ANTIOXIDANTS & REDOX SIGNALING
Volume 3, Number 6, 2001
Mary Ann Liebert, Inc.

Forum Review

Tea Flavonoids: Bioavailability *In Vivo* and Effects on Cell Signaling Pathways *In Vitro*

SHEILA WISEMAN, THEO MULDER, and ANTON RIETVELD

ABSTRACT

The elucidation of the potential health benefits of tea beverage continues to be a focus of research in many laboratories. Beneficial effects of tea have been particularly evident in animal tumorigenesis models, with green and black tea frequently demonstrating similar effectivity. Human data are now emerging to support a beneficial role for tea in cardiovascular disease, but the data with respect to cancer risk at various sites remain inconclusive. The constituent flavonoids of green and black tea beverage are known to be potent antioxidants, and although this may be a major factor in explaining their biological activity, it appears that the gallated flavonoids in particular (*e.g.*, epigallocatechin gallate and the gallated theaflavins) impact on a wide range of molecular targets that influence cell growth and more specifically pathways such as those involving angiogenesis. Data on the pharmacokinetic properties of tea flavonoids, primarily on the catechins and therefore related most closely to green tea, have provided indications of the plasma levels and circulating molecular forms that may be expected in humans following tea consumption. The structural complexity of black tea flavonoids, in particular the thearubigins, has hindered efforts to describe their bioavailability and to perform mechanistic studies. Recent studies on the effects of catechins and theaflavins on growth factor-, nuclear factor-κB-, and stress-mediated signal transductions are described in this review, where possible in relation to their bioavailability *in vivo*. These studies indicate that effects that may be relevant to both cancer and atherosclerosis are often observed at tea flavonoid levels that could realistically be encountered *in vivo*. However, more studies need to be performed using those molecular forms of tea flavonoids (methylated, sulfated, and glucuronidated conjugates) that are the major circulating species encountered following tea consumption. Such studies, combined with further human epidemiological and interventional data, should ultimately elucidate the full beneficial potential of tea beverage on human health. Antioxid. Redox Signal. 3, 1009–1021.

INTRODUCTION

TEA is a traditional beverage that is generally prepared by infusing the dried leaves of *Camellia sinensis* in hot water to achieve a desirable taste profile. Both green and black tea leaves are obtained from *C. sinensis*, and although they are both characterized by their high content of antioxidant polyphenols called flavonoids (~30% by dry weight), the precise chemical natures of green and black tea are quite different. Green tea contains primarily monomeric flavonoids termed catechins or flavanols, predominantly (−)-epigallocatechin-3-gallate (EGCG), (−)-epigallocatechin (EGC), (−)-epicatechin-3-gallate (ECG), and (−)-epicatechin (EC). Black tea additionally contains complex oligomeric and polymeric polyphenols, theaflavins and thearubigins, which are formed during black tea manufacture as products of polyphenol oxidase-mediated catechin oxidation (Fig. 1). Sixty to 80% of the catechins

Unilever Health Institute, Unilever Research, P.O. Box 114, 3130 AC Vlaardingen, The Netherlands.

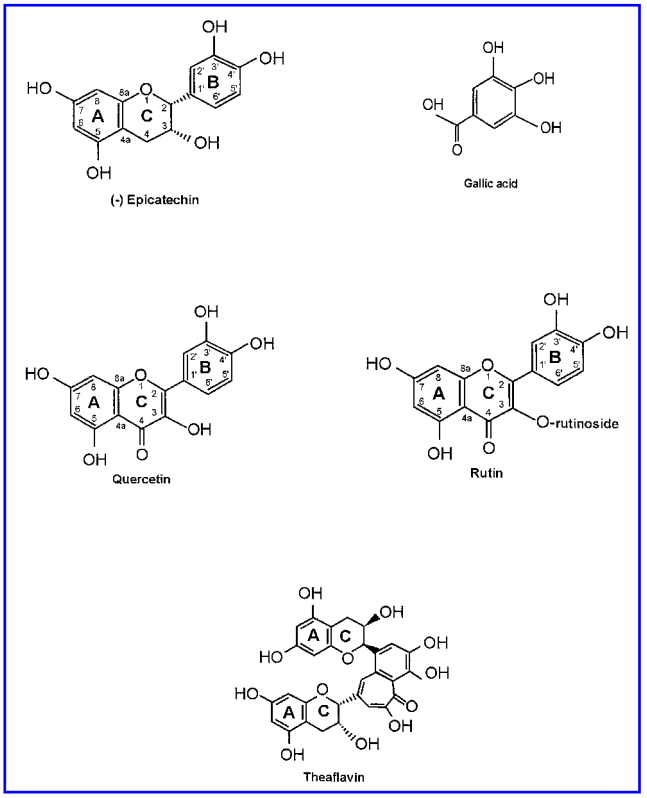

FIG. 1. Primary structure of mono- and dimeric tea flavonoids. Gallocatechins contain an extra hydroxyl group at the 5′ position. Catechin gallates are conjugated with gallic acid at the 3 position. Theaflavin can be conjugated with gallic acid at the 3 and the 3′ position.

present in the tea leaf are transformed in this way during black tea processing (6). All flavonoids possess C6-C3-C6 ring structures corresponding to phenyl-substituted benzpyrans and benzpyrones (25), and the dimeric theaflavins are included as flavonoids under this definition. Classification of thearubigins is less straightforward due to the analytical difficulties associated with their precise structural elucidation. Both green and black tea also contain a small amount of flavonol glycosides. Figure 2 shows the composition of typical green and black tea beverages.

Tea flavonoids are extracted rapidly from the leaf into hot water to give total flavonoid concentrations in average U.K. consumer black tea brews (water/leaf ratio of 75:1 and 40–60-s infusion) of ~65 mg per 100-ml serving (32), although it is possible that current analytical methodologies underestimate the contribution of the thearubigin fraction. Typical per capita tea consumption levels for a number of countries are shown in Fig. 3. Of these selected countries, the U.K. is the largest per-capita consumer of tea at 2.6 kg per year (or 3.3 cups per day), which is equivalent to a daily tea flavonoid consumption of ~430 mg (based on a cup size of 200 ml).

All tea flavonoids investigated so far have been identified as powerful antioxidants in a range of chemical and biological *in vitro* assay systems (62). Reaction rate constants for the scavenging by EGCG of superoxide and hydroxyl radicals have been determined as $7.2 \times 10^5\ M^{-1}\ s^{-1}$ (29) and $4.6 \times 10^{11}\ M^{-1}\ s^{-1}$ (55) respectively, and the rate of hydroxyl radical scavenging by EGCG has been shown to be an order of magnitude higher than that of other well established antioxidants such as ascorbate ($1.2 \times 10^{10}\ M^{-1}\ s^{-1}$), reduced glutathione ($1.5 \times 10^{10}\ M^{-1}\ s^{-1}$), and cysteine ($1.3 \times 10^{10}\ M^{-1}\ s^{-1}$) (55). Largely as a consequence of their high antioxidant potential, tea beverage and tea flavonoids have been the focus of numerous studies investigating their potential health benefits. This has resulted in a substantial body of data showing inhibitory activity of green and black tea in animal tumorigenesis models and human epidemiological data that associate primarily black tea consumption with a moderately reduced risk of cardiovascular disease (50, 58) and both green and black tea with reduced risk of certain cancers (18), albeit with varying degrees of consistency (9). The less conclusive data observed in human cancer studies in contrast to the animal data may be due to the predominance of retrospective epidemiological studies, the difficulty in accurately estimating tea flavonoid consumption in humans, and the relative difference in amounts of tea consumed in human and animal studies. Species differences related to the bioavailabilities and mechanisms of action of tea flavonoids may be an additional confounding factor. Indeed the biological impact of tea flavonoids on disease processes such as atherosclerosis and cancer will be dependent on absorption from the gut and subsequent distribution of the parent compound or active metabolite(s) to relevant tissue, cellular, and molecular sites. Knowledge on the bioavailability of tea flavonoids in humans is increasing, and combining this information with recent data on their ef-

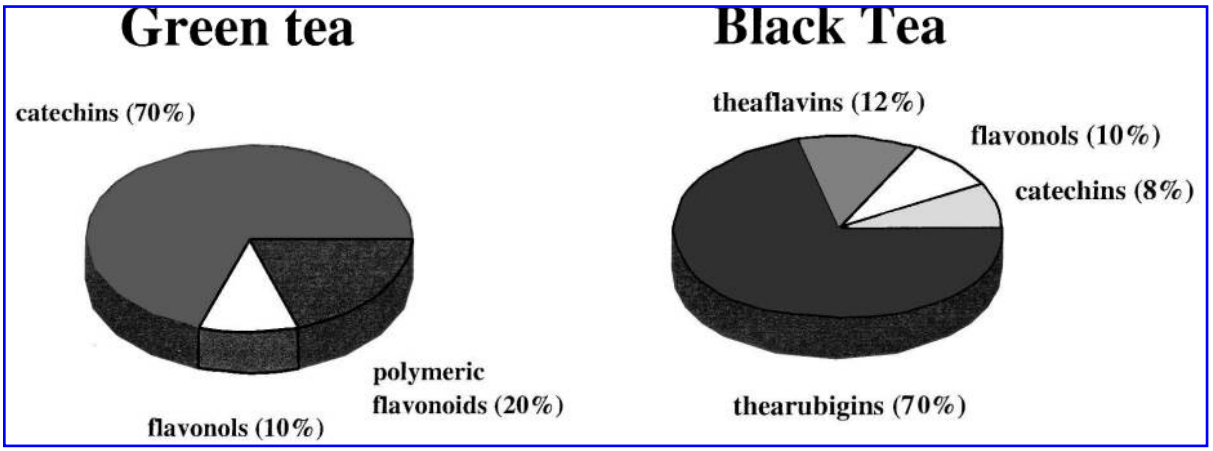

FIG. 2. Typical flavonoid compositions of green and black tea beverage.

fects on cellular processes *in vitro* should give insight into the most important mechanisms of action and provide information relevant to their potential for delivering health benefits *in vivo*. This review will address a number of these aspects.

WHAT LEVELS AND FORMS OF TEA FLAVONOIDS ARE FOUND IN PLASMA?

Most data on the bioavailability of tea flavonoids relate to catechins, flavonols, and their glycosides, due to the lack of analytical methods for the black tea polyphenols (theaflavins and thearubigins) in plasma. This implies that the published data refer primarily to green tea components, although black tea does contain a small amount of catechins, ~8% of the flavonoid content of black tea beverage (32). Ingestion of both green and black tea results in a rapid appearance of catechins and flavonols into plasma (16, 59). Maximum plasma levels for catechins have been observed between 1.5 and 2.5 h post tea ingestion in studies using green and black tea at various doses

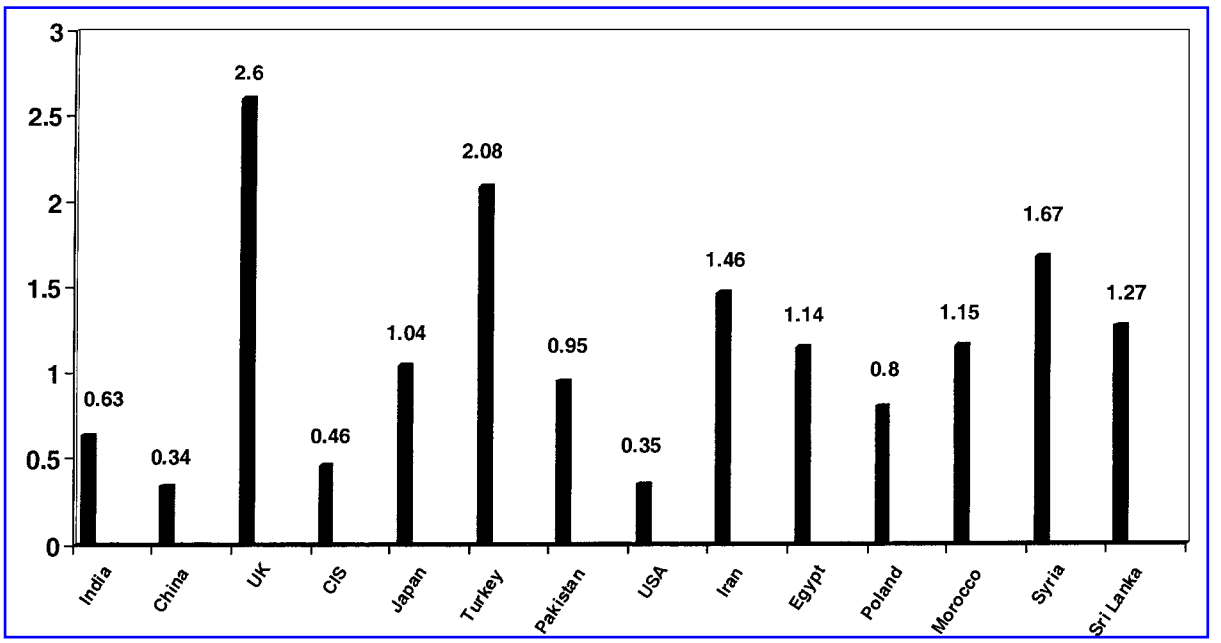

FIG. 3. Per capita tea consumption (kg) in selected countries. Source: International Tea Committee Ltd., *Annual Bulletin of Statistics*, Tea Broker's Publications, London, U.K. (1995).

(59, 63). Yang *et al.* (63) observed similar times to maximum plasma levels for three doses of green tea catechins derived from decaffeinated green tea extract (309, 618, and 927 mg of total catechins), suggesting that the dose may not affect the absorption or the access of tea catechins to the plasma compartment. The highest plasma concentrations were observed after the highest catechin dose (927 mg) when levels of 0.7 μM EGCG, 1.8 μM EGC, and 0.6 μM EC were measured. Repeated green tea consumption (8 cups per day), which reflects a normal pattern of tea consumption albeit at high intake levels, resulted in maximum plasma levels of total catechins in the range of 1 μM (60). This repeated pattern of tea consumption also significantly increased baseline plasma catechin levels to $\sim$0.4 μM after an overnight fast. It appears unlikely, however, that realistic levels of tea consumption will increase plasma total catechin concentrations far beyond the low micromolar levels that have so far been observed in humans, although a degree of flavonoid binding to red blood cells prior to plasma preparation, leading to an underestimation of true plasma levels, cannot yet be ruled out.

An important aspect related to defining the effects of tea flavonoids on cellular processes is that the majority of studies performed in *in vitro* cell models have involved those compounds occurring in the tea beverage and not the conjugated forms that are now known to be the major circulating forms *in vivo* (27). Lee *et al.* (33) quantified the amounts of free and unconjugated catechins in plasma following green tea consumption in four male subjects. At 1 and 4 h post tea consumption, EGC was reported to be circulating mostly in the glucuronide form (57–71%) followed by the sulfate (23–36%) and the free form (3–13%). EC was found to be exclusively in the conjugated form (66% sulfated and 33% glucuronidated). EGCG was mostly in the sulfated form (58–72%) with 12–28% in the free form and 8–19% in the glucuronidated form. ECG could not be detected in plasma samples after consumption of green tea. *O*-Methylated catechins were not measured in this study, but even without accounting for the contribution of methylated catechins, only $\sim$10% of catechins circulating in plasma 1–4 h after green tea consumption were in the free, unconjugated form.

ARE SIGNIFICANT LEVELS OF TEA FLAVONOIDS FOUND IN TISSUES?

There is currently little data on the tissue distribution of tea flavonoids after tea consumption. A recent study carried out in rats and mice has investigated blood and tissue levels of catechins after chronic consumption of 0.6% green tea polyphenols (GTP) via the drinking water (30). Peak plasma catechin levels in rats were achieved after 2 weeks of GTP consumption, with EGCG concentrations being much lower than those of EGC or EC. Higher levels of EGC and EC were also detected in urine, whereas high levels of EGCG were found in feces, most likely indicating a lower rate of intestinal absorption of EGCG or possibly excretion of absorbed EGCG into the bile. Remarkably, plasma levels of tea catechins actually decreased after 14 days of GTP consumption in rats to reach baseline levels by day 28, perhaps due to induction of conjugating enzymes and increased conversion of catechins to unknown metabolites, such as 3′-*O*-methyl conjugates, which would not be detected in the assay systems used. After 8 days of GTP consumption, substantial amounts of EGC and EC were detectable in rat bladder (800–810 ng/g of tissue), kidney (400–500), large intestine (300–930), esophagus (185–195), lung (190–230), and prostate (240–250). Only low levels of EGC and EC could be detected in liver, spleen, heart, and thyroid gland. EGCG levels were highest in the large intestine and esophagus, perhaps arising from direct contact of EGCG with intestinal tissue (64). Lower EGCG levels were found in other organs. Following administration of ^{3}H-EGCG into mouse stomach, radioactivity was detected in blood after 1 h, reaching a maximum of 2% of the administered dose at 6 h (56). At 24 h, significant amounts of radioactivity were recovered in a wide range of organs, with the bulk (14.5%) being recovered in digestive tract tissues (stomach, small intestine, and colon) and 2.1% being distributed over lung, brain, heart, liver, kidney, spleen, pancreas,

bladder, uterus, and ovary. However, the possibility of tritium exchange with protons from biological fluids (*e.g.*, gastric acid) cannot be excluded, and more studies using carbon-labeled catechins are required to identify conclusively pathways of postabsorptive distribution and metabolism.

Significant levels of tea flavonoids were detected in human colorectal tissue after ingestion of green tea extract (equivalent to 2–3 cups of tea) by human subjects (5). Increased tissue catechin levels were evident 4 h after tea ingestion and were still raised above baseline levels after 24 h. Interestingly, rectal tissue prostaglandin E_2 levels decreased in this study following green tea consumption, indicating a cellular biological response at relevant tea consumption levels.

ARE TEA FLAVONOID METABOLITES AN IMPORTANT SOURCE OF BIOLOGICALLY ACTIVE PHENOLICS?

Although absorption of the parent flavanol may be low, metabolic processing of catechins could result in a significant load of biologically active intermediates. Two catechin metabolites (ring fission products) were detected as major urinary metabolites after ingestion of green tea in humans (34). These metabolites were identified as (−)-5-(3′,4′,5′-trihydroxyphenyl)-γ-valerolactone and (−)-5-(3′,4′-dihydroxyphenyl)-γ-valerolactone and accounted for 6–39% of the ingested EGC and EC dose. Both metabolites appeared to be produced by intestinal microorganisms, with EGC and (−)-EC as the respective precursors, and were also detected in plasma, warranting investigation of their bioactivity. No studies have yet reported on the absorption of theaflavins or thearubigins into plasma, although indirect evidence of the bioavailability of black tea components is provided by both evidence of the efficacy of black tea in animal models of carcinogenesis (64) and recently reported studies in humans. Black tea consumption was associated with a highly significant increase in urinary hippuric acid excretion in human volunteers (14), and mass–balance calculations indicate that thearubigins (the dominant flavonoid fraction in black tea) are likely to be the major source. This implies that colonic microflora transformation of polymeric flavonoids is a route for the formation of significant amounts of metabolites with potential bioactivity. Flavonoids can reach the colon in two ways: either via nonabsorbed flavonoids entering from the small intestine, or as a result of excretion into the duodenum of bile containing conjugated flavonoids. In the colon, bacterial glycosidases, glucuronidases, and sulfatases liberate flavonoid aglycones that are less polar than their conjugated forms, thereby increasing the possibility of reabsorption. Otherwise bacterial ring fission of flavonoids forming a range of phenolic degradation products may occur (27), which may then undergo colonic absorption. A number of phenolic acids that could potentially be formed in this way are potent antioxidants (52), and they may also have significant effects on the colonic bacterial flora (24). In terms of elucidating the biological effects of tea consumption, it will be important to learn more about the structure and bioactivity of colonic flavonoid metabolites and their absorption.

ARE EFFECTS OF TEA FLAVONOIDS ON CELLULAR SIGNALING PROCESSES OBSERVED AT RELEVANT LEVELS?

Data from rodent models of carcinogenesis have consistently shown that green and black tea inhibit tumor development and growth to the same extent in a variety of models, but particularly in relation to cancer of the skin, lung, and esophagus (64). The effect of tea on cancer rates in humans is far less consistent than the body of animal data would suggest, but is sufficiently strong for the National Cancer Institute to fund chemoprevention trials with green tea and the major green tea catechin, EGCG (61). Additionally, there is a strengthening inverse association between tea consumption and incidence of cardiovascular disease (50), with the bulk of these data based on studies with black tea. Green and black tea are thus able to impact on disease processes, namely cancer and atherosclerosis, where the interplay be-

tween the signaling processes that regulate cell growth, cell proliferation, and apoptosis (controlled cell death) plays a fundamental role. Although the inhibitory effects of tea polyphenols on cell proliferation in numerous carcinoma cell types have been well documented (2, 45, 47, 57), the mechanism(s) of these antiproliferative effects remain to be established. Interpretation of these data also remains a complex issue, as it should be appreciated that the effective antiproliferative concentrations are frequently one to two orders of magnitude higher than the maximum plasma concentrations observed in humans at high levels of tea consumption. However, it is not yet known what levels of tea flavonoids or their active metabolites may occur within human tissues, and emerging data are showing profound effects of tea flavonoids on various aspects of cell proliferation and mitotic signal transduction, sometimes at low micromolar concentrations that may be feasible *in vivo*, for instance as a result of local accumulation.

CAN TEA FLAVONOIDS AFFECT GROWTH FACTOR-MEDIATED CELL SIGNALING?

Activation of the mitogen-activated protein kinase (MAP kinase) pathway by platelet-derived growth factor (PDGF-BB) is initiated by dimerization and autophosphorylation of the receptor tyrosine kinase (PDGF-Rβ) followed by phosphorylation of the MAP kinase isoforms p44/p42 [extracellular signal-regulated kinase (ERK) 1/2] (26). In this way extracellular mitogenic signals are transferred via phosphorylation cascades from the plasma membrane to the cell nucleus (Fig. 4), and activation of this pathway is critical for the expression of nuclear transcription factors and nonnuclear protein kinases involved in regulation of cell growth. In vascular smooth muscle cells preincubated with EGCG, PDGF-BB was unable to activate the MAP kinase pathway (4). Autophosphorylation of the PDGF-Rβ was inhibited in EGCG-treated vascular smooth muscle cells with an IC_{50} of 20 μM. However, at concentrations higher than 1 μM, EGCG already inhibited maximal phosphorylation of p44/p42 in a dose-dependent manner, consistent with the observation that maximal phosphorylation of p44/p42 occurs at low stimulation of the PDGF-Rβ. EGCG treatment also induced complete inhibition of the PDGF-BB induced increase in intracellular calcium, which is explained by the reduced ability of PDGF-Rβ to activate phospholipase C-γ1, responsible for the Ca^{2+} release, in the presence of EGCG. Increased intracellular calcium ion levels stimulate MAP kinase-as well as Src kinase-dependent signaling pathways. These effects of EGCG on downstream signaling events were not mediated via down-regulation of receptor (PDGF-Rβ) expression, but possibly via a conformational change in the PDGF-Rβ ATP-binding site, which could inhibit subsequent tyrosine phosphorylation (31). The EGCG-induced changes did indeed lead to a selective inhibition of the expression of transcription factors c-Fos and Egr-1, which could then account for the antiproliferative effect observed. Receptor tyrosine kinases such as the PDGF-Rβ have been implicated in several disorders including atherosclerosis and many cancers (26). If EGCG levels of 20–50 μM would indeed be achievable *in vivo*, then EGCG could potentially reduce cancers in which activation of PDGF-Rβ is causatively involved.

A separate study (53) showed similar inhibitory effects on the tyrosine kinase activity of PDGF-Rβ by 50 μM concentrations of gallated catechins (catechin gallate, ECG, and EGCG). Interestingly, the nongallated catechins, catechin and EC, had no inhibitory effects. Data from this study also suggested that enhanced activity of the phosphatase (MAP kinase phosphatase-1) was not responsible for loss of tyrosine kinase activity in the presence of gallated catechins. Liang *et al.* (35) found that EGCG inhibited epidermal growth factor (EGF) receptor phosphorylation in A431 epidermoid carcinoma cells, and the same authors recently reported (36) that although both theaflavin-3,3′-digallate, a constituent of black tea, and EGCG inhibited growth of A431 and NIH 3T3 cells, theaflavin-3,3′-digallate was more effective in inhibiting both EGF receptor and PDGF receptor autophosphorylation. The monogallated theaflavins and thearubigin were less effective than theaflavin-3,3′-digallate, which in-

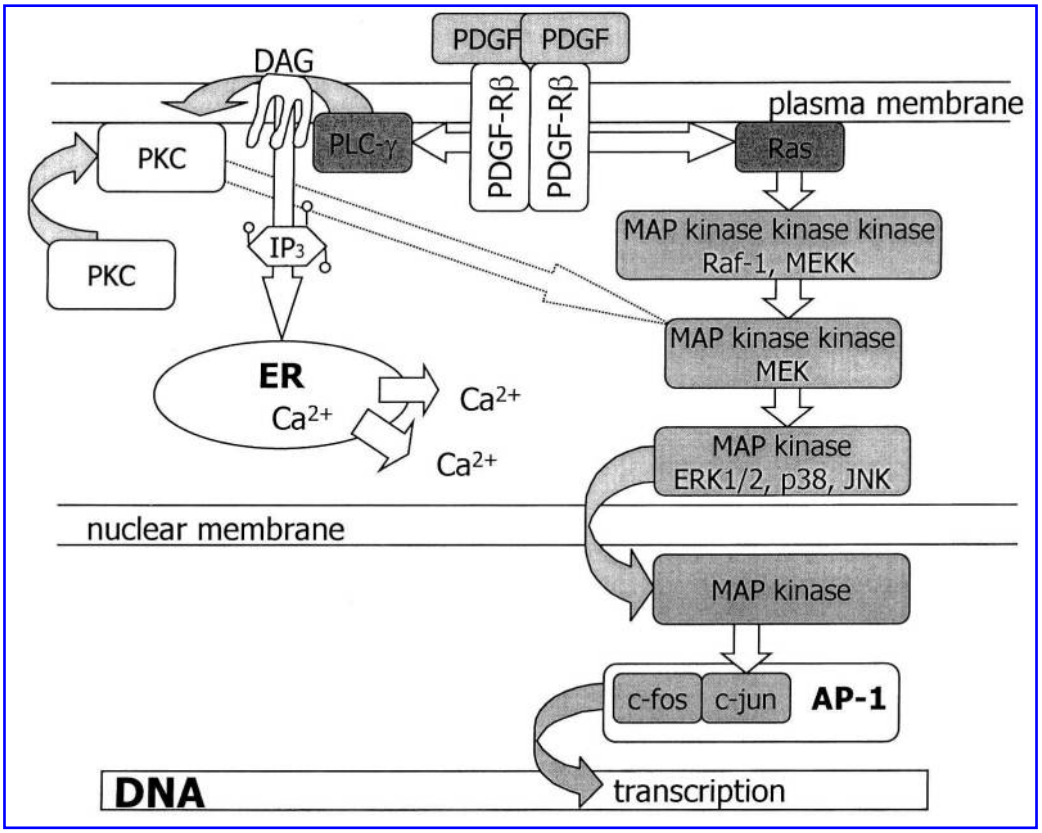

Figure 4. Components of the MAP kinase signaling pathway. Upon ligand binding, receptor tyrosine kinases such as the PDGF receptor activate a signaling cascade that leads to transcription of genes involved in proliferation and differentiation. The signal can be relayed through activation of either Ras, a monomeric GTPase, or phospholipase C (PLC). Ras activation leads to a series of subsequent serine/threonine phosphorylations by a family of MAP kinases, also called extracellular signal-regulated kinases (ERKs, *e.g.*, ERK 1/2 = p44/p42). These kinases can be switched on by a range of extracellular proliferation and differentiation signals. When activated, the MAP kinases migrate from the cytosol to the nucleus where they activate the transcription of the *fos* gene. In addition, MAP kinases phosphorylate the c-Jun protein, which dimerizes with the newly made c-Fos to form the active transcription regulator complex AP-1, which induces the transcription of additional genes. Alternatively, upon activation of PLC, phosphatidylinositol 4,5-biphosphate is cleaved into diacylglycerol (DAG) and inositol 1,4,5-triphosphate (IP_3). Protein kinase C (PKC) is then recruited to the membrane and activated by DAG while the IP_3 induces a Ca^{2+} release from the endoplasmic reticulum (ER). The signal is then relayed from PKC downstream to the nucleus through the MAP kinase pathway.

hibited EGF receptor-kinase activity by ~75% at a concentration of 5 μM.

CAN TEA FLAVONOIDS AFFECT CELL-CYCLE PROGRESSION?

EGCG was shown to inhibit dose-dependently the proliferation of breast epithelial cells (MCF10A) in culture at concentrations between 25 and 100 μM (38). At similar concentrations, EGCG also inhibited the phosphorylation of the retinoblastoma tumor suppressor protein (pRB). This phosphorylation is thought to be a prerequisite for passage through the so-called "restriction point" in the early/mid G1 phase of the cell cycle, which enables cells to complete the remaining phases of the growth cycle (51). EGCG appeared to act through induction of p21, a cyclin-dependent kinase inhibitor, an effect that would increase association of p21 with cyclin D1, thereby impairing cyclin-dependent kinase (CDK) activity and resulting in reduced pRB phosphorylation. Induction of p21 and the resulting decrease in cell proliferation rates has been observed for other flavonoids and flavonoid analogues and has been proposed as a potential chemopreventive mechanism (20). Consistent with the results observed by Liberto *et al.* (38) it was reported that 30 μM EGCG in-

hibited cell growth and induced G1 cell-cycle arrest in human breast carcinoma (MCF-7) cells (37). At this concentration, EGCG changed the phosphorylation state of the retinoblastoma protein from mainly hyperphosphorylated to hypophosphorylated after 9–24 h of incubation. Between 5 and 20 μM, EGCG was also able to shift the phosphorylation state to a more hypophosphorylated distribution, a situation that would favor decreased cell proliferation rates. Two classes of cyclin-dependent kinase inhibitor are found in mammalian cells—those that primarily inhibit Cdk2- and Cdk4-cyclin complexes (*e.g.*, p21, p27, and 57) and those with a preference for Cdk4- and Cdk6-cyclin complexes. EGCG at 30 μM inhibited the kinase activities of Cdk2 and Cdk4 by ~80% and induced a significant increase in p21. The nongallated catechins, EGC, EC, and catechin were not able to inhibit Cdk2 and Cdk4 kinase activities in MCF-7 cells, whereas 30 μM gallic acid showed slight inhibitory activity.

CAN TEA FLAVONOIDS AFFECT NUCLEAR FACTOR-κB-MEDIATED CELL SIGNALING?

Activation of the nuclear factor-κB (NF-κB) signaling pathway mediates a wide range of cellular responses involved in inflammation, proliferation, and apoptosis. NF-κB is an oxidative stress-sensitive nuclear transcription factor that controls the expression of many inflammatory genes including the tumor necrosis factor-α (TNFα) gene, and recent reports indicate how tea flavonoids may impact on this pathway. EGCG (40–80 μM) lowered NF-κB levels in NHEK (normal human epidermal keratinocyte) cells, but even lower levels (10 μM) effectively inhibited NF-κB expression in A431 cancer cells (3). This result concurs with earlier reports that EGCG treatment induced apoptosis and growth inhibition only in cancerous, and not in normal, cells (1, 12). Inhibition of NF-κB binding to DNA has been reported in RAW264.7 macrophages treated with EGCG (65), and the authors suggest this mechanism is also involved in the inhibition by GTP of lipopolysaccharide (LPS)-induced TNFα production in mice.

A major inflammatory pathway mediated by NF-κB is that involving the production of nitric oxide (NO) by induction of nitric oxide synthase (iNOS). A separate constitutive pathway of NO production is regulated by cyclic GMP and functions to maintain appropriate endothelial vasodilation. Green and black tea appear able to interact discriminately with the NO system, having a greater effect on the inducible, inflammatory pathway involving peroxynitrite formation than the constitutive pathway involved in endothelial vasodilation (49). In activated macrophages (39), theaflavin-3,3′-digallate inhibited NO generation and iNOS expression more effectively than EGCG, an effect that was mediated via blockage of IκB phosphorylation. IκB kinase (IKK) activity is required for phosphorylation of inhibitor proteins (IκBα, IκBβ, and IκBϵ), which results in liberation of NF-κB in the cytosol. NF-κB then translocates to the nucleus where it binds to DNA and initiates gene transcription. When a number of polyphenols (including EGCG, theaflavin-3,3′-digallate, a mixture of theaflavin-3-gallate and theaflavin-3′-gallate, thearubigins, procyanidin B-3, casuarinin, and geraniin) were compared at 30 μM concentrations (48), theaflavin-3,3′-digallate was found to be the most effective inhibitor of IKKs (IKK1 and IKK2) in LPS-activated macrophages. EGCG and monogallated theaflavins demonstrated some IKK inhibitory activity, whereas thearubigins showed very low activity. The level of IKK protein was not changed on incubation with tea polyphenols, suggesting either a direct effect on IKK activity or effects on upstream events. In the same study (48), theaflavin-3,3′-digallate also decreased LPS-mediated activation of iNOS by >90% and strongly inhibited inflammatory NO production.

CAN TEA FLAVONOIDS AFFECT STRESS-INDUCED CELL SIGNALING?

A group of MAP kinases, which includes the c-Jun N-terminal kinases (JNKs), regulate activator protein-1 (AP-1) transcriptional activity in response to environmental stress [such as ultraviolet (UV) irradiation] or to cytokines such as TNFα or interleukin-1. High AP-1 activity

has been shown to be involved in the promotion and progression of various types of cancers, and inhibition of AP-1 activation may be a relevant molecular target for potential chemopreventive agents (43). UVB-induced AP-1 activity was inhibited by EGCG in a dose range of 5 n*M* to 50 μM in human keratinocytes (7). The same group (11) also showed that EGCG inhibited UVB-induced transcription of the *c-fos* gene and expression of the c-Fos protein (a component of AP-1), via mechanisms that appeared to involve inhibition of the p38 MAP kinase, but not the MAP kinases ERK-1/2 or JNK. In a separate study, EGCG and theaflavins (range 5–20 μM) inhibited EGF- or 12-*O*-tetradecanoylphorbol 13-acetate-induced AP-1 activity (17). At concentrations that blocked AP-1 activity, both EGCG and theaflavin inhibited c-Jun (a component of AP-1 that forms a heterodimer with c-Fos) phosphorylation via pathways that appeared to be JNK-dependent and ERK-1/2-independent. The presence of a gallate moiety and the galloyl structure on the B ring of tea flavonoids appears to be important for AP-1 inhibitory activity in *ras*-transformed cells (13). EGCG with both a gallate moiety and the galloyl structure is a strong inhibitor of AP-1 activity (IC_{50} = 5 μM), and EC, which lacks both these structural features, is a poor inhibitor (IC_{50} = 100 μM). Catechin epimerization does not appear to be a major factor in the ability to inhibit AP-1. Theaflavins also showed a strong inhibitory effect on AP-1 activity with estimated IC_{50} values of 5 μM for both mono- and digallate forms. With an IC_{50} value of 20 μM, theaflavin appeared to be less effective than its gallate derivatives. Investigation of events upstream from AP-1 activation in *ras*-transformed cells showed that EGCG and theaflavin 3-3′-digallate decreased the phosphorylation of c-Jun and ERK-1/2, whereas phosphorylation of JNK was not affected. As both ERK-1/2 and JNK can phosphorylate c-Jun, binding of EGCG and theaflavin-3,3′-digallate to c-Jun could explain the inability of JNK to activate AP-1. To support this, the authors suggest that the well known association of (tea) flavonoids with proline-rich proteins could be the molecular basis for MAP kinase substrate binding. MAP kinases are known as "proline-directed protein kinases" because they catalyze phosphorylation of serine/threonine residues within proline-rich regions on proteins (23). In addition to this, the tea catechins affect the levels of AP-1 components in different ways: EGCG reduces the level of c-Jun, and theaflavin-3,3′-digallate reduces the level of Fra-1 (a member of the c-Fos family and a component of AP-1). The study by Chung *et al.* (13) observed another difference between EGCG and theaflavin-3,3′-digallate in the mechanism of AP-1 inhibition. Phosphorylation of MAP kinase p38 was inhibited by theaflavin-3,3′-digallate, but not by EGCG. p38 has been implicated in processes associated with cellular stress such as UV irradiation. Indeed, theaflavins and EGCG inhibited UVB-induced AP-1 activation in JB6 mouse epidermal cells (46), and theaflavins were found to be more effective than EGCG. EGC inhibited proliferative responses in a variety of cells including rat aortic smooth muscle (A7r5) cells, rabbit aortic smooth muscle cells, human coronary artery smooth muscle cells, and human CEM lymphocytes (41). IC_{50} concentrations of EGC were <4 μM for inhibition of cell proliferation in all cell types. Again the galloyl moiety was found to be important for full inhibitory activity, as catechin and EC were found to be less effective. EGC appeared to exert its antiproliferative activity via the JNK1 pathway.

One class of genes regulated by AP-1 is the matrix metalloproteinases (MMPs), which catalyze the proteolytic breakdown of extracellular matrix components such as collagen and play a role in the progression of human tumors (40) and macrophage-mediated pathologies such as atherosclerosis. The gelatinases, MMP-2 and MMP-9, are particularly involved in tumor invasion and metastasis and are the dominant MMPs released by most epithelial and endothelial cells (22), where they normally function to maintain extracellular matrix homeostasis. MMP-9 has recently been shown to be an important trigger of the angiogenic switch during carcinogenesis (8). Tea flavonoids have been shown to inhibit the activity of a number of MMPs, potentially through their inhibitory effects on AP-1 activation. Theaflavin, theaflavin-3,3′-digallate, and EGCG inhibited collagenases

(probably MMP-2 and MMP-9) from mouse lung carcinoma cells (54). No IC_{50} data were reported in this study, but inhibition could be observed at concentrations $<25\ \mu M$. In agreement with these results, EGCG dose-dependently inhibited the activities of MMP-2 and MMP-9 from HT1080 fibrosarcoma cells, with IC_{50} values of 20 and 50 μM, respectively (21). At significantly lower concentrations, which could be expected in plasma after tea consumption ($IC_{50} < 0.1\ \mu M$), EGCG was also effective in reducing tumor cell invasion. Green tea polyphenols (35 μg/ml) inhibited both MMP-2 and MMP-9 by 80%, an effect that was significantly greater than that of other natural compounds such as resveratrol, garlic-derived organosulfur compounds, limonene, and genistein at 100 μM concentrations (15). Of the green tea catechins, EGCG was the most potent inhibitor of MMP-2, MMP-9, and MMP-12 (macrophage elastase), followed by ECG. It appeared that the gallate moiety was essential for MMP inhibition, as EGC, EC, and catechin were not active.

Expression of vascular endothelial growth factor (VEGF) is crucial for the vascularization of tumors (angiogenesis). Tumors produce large amounts of VEGF, which stimulates the proliferation and migration of endothelial cells. Induction of VEGF expression is regulated by kinases (*e.g.*, ERK-1/2) and oncogenes that converge on AP-1 (44). Inhibition of tyrosine phosphorylation by EGCG could therefore be involved in the inhibition of VEGF-induced angiogenesis by green tea (10), an effect that was observed in a mouse model of corneal neovascularization. The EGCG concentration in the plasma of these mice was in the range 0.1–0.3 μM, a level that has been observed in humans after moderate tea drinking (63). EGCG has also been reported to inhibit urokinase activity (28), a proteolytic enzyme involved in tumor growth and metastasis. Like the MMPs, urokinase expression is also mediated by AP-1, but it appears that millimolar levels of EGCG are required for enzyme inhibition, levels that are not likely to be observed *in vivo*, whereas the effects of tea flavonoids on MMP activity could certainly be physiologically relevant.

SUMMARY

It is clear that a number of tea flavonoids are able to exert profound inhibitory effects on cellular proliferative processes, which may be of particular importance in the context of cancer and cardiovascular disease. Signal transduction pathways mediated by growth factors such as EGF and PDGF, stress factors such as UV, and inflammatory mediators such as LPS and 12-*O*-tetradecanoylphorbol 13-acetate are all inhibited by tea flavonoids. Downstream events related to these pathways (*e.g.*, angiogenesis and MMP activation) are also inhibited by tea flavonoids with angiogenesis inhibition also being observed in an animal model. Structural features that have been shown to be of importance in a range of studies for effective inhibitory activity are the galloyl moiety on the B ring of the flavonoid molecule and the gallate group. In the majority of *in vitro* mechanistic studies performed so far, the most effective inhibitory activity is displayed by EGCG and the gallated theaflavins, flavonoids that indeed contain the proposed structural requirements, and it appears that cancerous cells are more sensitive to the inhibitory effects of tea flavonoids than their normal counterparts.

Although the concentrations of tea flavonoids that are required for inhibitory activity are often far higher than can be expected in plasma or tissues on the basis of current knowledge, significant effects on cell proliferation pathways have also been observed at more realistic, low micromolar concentrations. The available pharmacokinetic data on tea flavonoids relate primarily to the flavan-3-ols (catechins) and flavonols and, although most relevant to green tea beverage, do show that low micromolar concentrations of catechins are present in plasma after green and black tea consumption. Conjugation to methylated, sulfated, and glucuronidated forms occurs rapidly as the flavonoids pass through gastrointestinal and hepatic tissues. Few data are currently available on the biological activity of these conjugated forms of tea flavonoids, in contrast to the unconjugated flavonoids, which indeed appear able to inhibit multiple cellular mechanisms involved in proliferation. The relative importance of these mechanisms to cancer and

cardiovascular disease still needs to be established, as does elucidation of the role of the major black tea polyphenolic fraction, the thearubigins, and the potential contribution to the biological activity of tea of colonic fermentation products.

Recent *in vivo* studies support some of the effects of tea flavonoids observed in mechanistic studies. For example, in SKH-1 mice orally pretreated with 0.6% green tea for 2 weeks (42), an enhancement in the UV-induced increase in the number of p53-positive cells, p21(WAF1/CIP1)-positive cells, and apoptotic sunburn cells in the epidermis was observed. These effects of green tea on early adaptive responses to UV demonstrate *in vivo* up-regulation of a tumor suppressor gene by a potential chemopreventive agent. In a fully randomized, placebo-controlled, cross-over trial, consumption of four cups of black tea per day significantly reversed endothelial dysfunction in patients with coronary artery disease as measured by flow-mediated dilation (19). Additional intervention trials of this kind, together with prospective epidemiological studies able to provide accurate assessment of tea flavonoid intake, will ultimately enable the true impact of tea beverage on human disease processes to be determined.

ABBREVIATIONS

AP-1, activator protein-1; CDK, cyclin-dependent kinase; EC, (−)-epicatechin; ECG, (−)-epicatechin-3-gallate; EGC, (−)-epigallocatechin; EGCG, (−)-epigallocatechin-3-gallate; EGF, epidermal growth factor; ERK, extracellular signal-regulated kinase; GTP, green tea polyphenols; IKK, IκB kinase; iNOS, inducible nitric oxide synthase; JNK, c-Jun N-terminal protein kinase; LPS, lipopolysaccharide; MAP kinase, mitogen-activated protein kinase; MMP, matrix metalloproteinase; NF-κB, nuclear factor-κB; NO, nitric oxide; PDGF, platelet-derived growth factor; pRB, retinoblastoma tumor suppressor protein; TNFα, tumor necrosis factor-α; UV, ultraviolet; VEGF, vascular endothelial growth factor.

REFERENCES

1. Ahmad N, Feyes DK, Nieminen A-L, Agarwal R, and Mukhtar H. Green tea constituent epigallocatechin-3-gallate and induction of apoptosis and cell cycle arrest in human carcinoma cells. *J Natl Cancer Inst* 89: 1881–1886, 1997.
2. Ahmad N, Cheng P, and Mukhtar H. Cell cycle dysregulation by green tea polyphenol epigallocatechin-3-gallate. *Biochem Biophys Res Commun* 275: 328–334, 2000.
3. Ahmad N, Gupta S, and Mukhtar H. Green tea polyphenol epigallocatechin-3-gallate differentially modulates nuclear factor κB in cancer versus normal cells. *Arch Biochem Biophys* 376: 338–346, 2000.
4. Ahn H-Y, Hadizadeh KR, Seul C, Yun Y-P, Vetter H, and Sachinidis A. Epigallocatechin-3 gallate selectively inhibits the PDGF-BB-induced intracellular signalling transduction pathway in vascular smooth muscle cells and inhibits transformation of *sis*-transfected NIH 3T3 fibroblasts and human glioblastoma cells (A172). *Mol Biol Cell* 10: 1093–1104, 1999.
5. August DA, Landau J, Caputo D, Hong J, Lee M-J, and Yang CS. Ingestion of green tea rapidly decreases prostaglandin E2 levels in rectal mucosa in humans. *Cancer Epidemiol Biomarkers Prev* 8: 709–713, 1999.
6. Balentine DA, Wiseman SA, and Bouwens LCM. The chemistry of tea flavonoids. *Crit Rev Food Sci Nutr* 37: 693–704, 1997.
7. Barthelman M, Bair WB, Stickland KK, Chen W, Timmermann BN, Valcic S, Dong Z, and Bowden GT. (−)-Epigallocatechin-3-gallate inhibition of ultraviolet B-induced AP-1 activity. *Carcinogenesis* 19: 2201–2204, 1998.
8. Bergers G, Brekken R, McMahon G, Vu TH, Itoh T, Tamaki K, Tanzawa K, Thorpe P, Itohara S, Werb Z, and Hanahan D. Matrix metalloproteinase-9 triggers the angiogenic switch during carcinogenesis. *Nat Cell Biol* 2: 737–744, 2000.
9. Blot WJ, Chow WH, and McLaughlin JK. Tea and cancer: a review of the epidemiologic evidence. *Eur J Cancer Prev* 5: 425–438, 1996.
10. Cao Y and Cao R. Angiogenesis inhibited by drinking tea. *Nature* 398: 381, 1999.
11. Chen W, Dong Z, Valcic S, Timmermann BN, and Bowden GT. 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: 79–84, 1999.
12. Chen ZP, Schell JB, Ho C-T, and Chen KY. Green tea epigallocatechin gallate shows a pronounced growth inhibitory effect on cancerous cells but not on their normal counterparts. *Cancer Lett* 129: 173–179, 1998.
13. Chung JY, Huang C, Meng X, Dong Z, and Yang CS. Inhibition of activator protein 1 activity and cell growth by purified green tea and black tea polyphenols in H-ras-transfomed cells: structure–activity relationship and mechanism involved. *Cancer Res* 59: 4610–4617, 1999.

14. Clifford MN, Copeland EL, Bloxsidge JP, and Mitchell LA. Hippuric acid as a major product associated with black tea consumption. *Xenobiotica* 30: 317–326, 2000.
15. Demeule M, Brossard M, Pagé M, Gingras D, and Béliveau R. Matrix metalloproteinase inhibition by green tea catechins. *Biochim Biophys Acta* 1478: 51–60, 2000.
16. de Vries JHM, Hollman PCH, Meyboom S, Buysman MNCP, Zock PL, van Staveren WA, and Katan MB. Plasma concentrations and urinary excretion of the antioxidant flavonols quercetin and kaempferol as biomarkers for dietary intake. *Am J Clin Nutr* 68: 60–65, 1998.
17. Dong Z, Ma W, Huang C, and Yang CS. Inhibition of tumour promotor-induced activator protein 1 activation and cell transformation by tea polyphenols, (−)-epigallocatechin gallate, and theaflavins. *Cancer Res* 57: 4414–4419, 1997.
18. Dresoti IE, Wargovich MJ, and Yang CS. Inhibition of carcinogenesis by tea: the evidence from experimental studies. *Crit Rev Food Sci Nutr* 37: 761–770, 1997.
19. Duffy SJ, Keaney JF, Holbrook M, Gokce N, Swerdloff P, Frei B, and Vita JA. Short- and long-term black tea consumption reverses endothelial dysfunction in patients with coronary artery disease. *Circulation* 104: 151–156, 2001.
20. Fischer PM and Lane DP. Inhibitors of cyclin-dependent kinases as anti-cancer therapeutics. *Curr Med Chem* 7: 1213–1245, 2000.
21. Garbisa S, Biggin S, Cavallarin N, Sartor L, Benelli R, and Albini A. Tumor invasion: molecular shears blunted by green tea. *Nat Med* 5: 1216, 1999.
22. Hanemaaijer R, Koolwijk P, le Clercq L, de Vree WJ, and van Hinsbergh VW. Regulation of matrix metalloproteinase expression in human vein and microvascular endothelial cells. Effects of tumour necrosis factor alpha, interleukin 1 and phorbol ester. *Biochem J* 296: 803–809, 1993.
23. Hanks SK and Hunter T. Protein kinases 6. The eukaryotic protein kinase superfamily: kinase (catalytic) domain structure and classification. *FASEB J* 9: 576–596, 1995.
24. Hara H, Orita N, Hatano S, Ichikawa H, Hara Y, Matsumoto N, Kimura Y, Terada A, and Mitsuoka T. Effect of tea polyphenols on fecal flora and fecal metabolic products of pigs. *J Vet Med Sci* 57: 45–49, 1995.
25. Harborne J (Ed). *The Flavonoids—Advances in Research Since 1986.* London, U.K.: Chapman and Hall, 1994.
26. Heldin C-H and Westermark B. Mechanism of action and in vivo role of platelet-derived growth factor. *Physiol Rev* 79: 1283–1316, 1999.
27. Hollman PCH and Katan MB. Absorption, metabolism and bioavailability of flavonoids. In: *Flavonoids in Health and Disease*, edited by Rice-Evans C and Packer L. New York, NY: Marcel Dekker Inc., 1998, pp. 483–522.
28. Jankun J, Selman SH, Swiercz R, and Skrzypczak-Jankun E. Why drinking green tea could prevent cancer. *Nature* 387: 561, 1997.
29. Jovanovic S, Hara Y, Steenken S, and Simic M. Antioxidant potential of gallocatechins. A pulse radiolysis and laser photolysis study. *J Am Chem Soc* 117: 9881–9888, 1995.
30. Kim S, Lee M-J, Hong J, Li C, Smith TJ, Yang G-Y, Seril DN, and Yang CS. Plasma and tissue levels of tea catechins in rats and mice during chronic consumption of green tea polyphenols. *Nutr Cancer* 37: 41–48, 2000.
31. Kovalenko M, Ronnstrand L, Heldin CH, Loubtchenkov M, Gazit A, Levitzki A, and Bohmer FD. Phosphorylation site-specific inhibition of platelet-derived growth factor beta-receptor autophosphorylation by the receptor blocking tyrphostin AG1296. *Biochemistry* 36: 6260–6269, 1997.
32. Lakenbrink C, Lapczynski S, Maiwald B, and Engelhardt UH. Flavonoids and other polyphenols in consumer brews of tea and other caffeinated beverages. *J Agric Food Chem* 48: 2848–2852, 2000.
33. Lee M-J, Wang Z-Y, Li H, Chen L, Sun Y, Gobbo S, Balentine DA, and Yang CS. Analysis of plasma and urinary tea polyphenols in human subjects. *Cancer Epidemiol Biomarkers Prev* 4: 393–399, 1995.
34. Li C, Mee M-J, Sheng S, Meng X, Prabhu S, Winnik B, Huang B, Chung J-Y, Yan S, Ho C-T, and Yang CS. Structural identification of two metabolites of catechins and their kinetics in human urine and blood after tea ingestion. *Chem Res Toxicol* 13: 177–184, 2000.
35. Liang Y-C, Lin-Shiau S-Y, Chen C-F, and Lin J-K. Suppression of extracellular signals and cell proliferation through EGF receptor binding by (−)-epigallocatechin gallate in human A431 epidermoid carcinoma cells. *J Cell Biochem* 67: 55–65, 1997.
36. Liang Y-C, Chen Y-C, Lin Y-L, Lin-Shiau S-Y, Ho C-T, and Lin J-K. Suppression of extracellular signals and cell proliferation by the black tea polyphenol, theaflavin-3,3′-digallate. *Carcinogenesis* 20: 733–736, 1999.
37. Liang Y-C, Lin-Shiau S-Y, Chen C-F, and Lin J-K. Inhibition of cyclin-dependent kinases 2 and 4 activities as well as induction of Cdk inhibitors p21 and p27 during growth arrest of human breast carcinoma cells by (−)-epigallocatechin-3-gallate. *J Cell Biochem* 75: 1–12, 1999.
38. Liberto M and Cobrinik D. Growth factor-dependent induction of $p21^{CIP1}$ by the green tea polyphenol, epigallocatechin gallate. *Cancer Lett* 154: 151–161, 2000.
39. Lin YL, Tsai SH, Lin-Shiau SY, Ho CT, Lin JK. Theaflavin-3,3′-digallate from black tea blocks the nitric oxide synthase by down-regulating the activation of NF-kappaB in macrophages. *Eur J Pharmacol* 367: 379–388, 1999.
40. Liotta LA. Metastatic potential correlates with enzymatic degradation of basement membrane collagen. *Nature* 284: 67–68, 1980.
41. Lu L-H, Lee S-S, and Huang H-C. Epigallocatechin suppresion of proliferation of vascular smooth muscle cells: correlation with *c-jun* and JNK. *Br J Pharmacol* 124: 1227–1237, 1998.
42. Lu YP, Lou YR, Li XH, Xie JG, Brash D, Huang MT and Conney AH. Stimulatory effect of oral adminis-

tration of green tea or caffeine on ultraviolet light-induced increases in epidermal wild-type p53, p21(WAF1/CIP1), and apoptotic sunburn cells in SKH-1 mice. *Cancer Res* 60: 4785–4791, 2000.
43. McCarty MF. Polyphenol-mediated inhibition of AP-1 transactivating activity may slow cancer growth by impeding angiogenesis and tumour invasiveness. *Med Hypotheses* 50: 511–514, 1998.
44. Mori K, Tani M, Kamata K, Kawamura H, Urata Y, Goto S, Kuwano M, Shibata S, and Kondo T. Mitogen-activated protein kinase, ERK1/2, is essential for the induction of vascular endothelial growth factor by ionizing radiation mediated by activator protein-1 in human glioblastoma cells. *Free Radic Res* 33: 157–166, 2000.
45. Morre DJ, Bridge A, Wu LY, and Morre DM. Preferential inhibition by (−)-epigallocatechin-3-gallate of the cell surface NADH oxidase and growth of transformed cells in culture. *Biochem Pharmacol* 60: 937–946, 2000.
46. Nomura M, Ma WY, Huang C, Yang CS, Bowden GT, Miyamoto K, and Dong Z. Inhibition of ultraviolet B-induced AP-1 activation by theaflavins from black tea. *Mol Carcinog* 28: 148–155, 2000.
47. Otsuka T, Ogo T, Eto T, Asano Y, Suganuma M, and Niho Y. Growth inhibition of leukemic cells by (−)-epigallocatechin gallate, the main constituent of green tea. *Life Sci* 63: 1397–1403, 1998.
48. Pan M-H, Lin-Shiau S-Y, Ho C-T, Lin J-H, and Lin J-K. Suppression of lipopolysaccharide-induced nuclear factor-κB activity by theaflavin-3,3′-digallate from black tea and other polyphenols through down-regulation of IκB kinase activity in macrophages. *Biochem Pharmacol* 59: 357–367, 2000.
49. Paquay JB, Haenen GR, Stender G, Wiseman SA, Tijburg LB, and Bast A. Protection against nitric oxide toxicity by tea. *J Agric Food Chem* 48: 5768–5772, 2000.
50. Peters U, Poole C, and Arab L. Does tea affect cardiovascular disease? A meta-analysis. *Am J Epidemiol* 154: 495–503, 2001.
51. Planas-Silva MD and Weinberg RA. The restriction point and control of cell proliferation. *Curr Opin Cell Biol* 9: 768–772, 1997.
52. Rice-Evans CA, Miller NJ, Bolwell PG, Bramley PM, and Pridham JB. The relative antioxidant activities of plant-derived polyphenolic flavonoids. *Free Radic Res* 22: 375–383, 1995.
53. Sachinidis A, Seul C, Seewald S, Ahn H-Y, Ko Y, and Vetter H. Green tea compounds inhibit tyrosine phosphorylation of PDGF β-receptor and transformation of A172 human glioblastoma. *FEBS Lett* 471: 51–55, 2000.
54. Sazuka M, Imazawa H, Shoji Y, Mita T, Hara Y, and Isemura M. Inhibition of collagenases from mouse lung carcinoma cells by green tea catechins and black tea theaflavins. *Biosci Biotechnol Biochem* 61: 1504–1506, 1997.
55. Shi X, Ye J, Leonard SS, Ding M, Vallyathan V, Castranova V, Rojanasakul Y, and Dong Z. Antioxidant properties of (−)-epicatechin-3-gallate and its inhibition of Cr(VI)-induced DNA damage and Cr(IV)- or TPA-stimulated NF-κB activation. *Mol Cell Biochem* 206: 125–132, 2000.
56. Suganuma M, Okabe S, Oniyama M, Tada Y, Ito H, and Fujiki H. Wide distribution of [3H](−)-epigallocatechin gallate, a cancer preventive tea polyphenol, in mouse tissue. *Carcinogenesis* 19: 1771–1778, 1998.
57. Tan X, Hu D, Li S, Han Y, Zhang Y, and Zhou D. Differences of four catechins in cell cycle arrest and induction of apoptosis in LoVo cells. *Cancer Lett* 158: 1–6, 2000.
58. Tijburg LB, Mattern T, Folts JD, Weisgerber UM, and Katan MB. Tea flavonoids and cardiovascular disease: a review. *Crit Rev Food Sci Nutr* 37: 771–785, 1997.
59. van het Hof KH, Kivits GA, Weststrate JA, and Tijburg LB. Bioavailability of catechins from tea: the effect of milk. *Eur J Clin Nutr* 52: 356–359, 1998.
60. van het Hof KH, Wiseman SA, Yang CS, and Tijburg LB. Plasma and lipoprotein levels of tea catechins following repeated tea consumption. *Proc Soc Exp Biol Med* 220: 203–209, 1999.
61. Webb T. Green tea experiments in lab, clinic yield mixed results. *J Natl Cancer Inst* 92: 1038–1039, 2000.
62. Wiseman SA, Balentine DA, and Frei B. Antioxidants in tea. *Crit Rev Food Sci Nutr* 37: 705–718, 1997.
63. Yang CS, Chen L, Lee MJ, Balentine D, Kuo MC, and Schantz SP. Blood and urine levels of tea catechins after ingestion of different amounts of green tea by human volunteers. *Cancer Epidemiol Biomarkers Prev* 7: 351–354, 1998.
64. Yang CS, Chung JY, Yang G, Chhabra SK, and Lee M-J. Tea and tea polyphenols in cancer prevention. *J Nutr* 130: 472S-478S, 2000.
65. Yang F, de Villiers WJ, McClain CJ, and Varilek GW. Green tea polyphenols block endotoxin-induced tumor necrosis factor-production and lethality in a murine model. *J Nutr* 128: 2334–2340, 1998.

Address reprint requests to:
Dr. Sheila A. Wiseman
Unilever Health Institute
Unilever Research
Olivier van Noortlaan 120
3133 AT Vlaardingen
The Netherlands

E-mail: sheila.wiseman@unilever.com

Received for publication December 8, 2000; accepted April 18, 2001.

This article has been cited by:

1. Maria Daglia. 2012. Polyphenols as antimicrobial agents. *Current Opinion in Biotechnology* **23**:2, 174-181. [CrossRef]
2. A. Imran, M. S. Butt, M. K. Sharif. 2011. PHYTOCHEMICAL DENSITY OF SOME PROMISING COMMERCIAL TEA BRANDS. *International Journal of Food Properties* **99999**:1, 1-1. [CrossRef]
3. Markus Schantz, Thomas Erk, Elke Richling. 2010. Metabolism of green tea catechins by the human small intestine. *Biotechnology Journal* **5**:10, 1050-1059. [CrossRef]
4. Daniele Del Rio, Luca Calani, Francesca Scazzina, Lucia Jechiu, Chiara Cordero, Furio Brighenti. 2010. Bioavailability of catechins from ready-to-drink tea. *Nutrition* **26**:5, 528-533. [CrossRef]
5. L. Lahiry, B. Saha, J. Chakraborty, A. Adhikary, S. Mohanty, D. M. S. Hossain, S. Banerjee, K. Das, G. Sa, T. Das. 2010. Theaflavins target Fas/caspase-8 and Akt/pBad pathways to induce apoptosis in p53-mutated human breast cancer cells. *Carcinogenesis* **31**:2, 259-268. [CrossRef]
6. Cheng Peng, Ho Yin Edwin Chan, Yuk Man Li, Yu Huang, Zhen Yu Chen. 2009. Black tea theaflavins extend the lifespan of fruit flies. *Experimental Gerontology* **44**:12, 773-783. [CrossRef]
7. Firas I. Kanaze, Aikaterini Termentzi, Chrysi Gabrieli, Ioannis Niopas, Manolis Georgarakis, Eugene Kokkalou. 2009. The phytochemical analysis and antioxidant activity assessment of orange peel (Citrus sinensis) cultivated in Greece-Crete indicates a new commercial source of hesperidin. *Biomedical Chromatography* **23**:3, 239-249. [CrossRef]
8. Marios Spanakis, Stamatis Kasmas, Ioannis Niopas. 2009. Simultaneous determination of the flavonoid aglycones diosmetin and hesperetin in human plasma and urine by a validated GC/MS method: in vivo metabolic reduction of diosmetin to hesperetin. *Biomedical Chromatography* **23**:2, 124-131. [CrossRef]
9. Demosthenes B. Panagiotakos, Christos Lionis, Akis Zeimbekis, Kyriaki Gelastopoulou, Natassa Papairakleous, Undurti N. Das, Evangelos Polychronopoulos. 2009. Long-Term Tea Intake is Associated with Reduced Prevalence of (Type 2) Diabetes Mellitus among Elderly People from Mediterranean Islands: MEDIS Epidemiological Study. *Yonsei Medical Journal* **50**:1, 31. [CrossRef]
10. Colin W Binns, Andy H Lee, Michelle L Fraser. 2008. Tea or coffee? A case study on evidence for dietary advice. *Public Health Nutrition* **11**:11. . [CrossRef]
11. Ting Sun, Fereidoon Shahidi, Chi-Tang HoBioavailability and Metabolism of Tea Catechins in Human Subjects **20083259**, 111-129. [CrossRef]
12. M. W. Laschke, C. Schwender, C. Scheuer, B. Vollmar, M. D. Menger. 2008. Epigallocatechin-3-gallate inhibits estrogen-induced activation of endometrial cells in vitro and causes regression of endometriotic lesions in vivo. *Human Reproduction* **23**:10, 2308-2318. [CrossRef]
13. Harvey Babich, Reena T. Gottesman, Emily J. Liebling, Alyssa G. Schuck. 2008. Theaflavin-3-Gallate and Theaflavin-3'-Gallate, Polyphenols in Black Tea with Prooxidant Properties. *Basic & Clinical Pharmacology & Toxicology* **103**:1, 66-74. [CrossRef]
14. Alyssa G. Schuck, Miriam B. Ausubel, Harriet L. Zuckerbraun, Harvey Babich. 2008. Theaflavin-3,3#-digallate, a component of black tea: An inducer of oxidative stress and apoptosis. *Toxicology in Vitro* **22**:3, 598-609. [CrossRef]
15. Evangelos Polychronopoulos, Akis Zeimbekis, Christina-Maria Kastorini, Natassa Papairakleous, Ioanna Vlachou, Vassiliki Bountziouka, Demosthenes B. Panagiotakos. 2008. Effects of black and green tea consumption on blood glucose levels in non-obese elderly men and women from Mediterranean Islands (MEDIS epidemiological study). *European Journal of Nutrition* **47**:1, 10-16. [CrossRef]
16. Amy R. Cameron, Siobhan Anton, Laura Melville, Nicola P. Houston, Saurabh Dayal, Gordon J. McDougall, Derek Stewart, Graham Rena. 2008. Black tea polyphenols mimic insulin/insulin-like growth factor-1 signalling to the longevity factor FOXO1a. *Aging Cell* **7**:1, 69-77. [CrossRef]

17. M. Singh, C. RamassamyBeneficial effects of phenolic compounds from fruit and vegetables in neurodegenerative diseases 145-181. [CrossRef]
18. Sharmila Basu-Modak, Dalia Ali, Matt Gordon, Tobias Polte, Anthie Yiakouvaki, Charareh Pourzand, Catherine Rice-Evans, Rex M. Tyrrell. 2006. Suppression of UVA-mediated release of labile iron by epicatechin—A link to lysosomal protection. *Free Radical Biology and Medicine* **41**:8, 1197-1204. [CrossRef]
19. Silvia Mandel, Tamar Amit, Lydia Reznichenko, Orly Weinreb, Moussa B. H. Youdim. 2006. Green tea catechins as brain-permeable, natural iron chelators-antioxidants for the treatment of neurodegenerative disorders. *Molecular Nutrition & Food Research* **50**:2, 229-234. [CrossRef]
20. R. Rodriguez-Proteau, J. E. Mata, C. L. Miranda, Y. Fan, J. J. Brown, D. R. Buhler. 2006. Plant polyphenols and multidrug resistance: Effects of dietary flavonoids on drug transporters in Caco-2 and MDCKII-MDR1 cell transport models. *Xenobiotica* **36**:1, 41-58. [CrossRef]
21. Hailin Zheng, Silvia Mandel, Tamar Amit, Moussa Youdim, Orly WeinrebThe Essentiality of Iron Chelation in Neuroprotection **20052039**, 277-299. [CrossRef]
22. Hong Liu, Xianliang Yang, Ren Tang, Jie Liu, Huibi Xu. 2005. Effect of scutellarin on nitric oxide production in early stages of neuron damage induced by hydrogen peroxide. *Pharmacological Research* **51**:3, 205-210. [CrossRef]
23. Silvia A. Mandel, Yael Avramovich-Tirosh, Lydia Reznichenko, Hailin Zheng, Orly Weinreb, Tamar Amit, Moussa B.H. Youdim. 2005. Multifunctional Activities of Green Tea Catechins in Neuroprotection. *Neurosignals* **14**:1-2, 46-60. [CrossRef]
24. Syed Ibrahim Rizvi, Mohd Abu Zaid, Rafat Anis, Neetu Mishra. 2005. Protective role of tea catechins against oxidation-induced damage of type 2 diabetic erythrocytes. *Clinical and Experimental Pharmacology and Physiology* **32**:1-2, 70-75. [CrossRef]
25. Feras Imad Kanaze, Melpomeni I. Bounartzi, Ioannis Niopas. 2004. A validated HPLC determination of the ?avone aglycone diosmetin in human plasma. *Biomedical Chromatography* **18**:10, 800-804. [CrossRef]
26. Min Zhang, Andy H. Lee, Colin W. Binns, Xing Xie. 2004. Green tea consumption enhances survival of epithelial ovarian cancer. *International Journal of Cancer* **112**:3, 465-469. [CrossRef]
27. Sidhartha Ray, Debasis BagchiRoles of Polyphenols, Flavonoids, and Oligomeric Proanthocyanidins in Cancer Chemoprevention **20041296**, . [CrossRef]
28. 2003. Trend of Most Cited Papers (2001-2002) in ARS. *Antioxidants & Redox Signaling* **5**:6, 813-815. [Citation] [Full Text PDF] [Full Text PDF with Links]
29. Jack N Losso. 2003. Targeting excessive angiogenesis with functional foods and nutraceuticals. *Trends in Food Science & Technology* **14**:11, 455-468. [CrossRef]
30. Ann M. Bode, Zigang Dong. 2003. Signal Transduction Pathways: Targets for Green and Black Tea Polyphenols. *Journal of Biochemistry and molecular biology* **36**:1, 66-77. [CrossRef]
31. L LEMARCHAND. 2002. Cancer preventive effects of flavonoids—a review. *Biomedecine & Pharmacotherapy* **56**:6, 296-301. [CrossRef]
32. Catherine A. Rice-Evans , Debasis Bagchi . 2001. Nutritional Proanthocyanidins, Flavonoids, and Related Phenols. *Antioxidants & Redox Signaling* **3**:6, 939-940. [Citation] [Full Text PDF] [Full Text PDF with Links]