cancer Epidemiol. Biomarkers Prev.* 1998, *7*, 351 – 354.

[42] Lambert, J. D., Lee, M. J., Diamond, L., Ju, J. *et al.*, Dose-dependent levels of epigallocatechin-3-gallate in human colon cancer cells and mouse plasma and tissues. *Drug Metab. Dispos.* 2006, *34*, 8 – 11.

[43] Chow, H. H., Hakim, I. A., Vining, D. R., Crowell, J. A. *et al.*, Effects of dosing condition on the oral bioavailability of green tea catechins after single-dose administration of Polyphenon E in healthy individuals. *Clin. Cancer Res.* 2005, *11*, 4627 – 4633.

[44] Perkins, S., Verschoyle, R. D., Hill, K., Parveen, I. *et al.*, Chemopreventive efficacy and pharmacokinetics of curcumin in the min/+ mouse, a model of familial adenomatous polyposis. *Cancer Epidemiol. Biomarkers Prev.* 2002, *11*, 535 – 540.

[45] Ireson, C. R., Jones, D. J., Orr, S., Coughtrie, M. W. *et al.*, Metabolism of the cancer chemopreventive agent curcumin in human and rat intestine. *Cancer Epidemiol. Biomarkers Prev.* 2002, *11*, 105 – 111.

[46] Ireson, C., Orr, S., Jones, D. J., Verschoyle, R. *et al.*, Characterization of metabolites of the chemopreventive agent curcumin in human and rat hepatocytes and in the rat *in vivo*, and evaluation of their ability to inhibit phorbol ester-induced prostaglandin E2 production. *Cancer Res.* 2001, *61*, 1058 – 1064.

[47] Pan, M. H., Huang, T. M., Lin, J. K., Biotransformation of curcumin through reduction and glucuronidation in mice. *Drug Metab. Dispos.* 1999, *27*, 486 – 494.

[48] Lin, J. K., Pan, M. H., Lin-Shiau, S. Y., Recent studies on the biofunctions and biotransformations of curcumin. *Biofactors* 2000, *13*, 153 – 158.

[49] Asai, A., Miyazawa, T., Occurrence of orally administered curcuminoid as glucuronide and glucuronide/sulfate conjugates in rat plasma. *Life Sci.* 2000, *67*, 2785 – 2793.

[50] Sharma, R. A., McLelland, H. R., Hill, K. A., Ireson, C. R. *et al.*, Pharmacodynamic and pharmacokinetic study of oral Curcuma extract in patients with colorectal cancer. *Clin. Cancer Res.* 2001, *7*, 1894 – 1900.

[51] Cheng, A. L., Hsu, C. H., Lin, J. K., Hsu, M. M. *et al.*, Phase I clinical trial of curcumin, a chemopreventive agent, in patients with high-risk or pre-malignant lesions. *Anticancer Res.* 2001, *21*, 2895 – 2900.

[52] Garcea, G., Jones, D. J., Singh, R., Dennison, A. R. *et al.*, Detection of curcumin and its metabolites in hepatic tissue and portal blood of patients following oral administration. *Br. J. Cancer* 2004, *90*, 1011 – 1015.

[53] Harborne, J. B., *The Flavonoids*. Chapman and Hall, Boca Raton 1993, p. 676.

[54] Gu, L., Kelm, M. A., Hammerstone, J. F., Zhang, Z. *et al.*, Liquid chromatographic/electrospray ionization mass spectrometric studies of proanthocyanidins in foods. *J. Mass Spectrom.* 2003, *38*, 1272 – 1280.

[55] Gu, L., Kelm, M. A., Hammerstone, J. F., Beecher, G. *et al.*, Screening of foods containing proanthocyanidins and their structural characterization using LC-MS/MS and thiolytic degradation. *J. Agric. Food Chem.* 2003, *51*, 7513 – 7521.

[56] Santos-Buelga, C., Scalbert, A., Proanthocyanidins and tannin-like compounds – Nature, occurrence, dietary intake and effects on nutrition and health. *J. Food Sci. Agric.* 2000, *80*, 1094 – 1117.

[57] Jimenez-Ramsey, L. M., Rogler, J. C., Housley, T. L., Butler, L. G. *et al.*, Absorption and distribution of ^{14}C-labeled condensed tannins and related sorghum phenolics in chickens. *J. Agric. Food Chem.* 1994, *42*, 963 – 967.

[58] Terrill, T. H., Waghorn, G. C., Woolley, D. J., McNabb, W. C. *et al.*, Assay and digestion of ^{14}C-labelled condensed tannins in the gastrointestinal tract of sheep. *Br. J. Nutr.* 1994, *72*, 467 – 477.

[59] Baba, S., Osakabe, N., Natsume, M., Yasuda, A. *et al.*, Cocoa powder enhances the level of antioxidative activity in rat plasma. *Br. J. Nutr.* 2000, *84*, 673 – 680.

[60] Holt, R. R., Lazarus, S. A., Sullards, M. C., Zhu, Q. Y. *et al.*, Procyanidin dimer B2 [epicatechin-(4beta-8)-epicatechin] in human plasma after the consumption of a flavanol-rich cocoa. *Am. J. Clin. Nutr.* 2002, *76*, 798 – 804.

[61] Rein, D., Lotito, S., Holt, R. R., Keen, C. L. *et al.*, Epicatechin in human plasma: *In vivo* determination and effect of chocolate consumption on plasma oxidation status. *J. Nutr.* 2000, *130*, 2109S – 2114S.

[62] Wang, J. F., Schramm, D. D., Holt, R. R., Ensunsa, J. L. *et al.*, A dose-response effect from chocolate consumption on plasma epicatechin and oxidative damage. *J. Nutr.* 2000, *130*, 2115S – 2119S.

[63] Richelle, M., Tavazzi, I., Enslen, M., Offord, E. A., Plasma kinetics in man of epicatechin from black chocolate. *Eur. J. Clin. Nutr.* 1999, *53*, 22 – 26.

[64] Zhu, Q. Y., Holt, R. R., Lazarus, S. A., Ensunsa, J. L. *et al.*, Stability of the flavan-3-ols epicatechin and catechin and related dimeric procyanidins derived from cocoa. *J. Agric. Food Chem.* 2002, *50*, 1700 – 1705.

[65] Spencer, J. P., Chaudry, F., Pannala, A. S., Srai, S. K. *et al.*, Decomposition of cocoa procyanidins in the gastric milieu. *Biochem. Biophys. Res. Commun.* 2000, *272*, 236 – 241.

[66] Rios, L. Y., Bennett, R. N., Lazarus, S. A., Remesy, C. *et al.*, Cocoa procyanidins are stable during gastric transit in humans. *Am. J. Clin. Nutr.* 2002, *76*, 1106 – 1110.

[67] Deprez, S., Brezillon, C., Rabot, S., Philippe, C. *et al.*, Polymeric proanthocyanidins are catabolized by human colonic microflora into low-molecular-weight phenolic acids. *J. Nutr.* 2000, *130*, 2733 – 2738.

[68] Scalbert, A., Williamson, G., Dietary intake and bioavailability of polyphenols. *J. Nutr.* 2000, *130*, 2073S – 2085S.

[69] Groenewoud, G., Hundt, H. K., The microbial metabolism of condensed (+)-catechins by rat-caecal microflora. *Xenobiotica* 1986, *16*, 99 – 107.

[70] Hou, Z., Lambert, J. D., Chin, K. V., Yang, C. S., Effects of tea polyphenols on signal transduction pathways related to cancer chemoprevention. *Mutat. Res.* 2004, *555*, 3 – 19.

[71] Yang, C. S., Lambert, J. D., Hou, Z., Ju, J. *et al.*, Molecular targets for the cancer preventive activity of tea polyphenols. *Mol. Carcinog.* 2006, *45*, 431 – 435.

[72] Yang, C. S., Sang, S., Lambert, J. D., Hou, Z. *et al.*, Possible mechanisms of the cancer-preventive activities of green tea. *Mol. Nutr. Food Res.* 2006, *50*, 170 – 175.

[73] Khan, N., Afaq, F., Saleem, M., Ahmad, N. *et al.*, Targeting multiple signaling pathways by green tea polyphenol (–)-epigallocatechin-3-gallate. *Cancer Res.* 2006, *66*, 2500 – 2505.

[74] Huang, M. T., Xie, J. G., Wang, Z. Y., Ho, C. T. *et al.*, Effects of tea, decaffeinated tea, and caffeine on UVB light-induced complete carcinogenesis in SKH-1 mice: Demonstration of caffeine as a biologically important constituent of tea. *Cancer Res.* 1997, *57*, 2623 – 2629.

[75] Lu, Y. P., Lou, Y. R., Xie, J. G., Peng, Q. Y. *et al.*, Topical applications of caffeine or (–)-epigallocatechin gallate (EGCG) inhibit carcinogenesis and selectively increase apoptosis in UVB-induced skin tumors in mice. *Proc. Natl. Acad. Sci. USA* 2002, *99*, 12455 – 12460.

[76] Lu, G., Liao, J., Yang, G., Reuhl, K. R. *et al.*, Inhibition of adenoma progression to adenocarcinoma in a 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone-induced lung tumorigenesis model in A/J mice by tea polyphenols and caffeine. *Cancer Res.* 2006, *66*, 11494 – 11501.

[77] Ju, J., Hong, J., Zhou, J. N., Pan, Z. *et al.*, Inhibition of intestinal tumorigenesis in Apcmin/+ mice by (–)-epigallocatechin-3-gallate, the major catechin in green tea. *Cancer Res.* 2005, *65*, 10623 – 10631.

[78] Tsuda, H., Sekine, K., Uehara, N., Takasuka, N. *et al.*, Heterocyclic amine mixture carcinogenesis and its enhancement by caffeine in F344 rats. *Cancer Lett.* 1999, *143*, 229 – 234.

[79] Lee, M. J., Lambert, J. D., Prabhu, S., Meng, X. *et al.*, Delivery of tea polyphenols to the oral cavity by green tea leaves and black tea extract. *Cancer Epidemiol. Biomarkers Prev.* 2004, *13*, 132 – 137.

[80] Khafif, A., Schantz, S. P., al-Rawi, M., Edelstein, D. *et al.*, Green tea regulates cell cycle progression in oral leukoplakia. *Head Neck* 1998, *20*, 528 – 534.

[81] Hsu, S. D., Singh, B. B., Lewis, J. B., Borke, J. L. *et al.*, Chemoprevention of oral cancer by green tea. *Gen. Dent.* 2002, *50*, 140 – 146.

[82] Yang, C. S., Liao, J., Yang, G. Y., Lu, G., Inhibition of lung tumorigenesis by tea. *Exp. Lung Res.* 2005, *31*, 135 – 144.

[83] Saleem, M., Adhami, V. M., Siddiqui, I. A., Mukhtar, H., Tea beverage in chemoprevention of prostate cancer: A mini-review. *Nutr. Cancer* 2003, *47*, 13 – 23.

[84] Gupta, S., Hastak, K., Ahmad, N., Lewin, J. S. *et al.*, Inhibition of prostate carcinogenesis in TRAMP mice by oral infusion of green tea polyphenols. *Proc. Natl. Acad. Sci. USA* 2001, *98*, 10350 – 10355.

[85] Nam, S., Smith, D. M., Dou, Q. P., Ester bond-containing tea polyphenols potently inhibit proteasome activity *in vitro* and *in vivo*. *J. Biol. Chem.* 2001, *276*, 13322 – 13330.

[86] Tachibana, H., Koga, K., Fujimura, Y., Yamada, K., A receptor for green tea polyphenol EGCG. *Nat. Struct. Mol. Biol.* 2004, *11*, 380 – 381.

[87] Ermakova, S., Choi, B. Y., Choi, H. S., Kang, B. S. *et al.*, The intermediate filament protein vimentin is a new target for epigallocatechin gallate. *J. Biol. Chem.* 2005, *280*, 16882 – 16890.

[88] Leone, M., Zhai, D., Sareth, S., Kitada, S. *et al.*, Cancer prevention by tea polyphenols is linked to their direct inhibition of antiapoptotic Bcl-2-family proteins. *Cancer Res.* 2003, *63*, 8118 – 8121.

[89] Shimizu, M., Deguchi, A., Lim, J. T., Moriwaki, H. *et al.*, (–)-Epigallocatechin gallate and polyphenon E inhibit growth and activation of the epidermal growth factor receptor and human epidermal growth factor receptor-2 signaling pathways in human colon cancer cells. *Clin. Cancer Res.* 2005, *11*, 2735 – 2746.

[90] Liao, J., Yang, G. Y., Park, E. S., Meng, X. *et al.*, Inhibition of lung carcinogenesis and effects on angiogenesis and apoptosis in A/J mice by oral administration of green tea. *Nutr. Cancer* 2004, *48*, 44 – 53.

[91] Chauhan, D. P., Chemotherapeutic potential of curcumin for colorectal cancer. *Curr. Pharm. Des.* 2002, *8*, 1695 – 1706.

[92] Mahmoud, N. N., Carothers, A. M., Grunberger, D., Bilinski, R. T. *et al.*, Plant phenolics decrease intestinal tumors in an animal model of familial adenomatous polyposis. *Carcinogenesis* 2000, *21*, 921 – 927.

[93] Huang, M. T., Lysz, T., Ferraro, T., Abidi, T. F. *et al.*, Inhibitory effects of curcumin on in vitro lipoxygenase and cyclooxygenase activities in mouse epidermis. *Cancer Res.* 1991, *51*, 813 – 819.

[94] Huang, M. T., Smart, R. C., Wong, C. Q., Conney, A. H., Inhibitory effect of curcumin, chlorogenic acid, caffeic acid, and ferulic acid on tumor promotion in mouse skin by 12-*O*-tetradecanoylphorbol-13-acetate. *Cancer Res.* 1988, *48*, 5941 – 5946.

[95] Li, N., Chen, X., Liao, J., Yang, G. *et al.*, Inhibition of 7,12-dimethylbenz[a]anthracene (DMBA)-induced oral carcinogenesis in hamsters by tea and curcumin. *Carcinogenesis* 2002, *23*, 1307 – 1313.

[96] Huang, M. T., Lou, Y. R., Ma, W., Newmark, H. L. *et al.*, Inhibitory effects of dietary curcumin on forestomach, duodenal, and colon carcinogenesis in mice. *Cancer Res.* 1994, *54*, 5841 – 5847.

[97] Huang, M. T., Wang, Z. Y., Georgiadis, C. A., Laskin, J. D. *et al.*, Inhibitory effects of curcumin on tumor initiation by benzo[a]pyrene and 7,12-dimethylbenz[a]anthracene. *Carcinogenesis* 1992, *13*, 2183 – 2186.

[98] Hecht, S. S., Kenney, P. M., Wang, M., Trushin, N. *et al.*, Evaluation of butylated hydroxyanisole, myo-inositol, curcumin, esculetin, resveratrol and lycopene as inhibitors of benzo[a]pyrene plus 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone-induced lung tumorigenesis in A/J mice. *Cancer Lett.* 1999, *137*, 123 – 130.

[99] Frank, N., Knauft, J., Amelung, F., Nair, J. *et al.*, No prevention of liver and kidney tumors in Long-Evans Cinnamon rats by dietary curcumin, but inhibition at other sites and of metastases. *Mutat. Res.* 2003, *523 – 524*, 127 – 135.

[100] Shukla, Y., Arora, A., Suppression of altered hepatic foci development by curcumin in wistar rats. *Nutr. Cancer* 2003, *45*, 53 – 59.

[101] Chuang, S. E., Kuo, M. L., Hsu, C. H., Chen, C. R. *et al.*, Curcumin-containing diet inhibits diethylnitrosamine-induced murine hepatocarcinogenesis. *Carcinogenesis* 2000, *21*, 331 – 335.

[102] Inano, H., Onoda, M., Inafuku, N., Kubota, M. *et al.*, Potent preventive action of curcumin on radiation-induced initiation of mammary tumorigenesis in rats. *Carcinogenesis* 2000, *21*, 1835 – 1841.

[103] Inano, H., Onoda, M., Inafuku, N., Kubota, M. *et al.*, Chemoprevention by curcumin during the promotion stage of tumorigenesis of mammary gland in rats irradiated with gamma-rays. *Carcinogenesis* 1999, *20*, 1011 – 1018.

[104] Huang, M. T., Lou, Y. R., Xie, J. G., Ma, W. *et al.*, Effect of dietary curcumin and dibenzoylmethane on formation of 7,12-dimethylbenz[a]anthracene-induced mammary tumors and lymphomas/leukemias in Sencar mice. *Carcinogenesis* 1998, *19*, 1697 – 1700.

[105] Woo, J. H., Kim, Y. H., Choi, Y. J., Kim, D. G. *et al.*, Molecular mechanisms of curcumin-induced cytotoxicity: Induction of apoptosis through generation of reactive oxygen species, down-regulation of Bcl-XL and IAP, the release of cytochrome c and inhibition of Akt. *Carcinogenesis* 2003, *24*, 1199 – 1208.

[106] Zhang, F., Altorki, N. K., Mestre, J. R., Subbaramaiah, K. *et al.*, Curcumin inhibits cyclooxygenase-2 transcription in bile acid- and phorbol ester-treated human gastrointestinal epithelial cells. *Carcinogenesis* 1999, *20*, 445 – 451.

[107] Goel, A., Boland, C. R., Chauhan, D. P., Specific inhibition of cyclooxygenase-2 (COX-2) expression by dietary curcumin in HT-29 human colon cancer cells. *Cancer Lett.* 2001, *172*, 111 – 118.

[108] Chan, M. M., Huang, H. I., Fenton, M. R., Fong, D., *In vivo* inhibition of nitric oxide synthase gene expression by curcumin, a cancer preventive natural product with anti-inflammatory properties. *Biochem. Pharmacol.* 1998, *55*, 1955 – 1962.

[109] Singh, S., Aggarwal, B. B., Activation of transcription factor NF-kappa B is suppressed by curcumin (diferuloylmethane) [corrected]. *J. Biol. Chem.* 1995, *270*, 24995 – 25000.

[110] Han, S. S., Keum, Y. S., Seo, H. J., Surh, Y. J., Curcumin suppresses activation of NF-kappaB and AP-1 induced by phorbol ester in cultured human promyelocytic leukemia cells. *J. Biochem. Mol. Biol.* 2002, *35*, 337 – 342.

[111] Chun, K. S., Keum, Y. S., Han, S. S., Song, Y. S. *et al.*, Curcumin inhibits phorbol ester-induced expression of cyclooxygenase-2 in mouse skin through suppression of extracellular signal-regulated kinase activity and NF-kappaB activation. *Carcinogenesis* 2003, *24*, 1515 – 1524.

[112] Sakagami, H., Sakagami, T., Yoshida, H., Omata, T. *et al.*, Hypochlorite scavenging activity of polyphenols. *Anticancer Res.* 1995, *15*, 917 – 921.

[113] Kashiwada, Y., Nonaka, G., Nishioka, I., Chang, J. J. *et al.*, Antitumor agents, 129. Tannins and related compounds as selective cytotoxic agents. *J. Nat. Prod.* 1992, *55*, 1033 – 1043.

[114] Eng, E. T., Ye, J., Williams, D., Phung, S. *et al.*, Suppression of estrogen biosynthesis by procyanidin dimers in red wine and grape seeds. *Cancer Res.* 2003, *63*, 8516 – 8522.

[115] Bagchi, D., Garg, A., Krohn, R. L., Bagchi, M. *et al.*, Oxygen free radical scavenging abilities of vitamins C and E, and a grape seed proanthocyanidin extract *in vitro*. *Res. Commun. Mol. Pathol. Pharmacol.* 1997, *95*, 179 – 189.

[116] Joshi, S. S., Kuszynski, C. A., Benner, E. J., Bagchi, M. *et al.*, Amelioration of the cytotoxic effects of chemotherapeutic agents by grape seed proanthocyanidin extract. *Antioxid. Redox Signal.* 1999, *1*, 563 – 570.

[117] Yamagishi, M., Natsume, M., Osakabe, N., Okazaki, K. *et al.*, Chemoprevention of lung carcinogenesis by cacao liquor proanthocyanidins in a male rat multi-organ carcinogenesis model. *Cancer Lett.* 2003, *191*, 49 – 57.

[118] Bomser, J. A., Singletary, K. W., Wallig, M. A., Smith, M. A., Inhibition of TPA-induced tumor promotion in CD-1 mouse epidermis by a polyphenolic fraction from grape seeds. *Cancer Lett.* 1999, *135*, 151 – 157.

[119] Gali, H. U., Perchellet, E. M., Gao, X. M., Karchesy, J. J. *et al.*, Comparison of the inhibitory effects of monomeric, dimeric, and trimeric procyanidins on the biochemical markers of skin tumor promotion in mouse epidermis *in vivo*. *Planta Med.* 1994, *60*, 235 – 239.

[120] Yamagishi, M., Natsume, M., Osakabe, N., Nakamura, H. *et al.*, Effects of cacao liquor proanthocyanidins on PhIP-induced mutagenesis *in vitro*, and *in vivo* mammary and pancreatic tumorigenesis in female Sprague-Dawley rats. *Cancer Lett.* 2002, *185*, 123 – 130.

[121] Karim, M., McCormick, K., Kappagoda, C. T., Effects of cocoa extracts on endothelium-dependent relaxation. *J. Nutr.* 2000, *130*, 2105S – 2108S.

[122] Schramm, D. D., Wang, J. F., Holt, R. R., Ensunsa, J. L. *et al.*, Chocolate procyanidins decrease the leukotriene-prostacyclin ratio in humans and human aortic endothelial cells. *Am. J. Clin. Nutr.* 2001, *73*, 36 – 40.

[123] Pearson, D. A., Paglieroni, T. G., Rein, D., Wun, T. *et al.*, The effects of flavanol-rich cocoa and aspirin on ex vivo platelet function. *Thromb. Res.* 2002, *106*, 191 – 197.

[124] Facino, R. M., Carini, M., Aldini, G., Berti, F. *et al.*, Diet enriched with procyanidins enhances antioxidant activity and reduces myocardial post-ischaemic damage in rats. *Life Sci.* 1999, *64*, 627 – 642.

[125] Mazur, A., Bayle, D., Lab, C., Rock, E. *et al.*, Inhibitory effect of procyanidin-rich extracts on LDL oxidation *in vitro*. *Atherosclerosis* 1999, *145*, 421 – 422.

[126] Ivanov, V., Carr, A. C., Frei, B., Red wine antioxidants bind to human lipoproteins and protect them from metal ion-dependent and -independent oxidation. *J. Agric. Food Chem.* 2001, *49*, 4442 – 4449.

[127] Osakabe, N., Baba, S., Yasuda, A., Iwamoto, T. *et al.*, Daily cocoa intake reduces the susceptibility of low-density lipoprotein to oxidation as demonstrated in healthy human volunteers. *Free Radic. Res.* 2001, *34*, 93 – 99.

[128] Chang, W. C., Hsu, F. L., Inhibition of platelet aggregation and arachidonate metabolism in platelets by procyanidins. *Prostaglandins Leukot. Essent. Fatty Acids* 1989, *38*, 181 – 188.

[129] Lee, Y. A., Cho, E. J., Tanaka, T., Yokozawa, T., Inhibitory activities of proanthocyanidins from persimmon against oxidative stress and digestive enzymes related to diabetes. *J. Nutr. Sci. Vitaminol. (Tokyo)* 2007, *53*, 287 – 292.

[130] Osakabe, N., Yamagishi, M., Natsume, M., Yasuda, A. *et al.*, Ingestion of proanthocyanidins derived from cacao inhibits diabetes-induced cataract formation in rats. *Exp. Biol. Med. (Maywood)* 2004, *229*, 33 – 39.

[131] Anderson, R. A., Broadhurst, C. L., Polansky, M. M., Schmidt, W. F. *et al.*, Isolation and characterization of polyphenol type-A polymers from cinnamon with insulin-like biological activity. *J. Agric. Food Chem.* 2004, *52*, 65 – 70.

[132] Lambert, J. D., Sang, S., Hong, J., Kwon, S. J. *et al.*, Peracetylation as a Means of enhancing *in vitro* bioactivity and bioavailability of epigallocatechin-3-gallate. *Drug Metab. Dispos.* 2006, *34*, 2111 – 2116.

[133] Lam, W. H., Kazi, A., Kuhn, D. J., Chow, L. M. *et al.*, A potential prodrug for a green tea polyphenol proteasome inhibitor: Evaluation of the peracetate ester of (–)-epigallocatechin gallate [(–)-EGCG]. *Bioorg. Med. Chem.* 2004, *12*, 5587 – 5593.

[134] Kuhn, D., Lam, W. H., Kazi, A., Daniel, K. G. *et al.*, Synthetic peracetate tea polyphenols as potent proteasome inhibitors and apoptosis inducers in human cancer cells. *Front. Biosci.* 2005, *10*, 1010 – 1023.

[135] Landis-Piwowar, K. R., Kuhn, D. J., Wan, S. B., Chen, D. *et al.*, Evaluation of proteasome-inhibitory and apoptosis-inducing potencies of novel (–)-EGCG analogs and their prodrugs. *Int. J. Mol. Med.* 2005, *15*, 735 – 742.

[136] Landis-Piwowar, K. R., Huo, C., Chen, D., Milacic, V. *et al.*, A novel prodrug of the green tea polyphenol (–)-epigallocatechin-3-gallate as a potential anticancer agent. *Cancer Res.* 2007, *67*, 4303 – 4310.

[137] Lambert, J. D., Kim, D. H., Zheng, R., Yang, C. S., Transdermal delivery of (–)-epigallocatechin-3-gallate, a green tea polyphenol, in mice. *J. Pharmacol. Pharm.* 2006, *58*, 599 – 604.

[138] Batchelder, R. J., Calder, R. J., Thomas, C. P., Heard, C. M., *In vitro* transdermal delivery of the major catechins and caffeine from extract of *Camellia sinensis*. *Int. J. Pharm.* 2004, *283*, 45 – 51.

[139] Fang, J. Y., Hung, C. F., Hwang, T. L., Wong, W. W., Transdermal delivery of tea catechins by electrically assisted methods. *Skin Pharmacol. Physiol.* 2006, *19*, 28 – 37.

[140] Shoba, G., Joy, D., Joseph, T., Majeed, M. *et al.*, Influence of piperine on the pharmacokinetics of curcumin in animals and human volunteers. *Planta Med.* 1998, *64*, 353 – 356.

[141] Lambert, J. D., Hong, J., Kim, D. H., Mishin, V. M. *et al.*, Piperine enhances the bioavailability of the tea polyphenol (–)-epigallocatechin-3-gallate in mice. *J. Nutr.* 2004, *134*, 1948 – 1952.

[142] Bajad, S., Bedi, K. L., Singla, A. K., Johri, R. K., Piperine inhibits gastric emptying and gastrointestinal transit in rats and mice. *Planta Med.* 2001, *67*, 176 – 179.

[143] Singh, J., Dubey, R. K., Atal, C. K., Piperine-mediated inhibition of glucuronidation activity in isolated epithelial cells of the guinea-pig small intestine: Evidence that piperine lowers the endogeneous UDP-glucuronic acid content. *J. Pharmacol. Exp. Ther.* 1986, *236*, 488 – 493.

[144] Reen, R. K., Jamwal, D. S., Taneja, S. C., Koul, J. L. *et al.*, Impairment of UDP-glucose dehydrogenase and glucuronidation activities in liver and small intestine of rat and guinea pig *in vitro* by piperine. *Biochem. Pharmacol.* 1993, *46*, 229 – 238.

[145] Galati, G., Lin, A., Sultan, A. M., O'Brien P, J., Cellular and *in vivo* hepatotoxicity caused by green tea phenolic acids and catechins. *Free Radic. Biol. Med.* 2006, *40*, 570 – 580.

[146] Bonkovsky, H. L., Hepatotoxicity associated with supplements containing Chinese green tea (*Camellia sinensis*). *Ann. Intern. Med.* 2006, *144*, 68 – 71.

[147] Galati, G., Lin, A., Sultan, A. M., O'Brien, P. J., Cellular and in vivo hepatotoxicity caused by green tea phenolic acids and catechins. *Free Radic. Biol. Med.* 2006, *40*, 570 – 580.

[148] Isbrucker, R. A., Edwards, J. A., Wolz, E., Davidovich, A. *et al.*, Safety studies on epigallocatechin gallate (EGCG) preparations. Part 2: Dermal, acute and short-term toxicity studies. *Food Chem. Toxicol.* 2005, *44*, 636 – 650.

