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Review

The relationship between the structure and biological actions of green tea catechins

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ABSTRACT

Catechins and their gallate esters are a class of polyphenolic compounds. The catechin subclass known as flavan-3-ols have recently attracted much attention with regards to their beneficial effect on human health. Their biological actions are dependent on the structure of the compounds and vary according to cell type. They are best known as powerful antioxidants; however depending on the doses they also exhibit prooxidant effects. The anti- or prooxidant effects of green tea catechins have been implicated in the modulation of several cellular functions often associated with strong chemoprotective properties. This review summarises the benefit catechins to human health, the main molecular pathways modulated by catechins. The relationship between the structure and activity of the catechins needs to be studied further. In the future, the structure of catechins could be modified so as to synthesise novel compounds with more specific beneficial properties and higher bioavailability.

Contents

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1. Introduction

1.1. Catechins as biological active compounds

Natural bioactive compounds such as the polyphenols have recently attracted much attention with regards to their beneficial effect on human health. The name of the catechin family of chemicals is derived from *catechu* which refers to the juice or boiled extract of *Mimosa catechu* (*Acacia catechu* L.f) (Zheng, Ryu, Kwon, Lee, & Suk, 2008). Catechins and their gallate esters are a class of polyphenols that includes the subclass known as flavan-3-ols; the latter include (−)-epigallocatechin gallate (EGCG), (−)-epicatechin gallate (ECG), (−)-epigallocatechin (EGC), (+)-gallocatechin gallate (GCG), (−)-epicatechin (EC), (+) gallocatechin (GC) and (+)-catechin (C). A cup of green tea prepared with one gram of tea leaves in 100 ml of boiling water in a three minute brew usually contains 250–350 mg of dry materials that are comprised of 30–42% catechins and 3–6% caffeine. Among the catechins, EGCG is the major component derived from green tea (48–55%), followed by EGC (9–12%), ECG (9–12%) and EC (5–7%) (Balentine, Wiseman, & Bouwens, 1997; Shahidi, 2000). Among these EGCG is believed to be the most bioactive (Kim, Ham, Brakes, Ma, & Han, 2011; Velayutham, Babu, & Liu, 2008). Other sources of polyphenols are grapes and grape seeds, chocolate, apples, and berries (Shahidi, 2000; Shimizu, Shirakami, & Moriwaki, 2008).

Green tea is a popularly consumed beverage, widely consumed in Japan and China and its polyphenolic components have a chemopreventive effect. A typical brewed green tea (2 g of tea leaves in 200 ml of hot water) contains 500–700 mg of water extractable materials, of which 30–40% (by dry weight) are catechins. Phase I trial in 17 patients with advanced lung cancer showed that the maximum tolerated dose of green tea extract was 3 g/m2 per day (Liu, Xing, & Fei, 2008). No adverse effects have been reported in association with the medicinal use of green tea (Ahmad, Feyes, Nieminen, Agarwal, & Mukhtar, 1997; Khan, Afaq, Saleem, Ahmad, & Mukhtar, 2006; Liu et al., 2008). Most of results achieved in different studies used catechin concentrations that are far from physiologically achievable levels (0.6–1.8 μM) in both humans and animals through dietary means (Velayutham et al., 2008).

Catechins contain two or more aromatic rings (called the A- and B-rings), each bearing at least one aromatic hydroxyl connected with a carbon bridge and a dihydropyran heterocycle (the C-ring) with a hydroxyl group on carbon 3 (Fig. 1). The A ring is similar to a resorcinol moiety, while the B ring is similar to a catechol moiety. The C-ring of catechin has no double bond; consequently the molecule has two additional atoms of hydrogen. Catechin has two mirror image forms, a positive (+) form and a negative (−) form. The (+)-catechin is an antioxidant, whereas the (−)-catechin induces oxidation and cellular death in root cells of neighbouring plants. Recent methods developed for the stereoselective total synthesis of structurally related catechins could provide new sources of these compounds for research and biomedical use. Catechins are characterised by multiple hydroxyl groups on the A and B rings. EC is an epimer containing two hydroxyl groups at the 3′ and 4′ position of the B-ring and a hydroxyl group at the 3 position of the C-ring (Fig. 1). The only structural difference between EGC and EC is that EGC possesses an additional hydroxyl group at the 5′ position of the B-ring. ECG and EGCG are ester derivatives of EC and EGC respectively, derived through esterification with gallate at three hydroxyl position of the C-ring.

Catechins have been shown to be effective antioxidants in neutralizing free radicals, (Rice-Evans, Miller, Bolwell, Bramley, & Pridham, 1995; Zaveri, 2006; Zheng et al., 2008), and their relative hierarchy of effectiveness as radical scavengers is ECG > EGCG > EGC > EC > C (Intra & Kuo, 2007; Zaveri, 2006Rice-Evans et al., 1995). The metal-chelating properties of green tea catechins are also important contributors to their antioxidative activity (Zaveri, 2006). A number of natural phenolic compounds display antioxidant and cell protective effects in cell culture models, whereas in some studies they show prooxidant and cytotoxic effects. Several studies have reported the oxidative effects of catechins, suggesting that catechins had a dual function of antioxidant and prooxidant potentials depending on dose, duration of administration and the interaction with other dietary components. In other words the picture is complex: the cellular effects of catechins are dose- and cell-type dependent and are not exclusively dependant on their antioxidant and radical-scavenging activities *in vivo* (Zaveri, 2006).

1.2. Catechins-beneficial effects based on preclinical and clinical trials studies

The so-called "*French paradox*" is the fact that despite consuming much fatty food, life expectancy is not as reduced as might be expected. The paradox might be explained in part by the regular consumption of red wine. Catechins from grapes and red wine have been extensively studied in order to define their chemical composition and their effect on human health (Shammas et al., 2006). Several epidemiological studies and clinical trials showed that

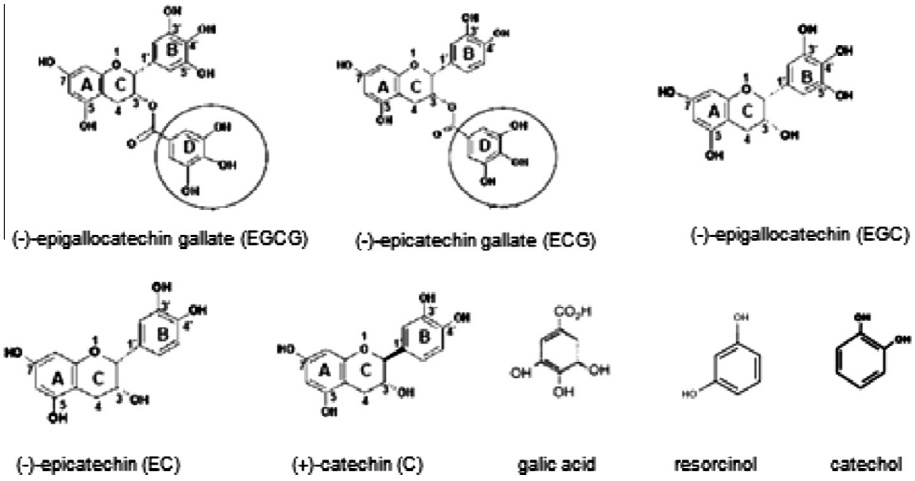

Fig. 1. Basic structure of catechins; the gallate moiety is circled. The A ring is similar to a resorcinol moiety while the B ring is similar to a catechol moiety and the C-ring is a dihydropyran heterocycle.

green tea may reduce the risk of many chronic diseases. This beneficial effect has been attributed to the presence of catechins, which are potent antioxidants at the appropriate dose (Chacko, Thambi, Kuttan, & Nishigaki, 2010). Oxidative stress has been linked to the development of various chronic diseases (Intra & Kuo, 2007). Numerous studies have shown that catechins are potent inhibitors of carcinogenesis in various cancer types, including models for cancers of the skin, lung, esophagus, stomach, liver, duodenum, small intestine, pancreas, colorectal, and mammary gland (Bode & Dong, 2009; Clark & You, 2006; Lu et al., 2006; Simons et al., 2009). In xenograft models, green tea catechins inhibited tumour growth and suppressed metastasis of metastasis-specific mouse mammary carcinoma 4T1 cells (Baliga, Meleth, & Katiyar, 2005) and reduced tumour blood vessel formation in estrogen receptor-negative breast cancer (Rodenberg & Brown, 2009). Recent evidence suggests that folate pathway inhibition may be one mechanism through which green tea protects against breast cancer in humans (Inoue et al., 2008; Rodenberg & Brown, 2009). A study conducted in southeastern China between 2004 and 2005, on female patients with histological confirmed breast cancer reveals that green tea consumption was associated with a reduced risk of breast cancer (Chen & Zhang, 2007; Zaveri, 2006; Zhang, Holman, Huang, & Xie, 2008). Catechins, however, produce health benefits in the context of diseases other than cancer. Research on the health promoting effects of catechins has provided evidence that they have beneficial effects on heart and liver diseases; that they cause a reduction of plasma oxidation stress; slow down aging processes and neurodegenerative processes; enhance weight loss; protect the skin from damage caused by ionising radiation; and they have also been described as preservatives against microbes (Mandel, Amit, Kalfon, Reznichenko, & Youdim, 2008; Nagle, Ferreira, & Zhou, 2006). They also possess anti-inflammatory, anti-hypertensive, anti-diabetic, anti-mutagenic, anti-bacterial and anti-viral effects. According to the free radical theory of aging, increased free radical generation and oxidative stress are the basis for phenotypic changes that lead to age-associated functional deterioration and neurodegeneration. Several age-associated diseases such as cancer, Parkinson's disease, Alzheimer's disease, cardiovascular diseases, and diabetes are linked to changes in oxidant/antioxidant balances and free radical damage (Shammas et al., 2006). Due to their antioxidant capacity, catechins may prevent these diseases or may improve the outcomes of the patient with such diseases (Zaveri, 2006).

As an anti-inflammatory and antioxidant agent, EGCG is considered a multimodal acting molecule, invoking various cellular neuroprotection mechanisms including iron-chelation, scavenging of oxygen and nitrogen radical species and activation of PKC signalling pathway and pro-survival genes (Weinreb, Amit, Mandel, & Youdim, 2009; Weinreb, Mandel, & Youdim, 2003). Recent studies have shown that misregulated iron metabolism may be a central pathological feature in Parkinson's disease and that the iron-chelating properties of EGCG are important for its protective effects in neurodegenerative diseases (Mandel, Maor, & Youdim, 2004).

Cardiovascular disease is multifactorial involving oxidative stress, abnormalities in lipid metabolism, disturbances in vascular tone, platelet aggregation, inflammation and proliferation of vascular cells. Catechins have been reported to beneficially impact the parameters associated with vascular dysfunction, including lipoprotein oxidation, blood platelet aggregation, vascular inflammation, vascular smooth muscle cell proliferation, altered lipid profile and vascular reactivity (Velayutham et al., 2008).

The effects of catechins on energy and fat metabolism have recently been examined in humans (Chen & Zhang, 2007; Nagao, Hase, & Tokimitsu, 2007). Catechins from green tea may lower blood pressure and thus reduce the risk of stroke and coronary heart disease (Chacko et al., 2010; Mandel et al., 2004). Chantre and Lairon (2002) reported that ingestion of 375 mg/d of catechins tended to decrease waist circumference in 70 subjects. Catechins led to a reduction in body fat, systolic blood pressure, and low-density lipoprotein cholesterol, suggesting that the ingestion of such an extract contributes to a decrease in obesity and cardiovascular disease risks (Nagao et al., 2007). Animal model studies suggested that green tea bioactive components might protect against the development of coronary heart disease by reducing blood glucose levels and body weight (Chacko et al., 2010). Before the use of green tea is widely adopted as health-promoting measure, it is necessary to understand the dose-related differences in the effects of EGCG in cancer versus neurodegenerative and cardiovascular diseases, as well as solving the discrepancy between doses used in *in vitro* studies and what is achievable in human plasma *in vivo* (Zaveri, 2006).

Some epidemiological and clinical studies have shown the health benefits of EGCG on obesity and diabetes (Chen & Zhang, 2007; Nagao et al., 2007; Rains, Agarwal, & Maki, 2011; Wolfram, Wang, & Thielecke, 2006). These mechanisms may be related to certain pathways, such as through the modulations of energy balance, endocrine systems, food intake, lipid and carbohydrate metabolism, and redox status (Chacko et al., 2010). The predominant hypothesis seems linked to the influences on sympathetic nervous system (SNS) activity, increasing energy expenditure and promoting the oxidation of fat or modifications in appetite, up-regulation of enzymes involved in hepatic fat oxidation, and decreased nutrient absorption (Rains et al., 2011). Green tea extract intake reduced these values in both Zucker rats, and rats fed a sucrose-rich diet. Green tea flavonoids were also shown to have insulin-like activities as well as insulin-enhancing activity. EGCG was found to inhibit intestinal glucose uptake by the sodium-dependent glucose transporter SGLT1, indicating its increase in controlling blood sugar (Tsuneki et al., 2004). Streptozotocin diabetic rats showed increased sensitivity to platelet aggregation and thrombosis, and this abnormality could be improved by dietary catechins. Catechins also reduced plasma triglyceride levels in normal rats (Chacko et al., 2010; Shahidi, 2000).

Experiments performed *in vitro* and in animal studies have begun to shed light on the underlying mechanisms that underpin the biological actions of green tea catechins (Yang & Wang, 2011; Yang, Wang, Lu, & Picinich, 2009). Several studies have demonstrated that non-physiologically high concentrations of catechins can interfere with many disease-related biochemical processes *in vitro* (Lim & Cha, 2011; Yang et al., 2009). However other reports show that at higher concentrations the effect of catechins on health can even be negative (Simons et al., 2009); and that furthermore, some catechin compounds are not readily absorbed (Mandel et al., 2008). As the human clinical evidence is still limited, future research needs to define the actual health benefits, establish the safe range of tea consumption associated with these benefits, and elucidate more precisely the mechanisms of action (Chacko et al., 2010).

The achievable tissue concentrations of these flavan-3-ols are in the low micromolar range, and therefore, beneficial effects observed with much higher concentrations *in vitro* may not be relevant *in vivo* (Bode & Dong, 2009). Flavan-3-ols have both direct and indirect effects. Numerous proteins that can directly bind with flavan-3-ols include the plasma, which may act as carrier proteins for EGCG (Bode & Dong, 2009; Sazuka, Isemura, & Isemura, 1998).

The many targets of flavan-3-ols that have been discovered and continue to be discovered are very likely dependent on the concentration of the polyphenol used and the specific cell, tissue, or organs (Shammas et al., 2006). Proteins that bind to flavan-3-ols in the lung, breast, colon, or skin might be very different from one another, and catechins very likely targets multiple proteins in each tissue (Bode & Dong, 2009; Dorai & Aggarwal, 2004); these differ-

ences explains the different results in different in *vitro* or *in vivo* systems.

1.3. Catechins exhibit both pro- and antioxidant activities

Much literature refers to the antioxidant activity of catechins as being one of their major health-promoting properties (Shahidi, 2000; Shimizu et al., 2008; Tachibana, 2009) by inducing important detoxifying enzymes (Chedea, Braicu, & Socaciu, 2010). This finding illustrates the point that the reactivity of catechins is modulated in cells through other molecules. Their stability can also be modulated *in vivo*: the stability of EGCG can be increased in Hat-29 colon adenocarcinoma cells due to stabilizing factors produced by the cells such as glutathione peroxidase and catalase (Hong et al., 2002).

Reactive oxygen species (ROS), as well as reactive nitrogen species (RNS) are key agents in the regulation of cell functions by acting as secondary messengers in intracellular signalling cascades. Moderate generation of ROS maybe selectively toxic to cancer cells and may even end up producing an antioxidant effect by activating the endogenous defence systems. Catechins are capable of both scavenging and generating free radicals and may exert their beneficial effects through a combination of both mechanisms (Valko et al., 2007). Catechins exhibit their antioxidant activity through scavenging ROS, chelating redox active transition- metal ions, inhibiting redox sensitive transcription factors, inhibiting pro-oxidant enzymes and inducing antioxidant enzymes (such as inducible nitric oxide synthase), induce the production of phase II detoxification enzymes (such as glutathione S-transferases) and antioxidant enzymes (such superoxide dismutases) (Youn et al., 2006).

The activities of redox-sensitive transcription factors NF-kB and activator protein-1 (AP-1), which play a critical role in responding to various oxidative stresses associated with pathogenesis, are modulated by this class of phytochemicals. NF-kB is activated by free radicals, inflammatory stimuli, cytokines, carcinogens, tumour promoters, endotoxins, γ-radiation, ultraviolet (UV) light, and X-rays (Aggarwal & Shishodia, 2006). Upon activation, NF-κB is translocated to the nucleus, where it induces the expression of target genes that have been shown to with different aspects of pathogenesis (Velayutham et al., 2008). Catechins are natural chemopreventive agents that have been found to be potent inhibitors of NF-κB. A number of studies have shown that catechins exert their anticancer effects through the suppression of NF-κB (Aggarwal & Shishodia, 2006; Velayutham et al., 2008).

The unique structure of these compounds contributes significantly to the beneficial health effects ascribed to them (Fig. 1) (Kanadzu, Yuquan, & Morimoto, 2006; Tachibana, 2009). The antioxidant properties are provided by the phenolic groups in their molecular structure. Literature suggests that there is a relationship between the percentage of pyrogallol and hydroxyl groups, scavenging super-oxide anion, whereas the galloyl moiety is responsible for quenching the hydroxyl radicals (Nanjo, Mori, Goto, & Hara, 1999). The antioxidative activity of catechins can be modified by the esterification of the hydroxyl group in position 3 by gallate acid. Esterification of the carboxylate group of gallic acid also decreases the antioxidant activity; this is potentially significant because the gallate acid residue reacts with lipids radicals and induces lipid peroxidation (Ostrowska & Skrzydlewska, 2006).

The antioxidative characteristics of these class of phenolic compounds are mainly ascribed to their free radical scavenging and metal chelating properties (Intra & Kuo, 2007), as well as their effects on antioxidant capacity against metal–ion-induced peroxidation than peroxyl–radical-induced peroxidation cell signalling pathways and on gene expression (Zaveri, 2006; Xia, Deng, Guo, Li, & & Li, 2010). The number of hydroxyls (-OH) groups and their position on the ring of the molecule determine the antioxidant capacity of flavonols (Intra & Kuo, 2007; Xia et al., 2010; Youn et al., 2006).

The prooxidant activity could also be due to the catechin–quinone redox system. Quinones, organic compounds containing carbonyl groups, are capable to produce new compounds through various coupling reactions that retain a number of hydroxyl groups. The simple o-quinones can act as prooxidants by forming reactive oxygen species through redox cycling. Quinones can generate also an antioxidant effect because they can be reduced to semiquinones by radicals and then back to diphenols; also the catechins o-quinones can react via phenolic coupling reactions to form dimers and oligomers, each retaining its original number of reactive hydroxyl groups, by enhancing its antioxidant capacity until a level is reached when the oligomers become insoluble and precipitate (Chedea et al., 2010). The formation of quinone metabolites is achieved through their scavenging with glutathione and identified as glutathione adducts. Quinone metabolites are also essential for some of the beneficial effects of catechins including the induction of gene transcription leading to increased expression of cancer protective enzymes, such as for example quinone reductase (Muzolf-Panek et al., 2008).

1.4. Catechins multiple uses in cancer therapy

The concept of cancer chemoprevention is to control the occurrence of cancer by slowing, blocking, or reversing the development of disease through the administration of naturally occurring or synthetic compounds. The chemopreventive proprieties of catechins were proved by interfering in the signal transduction pathways associated with cell death and survival. This class of flavan-3-ols may promote selective apoptosis and suppressing tumour growth by inhibiting angiogenesis or influencing the production of interleukins (Dorai & Aggarwal, 2004; Kanadzu et al., 2006; Shimizu et al., 2008; Tachibana, 2009).

The emergence and progression of cancer is a multistage process comprising: survival (gaining cellular immortality), uncontrolled proliferation, invasion at adjacent organs, metastasis (Zaveri, 2006). The identification of mechanisms involved in these interactions may contribute to the development of new methods to treat cancer (Rajamanickam & Agarwal, 2008). Studies in animal models have demonstrated that natural catechins can inhibit carcinogenesis at all stages (initiation, promotion and progression). This multifaceted inhibition of the tumourigenic process is attributed to a combination of antioxidative, antiproliferative and proapoptotic effects (Khan et al., 2006; Sutherland, Rahman, & Appleto, 2006; Zaveri, 2006). Catechins affect signal transduction pathways associated with cell death and cell survival (Zaveri, 2006). An interesting phenomenon that has been observed in different laboratories is the greater susceptibility of cancer cells to the inhibitory effect of catechins compared to normal and non-transformed cells. The anticancer effects of the various catechins in ovarian and prostate cancer cell lines varied with the type and stage of malignancy (Nihal, Ahmad, Mukhtar, & Wood, 2005; Ravindranath, Ramasamy, Moon, Ruiz, & Muthugounder, 2009). ECG is more effective than EGCG in suppressing the growth of these gender-specific carcinomas. In contrast, recent studies of human melanoma cell lines indicate that EGCG is more potent than other catechins, and that neither EGCG nor other catechins affect the growth of normal melanocytes (Nihal et al., 2005).

The flavan-3-ols and their metabolic products like gallic acid or pyrogallol can target several metabolic pathways of relevance to cancer. The difference between gallic acid and pyrogallol is only due to a carboxyl group. Carboxyl and methoxy groups of phenolic compounds seem to play an important role in selective cytotoxicity only in cancer cells, but not in normal cells. Galloil-containing mol-

ecules are known to work as tyrosine kinases receptors inhibitors (Kanadzu et al., 2006). The 5′(3′)-hydroxyl group in the B-ring and pyrogallol structure in a molecule is a minimum requirement for the induction of apoptosis by catechin compounds (Saeki, Hayakawa, Isemura, & Miyase, 2000; Yang, Ju, Lu, & Xiao, 2008).

The molecular mechanism of their antiproliferative effect may also involve the inhibition of prooxidant processes that causes tumour promotion. It is generally believed that the formation of ROS is a major catalyst of tumour promotion and progression that follow the initiation stages, including the metabolic activation of carcinogens (Ren, Qiao, Wang, Zhu, & Zhang, 2003). The harmful effects of oxidative processes in living organisms can be reduced by the dietary intake of flavan-3-ols through the inhibition of the prooxidant enzymes. These enzymes are activated by various tumour promoting agents, including mycotoxins, phorbol esters or arachidonate metabolising enzymes cyclooxigenase (COX) and lipoxygenase (LOX). To counteract these, catechins inhibit xanthine oxidase, COX and LOX (Chedea et al., 2010; Xia et al., 2010).

Cell differentiation involves a series of events in the development of a specialised cell having specific structural, functional, and biochemical properties – these events can be modulated by catechins. Adiponectin is an adipocyte-specific secretory hormone that can increase insulin sensitivity and promote adipocyte differentiation. Catechin stimulates adiponectin protein expression and secretion in adipocytes (Cho et al., 2007). Catechin promotes adipocyte differentiation and increased sensitivity to insulin in part by the direct activation of PPARγ, which could be at the basis of the observed pharmacological benefits of green tea intake in reducing the risk of type 2 diabetes (Shin et al., 2009). EGC has positive effects on bone metabolism through a double process of promoting osteoblastic activity and inhibiting osteoclast differentiation (Ko, Lau, Choy, & Leung, 2009). Other studies suggest that EGCG can exhibit anti-leukemic activity on a human eosinophilic cell line EoL-1 by suppressing the proliferation and by inducing the differentiation of the leukaemia cells (Lung, Wong, & Leung, 2002).

1.5. The use of catechins in therapy through the modulation of drug resistance

Catechins are potentially useful in therapy in two ways: by increasing the antitumoural effect of classical chemotherapeutics agents, or by reducing the doses required (Bode & Dong, 2009; Chacko et al., 2010). A recent paper shows that green tea catechins augment the antitumour activity of doxorubicin in mouse model of chemoresistant liver cancer. The chemosensitising effect of catechins may occur directly or indirectly through the reversal of multidrug resistance, involving the suppression of MDR1 expression; or via the enhancement of intracellular DOX accumulation involving the inhibition of the P-glycoprotein efflux pump function (Liang et al., 2010). EGCG and EGC were tested on doxorubicin-resistant murine sarcoma (S180-dox) and human colon carcinoma (SW620-dox) cell lines. Both substances showed a sensitising effect on the cell lines after the treatment with doxorubicin. These results suggest that protein kinase C may be inhibited by EGCG and EGC, leading to a reduced expression of drug resistance related proteins (Stammler & Volm, 1997). Tamoxifen (TAM) is a nonsteroidal triphenylethylene antiestrogenic drug widely used in the treatment and prevention of breast cancer. TAM causes a collapse of the mitochondrial membrane potential. The inhibitory effect of catechin on TAM-induced oxidative damage suggests that it may have potential benefits in prevention of human diseases where reactive oxygen species have a role as causative agents (Tabassum, Parvez, Rehman, Banerjee, & Raisuddin, 2007). Another study describes the interaction of green tea with the estrogen receptors pathways providing new mechanistic evidence that the combination of green tea and tamoxifen might be more potent than either agent alone in suppressing breast cancer growth. These results may lead to future improvements in breast cancer treatment and prevention (Sartippour et al., 2006).

2. Mechanisms of action of catechins in cancer

As outlined in this review, tea components may act at multiple molecular levels in cancer chemoprevention and chemotherapy (Fig. 2). In general, catechins induce the inhibition of tumour cell proliferation by affecting molecular pathways associated with apoptosis, cell cycle, angiogenesis, invasion, and growth factor-related proliferation (summarised in Fig. 2) (Sutherland et al., 2006; Zaveri, 2006). The mechanisms that underpin the beneficial effects of flavan-3-ols include: antioxidative, anti-inflammatory, antiproliferative, antithrombotic, and anti-angiogenic properties. Flavan-3-ols they are able to modulate the expression of proteins involved in detoxification, antioxidant enzymes, protein kinases, different growth factors or transcription factors and adhesions molecules.

2.1. Apoptosis and cell cycle

Apoptosis is the process of programmed cell death induced by extracellular stimuli or internal signalling pathways and play an important role in eliminating damaged or otherwise unwanted cells (Burz, Berindan-Neagoe, Balacescu, & Irimie, 2009). Apoptosis, in recent years, has attracted much attention in biomedical research (Béliveau & Gingras, 2007; Khan et al., 2006; Tachibana, 2009). Catechins have been shown to modulate apoptosis at various points in the process, by altering the expression of anti- and proapoptotic genes (Agarwal, Sharma, Zhao, & Agarwal, 2006; Chen & Zhang, 2007; Liu et al., 2008; Sutherland et al., 2006).

Catechins may have dual effects on apotptosis depending on the doses used. Low concentrations (1 μM) induce immediate expression of antiapoptotic bcl-xL and/or bcl-2 mRNAs whereas Bax expression is reduced (Béliveau & Gingras, 2007; D'Archivio et al., 2008; Liu et al., 2008; Yamauchi, Sasaki, & Yoshida, 2009). In contrast a proapoptotic pattern of gene expression was observed at high concentrations (50 μm) of the antioxidants. This pattern included the upregulation of caspases-3 and 10, Fas and Fas ligand, NF-κB p105 subunit, and p53 (Weinreb et al., 2003).

A perturbation in the cell cycle may also account for the anticarcinogenic effect of catechins. In an *in vitro* experiment on cervical cancer cells, cell cycle arrest was observed at the G1 phase with a concentration of 35 μM of EGCG triggering apoptosis; whereas exposure to 100 μM undergo apoptosis (Ahn et al., 2003). The inhibition of cyclin-dependent kinases (cdks) and the induction of cdk inhibitors p21 and p27 was also observed in breast and prostate cancer cells by using 75 μg/ml of the polyphenolic fraction isolated from grape seeds-treated cells (Agarwal et al., 2006).

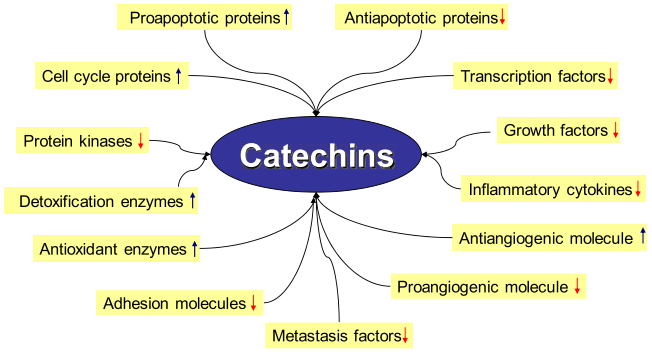

Fig. 2. Molecular target of catechins.

2.2. Inhibition of angiogenesis

Cancer cells induce an imbalance of pro- and anti-angiogenic factors, causing endothelial cell recruitment and proliferation (Carlson, Bauer, Vincent, Limburg, & Wilson, 2007). The effects of catechins were tested on *in vitro* models of angiogenesis, namely, growth, migration and neoangiogenesis in human umbilical vein endothelial cells at concentrations ranging from 1.56–100 μM (Kondo, Ohta, Igura, Hara, & Kaji, 2002).

Recent studies indicate that the receptor tyrosine kinases (RTKs) are one of the critical targets of EGCG to inhibit cancer cell growth. EGCG inhibits the activation of RTKs, including VEGF receptors, IGF-1, EGFR (erbB1), HER2 (neu/erbB2) and also HER3 (neu/erbB3), in various types of human cancer cells. EGCG alters membrane lipid organisation and thus inhibits the dimerization and activation of EGFR (Lim & Cha, 2011). Targeting RTKs and related signalling pathway with green tea catechins might prove to be a promising strategy for the prevention of human cancers (Kondo et al., 2002; Shimizu et al., 2008).

EGCG can suppress oxidant-induced production of the pro-angiogenic cytokine interleukin 8 (IL-8) and Il-12 (Carlson et al., 2007). EGCG inhibits the binding of vascular endothelial growth factor (VEGF), a major angiogenesis inducing factor, to endothelial cells. VEGF-induced stimulation of the tyrosine phosphorylation of VEGFR-2 in endothelial cells is inhibited in a dose and time dependent manner by green tea catechins (Lamy, Gingras, & Béliveau, 2002). EGCG can reduce the binding of VEGF to its receptors and thus affects downstream signalling (Kondo et al., 2002). It was reported that VEGF -induced vessel formation is inhibited by anti-VE-cadherin antibody in a dose-dependent manner by green tea catechins. The inhibition of tube formation by EGCG is in part mediated through suppression of VE-cadherin tyrosine phosphorylation and inhibition of Akt activation during VEGF-induced tube formation. Thus VE-cadherin and Akt, proteins known to be downstream of VEGFR-2-mediated cell signalling cascade, are the proteins through which green tea catechins can inhibit angiogenesis (Tang, Nguyen, & Meydani, 2003).

2.3. Anti-inflammatory effects of catechins

Flavan-3-ols are reported to possess anti-inflammatory properties *in vitro* and *in vivo*. Because of these properties, it is thought that EGCG may have therapeutic benefit in numerous inflammatory diseases such as atherosclerosis and arthritis (Cavet, Harrington, Vollmer, Ward, & Zhang, 2011). Several molecular targets have been recently identified from the *in vitro* cell culture experiments and *in vivo* animal studies together with the human intervention trials (Rimbach, Melchin, Moehring, & Wagner, 2009; Velayutham et al., 2008). These effects are induced by the suppression of several inflammatory factors including adhesion molecules, MAPKs pathways (mitogen activated protein kinases) (Agarwal et al., 2006), or nuclear factor-kappa B (NF-κB), a multipotential promoter of inducible nitric oxide synthase (iNOS). The redox-sensitive transcription factors NF-kB and activator protein-1 (AP-1) play a critical role in mediating various oxidative stress-regulated vascular inflammation. NF-kB activation plays a major role in the expression of pro-inflammatory molecules, including cytokines, chemokines and adhesion molecules (Velayutham et al., 2008). Catechins may alter the Th1/Th2 cytokine balance which regulates the inflammatory network; an increased Th1/Th2 ratio indicates an inflammatory process (Junkun, Selman, Swiercz, & Skrzypczak-Jankun, 1997; Suzuki, Ogawa, Sagesaka, & Isobe, 2006).

Flavan-3-ols may inhibit lipid peroxidation and the inflammatory mediators cyclo-oxygenase (COX)-1 and -2 (Pandey & Rizvi, 2009; Seeram, Cichewicz, Chandra, & Nair, 2003). Other anti-inflammatory effects as well as increasing HDL (high density lipoproteins), or improving endothelial function are observed in cardiovascular diseases by stabilization of the atheroma plaque (Pandey & Rizvi, 2009).

2.4. Catechins and alternative splicing

Alternative pre-mRNA splicing is an important aspect of eukaryotic gene expression, as most of the protein-coding genes use this process to generate multiple protein isoforms from a single gene. An increasing number of human diseases are now recognised to be caused by the selection of 'wrong' alternative exons (Sumanasekera, Watt, & Stamm, 2008).

The loss of survival motor neuron-1 gene (*SMN1*) is responsible for the development of the neurodegenerative disorder spinal muscular atrophy (SMA). A nearly identical copy of *SMN1* is present on the same chromosomal region called *SMN2*. While *SMN2* encodes a normal SMN protein, the majority of *SMN2*-derived transcripts are alternatively spliced, resulting in a truncated protein that lacks the 16 amino acids encoded by SMN exon 7. Numerous studies have shown that the *SMN2*-derived protein product, called SMN7, is unstable and dysfunctional. Therefore, identifying molecules that stimulate full-length SMN expression from the *SMN2* gene could lead to the development of effective therapies for a broad range of SMA patient populations (Sakla & Lorson, 2008). Similarly, the most prevalent causative mutation of familiar dysautonomia (FD) is a T to C transition at the donor splice site of intron 20 that results in the exclusion of exon 20 (Anderson, Qiu, & Rubin, 2003). Mutations in cis-acting splicing elements or changes in the activity of regulatory proteins that compromise the accuracy of either constitutive or alternative splicing could have a profound impact on human pathogenesis, in particular in tumour development and progression. Mutations in splicing elements, for example, have been found in genes such as *LKB1, KIT, CDH17, KLF6* and *BRCA1*, and changes in trans-acting regulators can affect the expression of genes such as *Ron, RAC1* and *CD44* (Srebrow & Kornblihtt, 2006).

Research during the last few years has identified a number of low-molecular-mass chemical substances that can change alternative exon usage. Most of these substances act by either blocking histone deacetylases or by interfering with the phosphorylation of splicing factors. How the remaining large number of these substances affect alternative splicing is not yet fully understood. The emergence of these low-molecular-mass substances provides not only probes for studying alternative pre-mRNA splicing, but also opens the door to the possible harnessing of these compounds as drugs to control diseases caused by the selection of 'wrong' splice sites.

Polyphenolic compounds have now been linked to the modulation of alternative splicing. EGCG was found to correct aberrant pre-mRNA splicing in FD and increase full-length RNA and protein levels (Ahmed et al., 2008; Sumanasekera et al., 2008). Sakla and Lorson (2008) reported that EGCG had effect in increasing SMN protein expression and increasing exon 7 inclusion from SMN2 mRNA in SMA type I patient fibroblast cells. EGCG enhances the synthesis of soluble gp130 protein (sgp130), an endogenous inhibitor of IL-6 signalling and transsignalling, by inducing alternative splicing in gp130 gene, resulting in enhanced sgp130 synthesis (Ahmed et al., 2008). The mechanism of action of EGCG in the context of alternative splicing appears to involve the down-regulation of hnRNP A2/B1, an abundant splice factor that regulates the alternative splicing of several genes.

3. Conclusion

In spite of the strong evidence for the chemopreventive activity of natural catechins in cell culture and animal models, such an

activity has not been consistently observed in studies on patients. Experimental investigations and epidemiological studies on the chemoprotective activities of catechins belong to a field of research which produces numerous controversial reports. It is still unclear whether or not the effects on molecular endpoints in signal transduction pathways are downstream of the modulation of pro-oxidant/antioxidant balance in cells; or instead due to the direct action of EGCG and other catechins directly on molecular targets. Thus, the relevance of the various mechanisms of action of catechins to the prevention of cancer and other diseases in humans is a question that has not yet been fully addressed. Furthermore, most of the putative molecular mechanisms that have been proposed are based on *in vitro* studies at concentrations far in excess of those achievable *in vivo*. Catechins are relatively unstable and are likely to be quantitatively and qualitatively modified during the time frame of the experiments.

Despite these caveats and challenges, which typically apply to naturally derived products, EGCG and other natural catechins and their derivatives do have a clear potential to be developed as new drugs. The rational design of catechin analogues will be based on the deciphering the relationship between the structure and activity of the various bioactive groups of catechins. Longer term the structure of catechins can be modified so as to synthesise novel compounds which exhibit a more disease-specific function and higher bioavailability and therefore greater effectiveness *in vivo*.

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