Review

Molecular targets of tea polyphenols in the cardiovascular system

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Abstract

Tea-derived polyphenols have attracted considerable attention in the prevention of cancer and cardiovascular diseases. In comparison to tumour cells, the elucidation of their molecular targets in cardiovascular relevant cells is still at the beginning. Although promising experimental and clinical data demonstrate protective effects for the cardiovascular system, little information is actually available on how these beneficial effects of tea polyphenols are mediated at the cellular level. By affecting the activity of receptor and signal transduction kinases, both catechins and theaflavins – the major ingredients of green and black tea, respectively – exert a variety of cardiovascular beneficial effects. In general, the number and positions of galloyl groups have major influence on the potency of polyphenols. Compared to their broad impact on cellular signal transduction, tea polyphenols reveal little transcriptional effects. However, more detailed and profound analysis of molecular actions in different cells of the cardiovascular system is necessary before safe clinical use of tea polyphenols for treatment of cardiovascular diseases will become possible.

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1. Introduction

Nutritional polyphenols are increasingly regarded as promising substances in disease prevention, as summarised in a number of recent reviews [e.g., 1,2]. These natural compounds possess diverse biological effects [3–5]. However, their molecular targets are generally less well understood.

Since tea represents, after water, the most widely consumed beverage in the world, and tea-derived polyphenols are extensively studied as anticancer and cardiovascular protective compounds, this review focuses on polyphenolic substances from tea. Compelling epidemiological, clinical and experimental evidence suggests that polyphenolic compounds contained in green and black tea are associated with beneficial effects in prevention of cardiovascular diseases, particularly of atherosclerosis and coronary heart disease. Although – compared to anticancer effects – the underlying mechanisms of action by which tea ingredients exert their beneficial cardiovascular effects are still sparsely known, an increasing number of publications have shed light on the molecular targets of tea ingredients in cardiovascular cells. Several mechanisms have been implicated, such as antioxidant, antiproliferative, anti-inflammatory, and antithrombogenic properties, as well as favourable effects on endothelial function. Evidence is accumulating that catechins, and to a lesser extent theaflavins – the main polyphenolic compounds of green and black tea, respectively – are the substances responsible for the physiological effects of tea. This review focuses on molecular targets of naturally-occurring compounds of tea in the cardiovascular system, and attempts to correlate their functional effects to molecular signal transduction pathways.
2. Tea ingredients

Tea is made from the leaves of *Camellia sinensis* and prepared mainly in two different modes: green tea by gradually drying the leaves and black tea by fermentation, during which some tea compounds are converted to more complex structures. Both teas are rich in polyphenols but differ in composition and types of these compounds. Tea contains large amounts of various flavonoids, which are characterized as containing two or more aromatic rings, each bearing at least one aromatic hydroxyl connected with a carbon bridge (for chemical structures see Fig. 1). The major flavonoids are catechins, which include epicatechin (EC), epigallocatechin (EGC), epicatechin-3-gallate (ECG), and epigallocatechin-3-gallate (EGCG) [6]. The catechins – of which EGCG is the most potent physiologically active compound – predominantly account for the biological effects of green tea. During the fermentation process to produce black tea, the catechins are oxidized by polyphenol oxidase to higher molecular components, the theaflavins [7]. The group of theaflavines includes theaflavin (TF1), theaflavin-3-monogallate (TF2A), theaflavin-3′-monogallate (TF2B), and theaflavin-3,3′-digallate (TF3). In addition to theaflavins, the catechins are converted to another group of compounds, the thearubigins [8]. Both green and black teas contain numerous additional constituents: e.g., caffeine, gallic acid, theobromine, theogallin, as well as rutin, quercetin, kaempferol, and several other polyphenols [9,10], which are all less characterized in terms of their biological effects.

3. Molecular mechanisms of antioxidant effects

Current models of the pathophysiological mechanisms that cause atherosclerosis and myocardial ischaemia reperfusion injury involve the generation of supraphysiological levels of reactive oxygen species (ROS). These partially reduced metabolites – e.g., superoxide anions and hydroxyl radicals – cause cellular oxidative stress and damage proteins, DNA, and lipids [11]. Accordingly, the beneficial cardiovascular effects of tea ingredients have been mainly attributed to their potent antioxidant properties.

Due to the number and arrangement of their phenolic hydroxyl groups catechins and theaflavins are excellent electron donors and efficient scavengers of free radicals such as superoxide anions, singlet oxygen, nitric oxide, and peroxyxinitrite [12,13]. In an ischaemia reperfusion injury model of the gastrocnemius muscle, these data were confirmed in vivo by Büttemeyer et al., who demonstrated a significant decrease in singlet oxygen levels by simultaneous intravenous treatment with 4 mg/kg EGCG [14]. Leung et al. found theaflavins and catechins to be equally effective in preventing

![Fig. 1. Chemical structures of catechins and theaflavins indicating the numbers and positions of the galloyl groups in the compounds.](image-url)
It should be noted that the antioxidant potency of the polyphenols was in the following order: TF3 > ECG > EGCG ≥ TF2B ≥ TF2A > TF1 ≥ EC > EGC [15]. Consistently, Lee et al. reported a decrease in oxidized LDL plasma levels after 4 weeks of green tea consumption (600 ml/d) in smokers [16]. Although their direct antioxidant capacity is much higher, it should be noted that – after oral consumption – plasma levels of tea polyphenols remain 100 to 1000 times lower than those of other physiological antioxidants: e.g., ascorbate and glutathione [17]. This finding possibly explains conflicting data on the effect of oral consumption of tea polyphenols on total plasma antioxidant capacity [18,19].

In contrast to the evidence of antioxidant properties of tea polyphenols, a more recent report suggests that EGCG itself may contribute to the formation of ROS by spontaneous generation of hydrogen peroxide upon dissolution [20]. Since, however, repetitive induction of moderate oxidative stress is known to induce cellular resistance to subsequent exposure to high concentrations of ROS, this phenomenon could eventually contribute to the beneficial effects of EGCG. In addition to their ability to directly scavenge free radicals, tea polyphenols also act as metal chelators [21,22], thereby protecting cells against metal ion-induced oxidative stress.

Numerous studies in cancer cells suggest a modulation of redox-sensitive transcription factors such as nuclear factor kappa B (NFκB) and activator protein-1 (AP-1) by tea polyphenols [23]. However, data on primary cells of the cardiovascular system are rather limited. Anjaj et al. observed an attenuation of myocardial ischaemia reperfusion injury after treatment with EGCG. Intravenous application of 10 mg/kg EGCG during reperfusion significantly decreased IkB kinase activity resulting in reduction of IkBα degradation and NFκB activity. Moreover, EGCG treatment diminished phosphorylation of c-Jun and, consequently, AP-1 activity [24]. These results were confirmed by Suzuki et al. who reported a decreased activity of NFκB in murine cardiac transplants after oral administration of green tea polyphenols (20 mg/kg/d) for 60 d [25]. Whereas the effect of theaflavins on NFκB activity has not until now been studied on primary cardiovascular cells, data from macrophages indicate an inhibition of NFκB by TF3 [26]. Another potential mechanism of protection against myocardial ischaemia reperfusion injury was suggested by Townsend et al., who reported a reduction of STAT-1 activation and Fas receptor expression by EGCG and green tea extracts [27].

The ability of tea polyphenols to inhibit ROS-generating enzymes may also contribute to their beneficial antioxidant effects. Catechins as well as theaflavins suppress the expression of inducible nitric oxide synthase (iNOS) which is responsible for the production of large quantities of nitric oxide upon stimulation of inflammatory cells by bacterial endotoxins or cytokins [13,26,28]. Another physiological source of ROS is the enzymatic oxidation of hypoxanthine and xanthine to uric acid. The reaction is catalyzed by xanthine oxidase and has been found to be inhibited by catechins [29] and theaflavins [30]. Both lipoxygenases and cyclooxygenases are able to oxidize proteins other than their regular substrates, thereby aggravating oxidative damage. Inhibition of both types of oxygenases by catechins and theaflavins, as observed in human colon mucosa by Hong et al., may complement the antioxidant capacity of tea polyphenols [31].

Several studies have found that catechins are able to induce enzymes that play important roles in cellular antioxidant defence mechanisms. Negishi et al. reported an induction of catalase in aortas of spontaneously hypertensive rats after oral consumption of green tea polyphenols for 2 weeks [32]. More recently, Wu et al. presented evidence for a marked induction of heme oxygenase-1 via activation of Akt and Nrf2 by EGCG in endothelial cells, resulting in significant protection against hydrogen peroxide-mediated oxidative stress [33].

Taken together, there is considerable evidence for direct and indirect antioxidant effects of both green and black tea polyphenols in the cardiovascular system. However, most in vitro studies employed much higher concentrations than those found in vivo after oral consumption. Whereas this aspect provides a possible explanation for conflicting results of in vivo studies, it also raises questions on the biological relevance of effects observed in vitro.

### 4. Molecular basis of antiproliferative effects of tea ingredients

Proliferation and migration of vascular smooth muscle cells (SMC) are key events in the development and progression of atherosclerosis, restenosis after interventional vascular procedures, and transplant vasculopathy [34,35]. A number of studies have reported that tea catechins are capable of inhibiting SMC proliferation and migration in vitro and in vivo [36–38]. Among the catechins, EGC, EC, and ECG are apparently more effective in preventing proliferation than catechin and epicatechin, which suggests that the galloyl group is essential for full inhibitory activity. EGCG (20–80 μg/ml) inhibited vascular SMC proliferation and arrested the cells in the G1 phase [38,39]. PCNA, a marker of cell growth, was also inhibited by EGCG [38]. Kim and Moon reported that the EGCG-induced vascular SMC arrest is attributed to the inhibition of cyclin D1/CDK4 and cyclin E/CDK2 complexes that control G1 to S cell cycle progression. In addition, the authors suggested that increased expression of the CDK inhibitor p21(WAF1) may be part of the mechanisms by which EGCG induces cell-cycle arrest in the late G1 phase and inhibits cell proliferation in vascular SMC [39]. Similarly, Hofmann and Sonnenschein reported growth arrest in aortic vascular SMC with EGCG at a dose range of 40–50 μg/ml. Higher doses of EGCG (80 μg/ml), however, resulted in apoptosis, mediated via the induction of p53 and downstream target p21(CIP1) CK1, as well as activation of the transcription factor NFκB [40]. In contrast to these data, a recent study by Han et al. described that EGCG lowered the expression of...
NFκB/p65 nuclear and cytosolic protein in rat aortic SMC. These divergent effects of EGCG on NFκB are unclear, and may be possibly explained by different applied doses [41].

Other mechanisms for the antiproliferative effects of tea components involve interactions with growth factors linked to proliferation and migration of vascular SMCs. Sachinidis et al. have demonstrated that tea catechins interfere with platelet-derived growth factor (PDGF)-induced mitogenic pathways [42]. It has been reported that catechins with a galloyl group in the 3-position of the catechin structure, selectively suppress PDGF-BB-induced stimulation of the PDGF-Rβ-mediated signal transduction pathway in vascular SMC by inhibiting tyrosine phosphorylation and its downstream signalling targets, extracellular signal-regulated kinase (ERK1/2) at 20–50 μM [40,42,43]. In addition, PDGF-induced mRNA expression of c-fos and egr-1 was completely inhibited in EGCG-treated vascular SMCs [43].

The underlying molecular mechanism for the inhibitory effects of catechins involves incorporation of EGCG into various cellular compartments, including cell surface membranes. This leads to binding of PDGF to non-receptor binding sites, resulting in reduced PDGF binding to the respective receptors [44]. Thus, the non-competitive inhibition of PDGF-induced mitogenesis by EGCG, incorporated into cell membranes, would result in a sustained inhibition of cell proliferation, even at low, physiological concentrations.

Whereas there is some evidence in tumour cells that TF3 is more effective than EGCG in inhibiting PDGF-induced phosphorylation of the PDGF-receptor, no experimental data have until now become available for cells of cardiovascular relevance [45].

EGCG has also been shown to interact with basic fibroblast growth factor (bFGF), which plays a role in cell proliferation and migration, and has been linked to the pathogenesis of atherosclerosis [46]. In rat aortic SMCs, EGCG at a concentration of 30 μM inhibited the proliferative response stimulated by fetal bovine serum and also prevented the migration of bFGF-stimulated rat vascular SMCs — effects apparently mediated partly through the MAP kinase signalling pathway [47]. Indeed, EGCG markedly inhibited Ras activation and c-Jun N-terminal kinase (JNK) activity (but not expression levels) in rat vascular SMC, without affecting protein kinase C expression. In addition, induction of c-Jun mRNA stimulated by bFGF was significantly dose-dependently reduced by EGCG. The authors suggested that the antiproliferative effects of EGCG in SMC are partly Ras/JNK-mediated [47].

Potential mechanisms for the antiproliferative effects exhibited by EGCG also include its ability to inhibit redox-sensitive signal-transduction pathways. EGCG significantly inhibited the nuclear translocation of c-Jun and AP-1 binding activity and reduced expression of iNOS [38,48]. Correspondingly, Lu et al. have shown that the inhibition of serum-stimulated cell proliferation of vascular SMC by EGC (10–100 μM) is due to suppression of protein tyrosine kinase activity, reducing c-Jun activity [37].

Another mechanism by which tea components may exert antiproliferative effects is their interaction with the matrix metalloproteinase (MMP) system. These MMPs play relevant roles in atherosclerosis and restenosis by promoting smooth muscle cell migration, proliferation, and neointima formation after vascular injury. Recently, Cheng et al. have demonstrated the ability of green tea catechins to inhibit neointima hyperplasia in a rat carotid arterial injury model by reducing MMP-2 gelatinolytic activity in the injured vessels [49]. In vascular SMC and endothelial cells, EGCG reduced the gelatinolytic activity of MMP-2 at physiological concentrations: EGCG inhibited MMP-2 — which is constitutively present in the arterial wall and further upregulated after vascular injury — via modulation of membrane type-1-MMP-dependent pro-MMP activation and by direct inhibition of MMP-2 and MT1-MMP [49–52]. In addition, EGCG also significantly stimulated the production of tissue inhibitor of MMP-2 (TIMP-2) protein in VSMC and balloon-injured carotid arteries in a concentration-dependent manner, but had no effect on TIMP-1 [52,53]. Catechins have also been shown to exert an effect on MMP-9 gelatinolytic potential by reducing MMP-9 release from macrophages [54]. This catechin-induced reduction in MMP-9 secretion correlated with decreased MMP-9 promoter activity and mRNA levels. Kim and Moon reported that TNFα induced MMP-9 expression was abolished by EGCG (20–80 μg/ml) in vascular SMCs via inhibition of NFκB and AP-1 binding activities [39].

Taken together, the molecular mechanisms of antiproliferative and anti-inflammatory effects of tea catechins involve induction of cell cycle arrest by inhibition of CDKs and increased expression of the CDK inhibitor p21^WAF1, inhibition of PDGF-Rβ, and bFGF-mediated signal transduction — primarily by blocking of tyrosine kinase activity, prevention of Ras/JNK activation, as well as inhibition of MMP-2 and -9 activities. In contrast to these data on molecular targets of catechins in cardiovascular cells, no studies are yet available for antiproliferative or anti-inflammatory effects of theaflavins in the cardiovascular system.

5. Molecular mechanisms of anti-inflammatory effects of tea ingredients

It has been widely shown for cancer cells that tea flavonoids have the capacity to modulate the immune response and exert putative anti-inflammatory activity. In contrast, only limited data are available regarding anti-inflammatory effects of tea catechins in cardiovascular cells. Chronic inflammation plays a key role in atherogenesis: inflammation of the vessel wall, activation of the vascular endothelium, increased adhesion of mononuclear cells to the injured endothelial layer, and their subsequent extravasation into the vessel wall, are initial events in this process [55].

Catechins inhibit neutrophil adhesion and migration through endothelial monolayers by several mechanisms: Hofbauer et al. and Takano et al. reported direct action of
EGCG on neutrophils, including the suppression of chemokine production at the inflammatory site [56,57]. In addition, evidence exists that EGCG modulates adhesion-molecule expression: we have recently shown that EGCG – and to a lesser extent ECG – selectively prevented cytokine-induced VCAM-1 expression in endothelial cells and reduced monocyte adhesion to the endothelial monolayer independently of NFκB activation [58]. In addition, EGCG treatment significantly decreased the expression of CD11b on both monocytes and granulocytes [59].

Monocytes play an important role in initiation, development, and outcome of the immune response in atherogenesis. EGCG and ECG strongly induced apoptosis of monocytes by dose-dependent activation of caspase 8 and 9, and further downstream caspase 3, whereas EC and EGC had no effect [60]. Because only the former two isomers possess a galloyl group, this group was assumed to play an important role in apoptosis induction.

Another suggested mechanism for the anti-inflammatory properties of tea polyphenols is associated with inhibition of the activity of the transcription factor NFκB. It has been reported that both EGCG and TF3 reduced LPS-induced TNFα production in macrophages by blocking NFκB activation via inhibition of IkB kinase activity [61,62]. EGCG also prevented LPS-induced IL-12p40 production in murine macrophages by inhibiting p38 MAPK, while activating ERK1/2, leading to prevention of IkBα degradation and NFκB activation [63]. Lin et al. demonstrated that EGCG (5 μM) as well as TF3 inhibited iNOS expression by blocking NFκB activation [26,28].

In summary, tea polyphenols, primarily EGCG, exert important anti-inflammatory activities partly related to prevention of NFκB activation, with subsequent reduction of the expression of target genes indispensable to the inflammatory process.

6. Molecular actions of tea ingredients on endothelial function

Many pathophysiological conditions in the cardiovascular system are characterized by attenuated production of protective vasoactive substances and deregulated gene expression in endothelial cells, known as endothelial dysfunction. The major vasoprotective molecule produced by endothelial cells is nitric oxide (NO). In addition to relaxation of blood vessels, it contributes to a plethora of anti-atherogenic effects. All four tea catechins (30–500 μM) evoke vasorelaxation in precontracted rat mesenteric arteries, with EGCG being the most potent [64]. Consumption of tea, furthermore, mediates flow-mediated dilation in humans [65]. The molecular mechanisms for tea-induced vasorelaxation are controversially discussed. Huang et al. found that the vasorelaxing effects of epicatechin (100–500 μM) in rat mesenteric arteries were attenuated by the NOS inhibitor L-NAME, and by removal of the endothelium. Moreover, the tissue content of cyclic GMP – the second messenger mediating the vasorelaxant effects of NO in smooth muscle cells – was increased in these vessels after treatment with 100 μM epicatechin [66]. However, a recently published paper suggests that EGCG-induced vasorelaxation in rat aorta is attributed to its action as nonselective inhibitor of phosphodiesterase activity, albeit above 30 μM [67]. Besides vasorelaxation, also vasoconstricting effects of tea catechins have been reported at concentrations beyond 30 μM [68]. EGCG induced – after an initial transient contraction – a cumulative vasorelaxation in isolated rat aortic rings, which was completely prevented by L-NAME up to the dose of 25 μM EGCG. Higher concentrations of EGCG led to NO-and endothelium-independent vasorelaxation [69]. The time of monitoring changes in vasomotor tone and the concentrations of different molecular actions of tea ingredients on endothelial cells after treatment with a black tea polyphenol fraction [70]. The effect was accompanied by enhanced phosphorylation of eNOS Ser1177 and concomitant dephosphorylation at the Thr195 site. By using pharmacological inhibitors and dominant-negative kinases, the authors identified the PI3K-Akt pathway as being involved in eNOS activation by black tea polyphenols. Overexpression of dominant-negative p38 MAPK attenuated both Akt activation and eNOS phosphorylation, indicating p38 MAPK as an upstream component in eNOS activation induced by tea polyphenols. Upregulation of the antioxidant enzyme heme oxygenase-1 by EGCG (50–100 μM) in bovine aortic endothelial cells was mediated via PI3K activation and phosphorylation of Akt and ERK1/2, further confirming the activation of these kinases in endothelial cells by tea polyphenols [33]. Again, of all catechins tested, EGCG was the most potent in the induction of heme oxygenase-1 expression. The activation of the PI3K-Akt pathway in endothelial cells is in marked contrast to the situation in tumour cells and EGCG-pretreated cells (see below) in which tea catechins suppress this signalling pathway (reviewed in Ref. [71]).

Tea polyphenols have been discussed to possess estrogen-like properties and binding of EGCG to ERα and ERβ with concomitant induction of ER-mediated gene expression has been reported [72]. It was recently shown that activation and phosphorylation of eNOS by tea polyphenols in endothelial
cells involves an estrogen receptor α-dependent pathway [73]. Silencing of ERα by siRNA prevented eNOS activation, and cotransfection of Ser118-phosphorylated ERα was crucial for polyphenol-mediated increase in eNOS activity in COS-7 cells. It should be noted that p38 MAPK was located upstream of ERα, indicating that the estrogen receptor was part of the signalling cascade and was activated in a ligand-independent manner [73]. A receptor involved in the effects of catechins and tea polyphenols in cells of the cardiovascular system remains still to be identified.

Compared to the activation of cellular signalling cascades, the transcriptional effects of tea compounds in cardiovascular cells are limited. In human umbilical vein endothelial cells (HUVEC), only 65 of 12,500 genes were regulated more than 1.4-fold after treatment with 50 μM EGCG, as determined by microarray analysis [74]. The lesser transcriptional effects of tea catechins are probably attributed to their short half-life in media and blood plasma [75].

In summary, despite strong evidence for the involvement of nitric oxide in tea polyphenol-induced vasoralexation, the mechanism(s) of tea-induced vasodilation in blood vessels have not been definitely clarified. Studies in non-activated endothelial cells point to an activation of signalling kinases that involve the PI3K-Akt and the MAPK-ERK1/2 pathway.

7. Molecular mechanisms of anti-angiogenic properties

In contrast to cancerogenesis, the role and causality of angiogenesis in the development of cardiovascular diseases is a topic of much debate (reviewed in [76]). The prevalence of neovascularization in atherosclerotic plaques has been positively correlated with the pathogenesis of unstable angina [77]. A study in humans showed that the density of microvessels was increased in ruptured plaques and in lesions with macrophage infiltration. In this study, the plaque base microvessel density appeared to be an independent marker for plaque rupture [78]. Endothelial cells produce essential factors for angiogenesis. Tea compounds have been demonstrated to effectively impair growth factor signalling pathways and inhibit receptor tyrosine kinase activities, thereby affecting angiogenic processes in endothelial cells [79]. EGCG (1.5–100 μM) inhibited VEGF-induced cell proliferation, migration and tube formation in endothelial cells by blocking the binding of VEGF to its receptor and by decreasing VEGF receptor-1 and -2 autophosphorylation. Among the different catechins tested, EGCG was the most potent in angiogenesis inhibition [80,81]. In human microvascular endothelial cells and HUVEC, pre-incubation of cells with EGCG (0.5–20 μM) for 24 h prevented VEGF-induced VE (vascular endothelial)-cadherin tyrosine phosphorylation and the formation of a multicomponent receptor complex composed of VEGFR-2, β-catenin, VE-cadherin, and PI3K [82,83]. In rabbits fed with a hypercholesterolemic diet for 17 weeks, supplementation with green tea led to a lower number of VEGF positively stained foam and smooth muscle cells, and decreased the percentage of atherosclerotic lesions compared to the control group [84]. Additional targets for EGCG-mediated inhibition of angiogenesis represent further angiogenic factors. Treatment of cells for 24–48 h with EGCG (40 μg/ml) decreased the transcript...
levels of bFGF and acidic fibroblast growth factor (aFGF) in HUVECs [85]. Suppression of radiation-induced tube formation in HUVEC by pre-treatment of cells with 5 μM EGCG for 8 h involved downregulation of beta(3) integrin [86].

MMPs play a major role during angiogenesis. Prevention of tube formation and cell migration by EGCG (30–100 μg/ml) in HUVEC was associated with inhibition of proteolytic activities of metalloproteinases [87]. EGCG (25–100 μM) decreased MMP-2 activity in HUVEC and restrained tumour growth by anti-angiogenic activities in vivo in mice [88]. Taken together, the molecular targets for the antiangiogenic effects of tea polyphenols in endothelial cells are evidently associated with inhibition of growth factor signalling pathways, either by prevention of growth factor binding to their receptors or by impairment of receptor tyrosine kinase activities. Whether these properties of tea polyphenols translate into protective effects in prevention of cardiovascular diseases remains to be proven.

8. Molecular mechanisms of antithrombotic properties

Platelet activation and subsequent thromboembolism are important pathophysiological mechanisms of ischaemic cardiovascular events. In an in vivo model of pulmonary thrombosis, Kang et al. reported markedly improved survival of mice fed green tea catechins or EGCG alone. Consistently, intraperitoneal application of green tea extract (GTC) or EGCG significantly prolonged tail bleeding time. In vitro GTC and EGCG dose-dependently inhibited human platelet aggregation induced by ADP, collagen, epinephrine or calcium ionophore. In contrast, coagulation parameters of aggregation induced by ADP, collagen, epinephrine or GTC and EGCG dose-dependently inhibited human platelet aggregation [89]. As a possible mode of action, Sugatani et al. reported reduced production of platelet activating factor (PAF) by inhibition of acetyl-CoA:lysoPAF acetyltransferase by catechins and theaflavins [90]. However, Duffy et al. found no significant decrease of human platelet aggregating ex vivo after consumption of 900 ml of black tea per day for 4 weeks [91]. More conflicting data were presented by Lill et al., who showed that only EGCG but no other catechin (i.e., C, EC, EGC, ECG, or CG) was able to inhibit thrombin-induced platelet aggregation in vitro. Investigation of platelet signalling cascades revealed an inhibition of thrombin-induced phosphorylation of p38 MAPK and ERK1/2 by EGCG. Further, EGCC inhibited thrombin-induced tyrosine phosphorylation of several platelet proteins (e.g., p115, FAK), providing a possible explanation for its inhibitory effect on platelet aggregation. However, tyrosine phosphorylation of other proteins (p70, p76) was potentiated by EGCG and other catechins containing a 3 galloyl group (CG, ECG). Interestingly, prolonged incubation of stirred platelets with one of these compounds alone also induced platelet aggregation [92]. Another antithrombotic mechanism of tea polyphenols has been suggested by Kang et al. They observed catechin-mediated reduction of intracellular calcium levels in platelets, which subsequently resulted in an inhibition of fibrinogen-GPIIb/IIIa binding via the activation of Ca2+-ATPase and inhibition of IP3 production [93]. In addition, a

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<th>Table 1</th>
<th>Summary of molecular targets of tea polyphenols</th>
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<tr>
<td>Molecular mechanisms</td>
<td>Catechins/ green tea</td>
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<tr>
<td>Antioxidant</td>
<td></td>
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<tr>
<td>Free radical scavenging</td>
<td>+ [12,13]</td>
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<tr>
<td>Chelating of metal ions</td>
<td>+ [22]</td>
</tr>
<tr>
<td>Inhibition of LDL oxidation</td>
<td>+ [15]</td>
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<tr>
<td>Inhibition of redox-sensitive transcription factors (NFκB, AP-1)</td>
<td>+ [24,25]</td>
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<tr>
<td>Inhibition of ROS generating enzymes (iNOS, XO, COX, LOX)</td>
<td>+ [13,28–31]</td>
</tr>
<tr>
<td>Induction of ROS scavenging enzymes (CAT, HO-1)</td>
<td>+ [32,33]</td>
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<tr>
<td>Antiproliferative</td>
<td></td>
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<tr>
<td>Cell cycle arrest via inhibition of cyclins in SMC</td>
<td>+ [39]</td>
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<tr>
<td>Induction of p53</td>
<td>+ [40]</td>
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<tr>
<td>Dose-dependent activation or inhibition of NFκB in SMC</td>
<td>+ [39–41]</td>
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<tr>
<td>Inhibition of AP-1 activity in SMC</td>
<td>+ [38]</td>
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<tr>
<td>Inhibition of PDGF-BB mediated tyrosine phosphorylation and ERK1/2 activation</td>
<td>+ [40,42,43]</td>
</tr>
<tr>
<td>Inhibition of MMP-2/9</td>
<td>+ [39,49–52,54]</td>
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<tr>
<td>Induction of TIMP-2</td>
<td>+ [49,53]</td>
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<tr>
<td>Anti-inflammatory</td>
<td></td>
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<tr>
<td>Inhibition of neutrophil adhesion and migration</td>
<td>+ [56]</td>
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<tr>
<td>Suppression of chemokine and cytokine (IL-8, IL-12) production</td>
<td>+ [56,57,63]</td>
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<tr>
<td>Suppression of adhesion molecule expression in endothelial cells (VCAM-1) and monocytes (CD11b)</td>
<td>+ [58,59]</td>
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<tr>
<td>Induction of monocyte apoptosis</td>
<td>+ [60]</td>
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<tr>
<td>Inhibition of IκB kinase and NFκB activity in inflammatory cells</td>
<td>+ [61,63]</td>
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<tr>
<td>Endothelial function</td>
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<tr>
<td>Vasorelaxation</td>
<td>+ [64,67,69]</td>
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<tr>
<td>Inhibition of phosphodiesterase</td>
<td>+ [67]</td>
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<tr>
<td>Activation of eNOS via PI3K/Akt</td>
<td>+ [69]</td>
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<tr>
<td>Anti-angiogenic</td>
<td></td>
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<tr>
<td>Inhibition of VEGF receptor binding</td>
<td>+ [80,81]</td>
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<tr>
<td>Downregulation of FGF</td>
<td>+ [85]</td>
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<tr>
<td>Downregulation of beta-integrin</td>
<td>+ [86]</td>
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<tr>
<td>Inhibition of receptor tyrosine kinase activity</td>
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<td>Antithrombotic</td>
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<tr>
<td>Inhibition of platelet aggregation</td>
<td>+ [89]</td>
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<tr>
<td>Inhibition of PAF production</td>
<td>+ [90]</td>
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<tr>
<td>Inhibition of thromboxane A2 and prostaglandin D2 production</td>
<td>+ [94]</td>
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+ Effect shown; ○ no data available.
study by Son et al. reported evidence for an interaction of catechins with the arachidonic acid pathway. Treatment with catechins significantly inhibited the release of arachidonic acid, as well as the activity of thromboxane A2 synthase, resulting in a diminished generation of thromboxane A2 and prostaglandin D2 [94].

In summary, there are convincing data on the inhibition of platelet aggregation by tea polyphenols in vitro. However, the biological relevance of this effect remains to be shown in humans, and further studies are necessary to confirm existing data on the molecular mechanisms that cause polyphenol-mediated platelet inactivation.

9. Conclusion

Substances retrieved from foods and plants have recently attracted much attention with regard to human health, due to their low toxicity, limited costs, and broad availability. The potential underlying physiological mechanisms of many of these natural compounds, however, are scarcely understood, especially in the cardiovascular system. As outlined in this review, tea components may act at multiple molecular levels in cardiovascular relevant cells (Fig. 2). The pathophysiology of cardiovascular diseases is multifactorial and comprises processes which appear to be affected by tea ingredients: endothelial dysfunction, inflammation, migration and proliferation of smooth muscle cells, extracellular matrix formation, as well as thrombus formation. The effects of catechins and theaflavins may therefore be of potential therapeutic impact in protection and treatment of cardiovascular disease.

A number of unresolved questions, however, hamper the clinical use of tea polyphenols at present. Open questions particularly exist concerning dose, specificity, potency, feasibility, as well as short- and long-term side effects in humans. It must be critically noted that most of the above-described molecular effects of EGCG and other tea ingredients in cell culture systems are obtained with rather high doses of these compounds. Although naturally occurring polyphenols are generally considered to be pharmacologically safe, it is necessary to be aware that these compounds can have detrimental effects in the body, depending on the localisation and cell type upon which they are acting.

Experimental evidence for sites of molecular action within cells is more comprehensive for catechins, mainly EGCG, whereas data on theaflavins are limited (Table 1). There are some indications that theaflavins may be physiologically more potent than catechins, but compelling data are still lacking. Differences in dose-effects and molecular targets between these two groups of polyphenols remain to be elucidated. It is of considerable interest that the galloyl group (at the 3 position) in the catechin and theaflavin structure appears to be essential for the physiological activity of both compound groups. Isoforms without

Fig. 3. Suggested model of differential molecular actions of tea polyphenols in vascular cells. Depending on the state of the cell, tea polyphenols exert opposite effects on intracellular signal transduction kinases. Whereas in activated cells (pre-stimulated; left) treatment with tea polyphenols leads to inhibition of a variety of signalling kinases, e.g. extracellular signal-regulated kinase (ERK1/2), protein kinase B (Akt), phosphatidylinositol-3-OH-kinase (PI3K), and p38 MAPK (p38), these kinases are activated and phosphorylated by tea polyphenols in resting (non-stimulated; right) cardiovascular cells.
a galloyl group in this position exert less or no biological effects.

The modification of the activity of various kinases (e.g., tyrosine kinases) is a common mechanism involved in the molecular effects of tea polyphenols. Since enhanced activities of receptor tyrosine kinases are implicated in the development of cardiovascular diseases, galloyl-containing tea components may be natural tyrosine kinase inhibitors for clinical application. On the other hand, data for induction of eNOS activity in endothelial cells suggest that, in initially resting cells, activation of protein kinases may be a crucial mechanism involved. We therefore hypothesize that tea components—best shown for catechin—act either as activators or as inhibitors of signal transduction kinases, depending on the status (i.e., activated or resting) of the cells (Fig. 3).

In conclusion, evidence is accumulating that tea polyphenols with a galloyl group can interfere with multiple pathways of signal transduction in cardiovascular relevant cells, primarily by modifying kinase activities. The induction of multiple effects may play crucial roles in the prevention and treatment of cardiovascular diseases. However, caution is still warranted for clinical application, and further work is needed to better understand the molecular effects of tea compounds in various cells.

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