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Epicatechin: Endothelial Function and Blood Pressure

Rosario Jiménez,[†] Juan Duarte,[†] and Francisco Perez-Vizcaino*[,§,#]

[†]Department of Pharmacology, School of Pharmacy, Universidad de Granada, 18071 Granada, Spain

[§]Department of Pharmacology, School of Medicine, Universidad Complutense de Madrid, Instituto de Investigación Sanitaria del Hospital Clínico San Carlos (IdISSC), 28040 Madrid, Spain

[#]Ciber Enfermedades Respiratorias (Ciberes), Palma de Mallorca, Spain

ABSTRACT: Epidemiological studies indicate an inverse relationship between flavanol intake and the risk of cardiovascular disease. Potential mechanisms include their effects on endothelial function and hypertension. A number of studies have shown that flavanol-rich cocoa reduces blood pressure and endothelial dysfunction, whereas black tea may have opposite effects. These results highlight the importance of the different effects of the multitude of phytochemical constituents in these foods and the need for studying the individual flavanols. Epicatechin seems to be a major bioactive constituent of cocoa and other flavanol-rich foods and beverages. It has been shown to improve endothelial function in animals and humans. In salt-sensitive animal models of hypertension, epicatechin lowers blood pressure and the associated end-organ damage. Nitric oxide (NO) seems to play a key role in the protection of both hypertension and endothelial dysfunction.

KEYWORDS: *epicatechin, endothelium, vasodilation, blood pressure*

INTRODUCTION

Flavanols (flavan-3-ols), such as epicatechin and catechin, and their oligomers, the procyanidins, represent a major class of flavonoids widely distributed in plants. They are found at high concentrations in certain foods, such as wine, grapes, tea, and cocoa. The profile of flavanols in these foods is variable, and it can be modified as a consequence of food processing.

Epidemiological studies have found an inverse relationship between flavanol intake (or green tea and cocoa consumption) and the risk of cardiovascular disease.[1−5] A very wide range of biological actions of flavanol-rich foods support the potential cardiovascular protection including effects on vascular tone and endothelial function,[6−11] blood pressure,[12−14] insulin resistance and glucose tolerance,[15] platelet reactivity,[16] and the immune and antioxidant defense systems.[17] In contrast, some epidemiological studies have shown that black tea does not modify or even increases blood pressure and has no effect on platelet aggregation, several markers of oxidative stress, or inflammation in coronary artery disease patients.[18]

The molecular mechanisms involved in the effects of these foods are not completely understood. The reasons for these shortcomings are, at least in part, based on the fact that food matrices contain a multitude of phytochemical constituents, of which an unknown number may exert physiological effects. Most studies attribute the reported effects of cocoa, green tea, and wine to the food content of flavanols and other flavonoids present. In some studies the effects of a high-flavanol cocoa were compared with an oral intake of pure epicatechin in humans.[11] The maximum effect on endothelial function of cocoa coincided with the peak of the plasma level of epicatechin metabolites, and pure epicatechin produced an effect similar to that of cocoa. From this indirect evidence, the authors concluded that epicatechin might be a major bioactive constituent of cocoa and other flavanol-rich foods and beverages. However, few human and animal studies have focused on the protective effects of pure epicatechin in the cardiovascular system. In this paper, we review the continuously growing evidence supporting a beneficial role of epicatechin on endothelial function and hypertension and the potential molecular targets involved.

EFFECTS ON ENDOTHELIAL FUNCTION

Endothelial dysfunction is characterized by a reduced capacity of endothelial cells to induce vasodilatation via the release of nitric oxide (NO). It is an early manifestation common to several cardiovascular risk factors such as arterial hypertension, arteriosclerosis, smoking, diabetes, or aging,[19−21] and it has prognostic value independent of these traditional risk factors. One of the mechanisms involved in endothelial dysfunction involves the synthesis of superoxide ($O_2^{\bullet-}$), a highly reactive oxygen species that inactivates NO.[22]

Clinical Trials with Flavanol-Rich Foods and Epicatechin. During the past decade, a number of clinical trials have analyzed the short-term effects of flavanol-rich foods on endothelial function by measuring flow-mediated vasodilation (FMD) of the brachial artery and peripheral artery, tonometry of the fingertips, or the cold pressor test during coronary angiography. These studies show that these foods induce an acute increase of endothelium-dependent vasodilation in healthy subjects, in smokers, and in patients with obesity, coronary artery disease, or renal disease.[6,7,10,15,23−25] The positive effects on FMD were dose-dependent and related to the increments in the plasma concentrations of the flavanols,

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particularly epicatechin. The genotype of catechol-*O*-methyltransferase (COMT) may affect the effects of green tea.[26] In contrast, in another paper, longer term studies with cocoa for several weeks did not show an increase in endothelium-dependent vasodilation.[27] Studies with pure epicatechin on endothelial function in humans are scarce. In healthy subjects epicatechin acutely enhanced FMD in conduit arteries and augmented microcirculation.[11] In another study, improved endothelial function by pure epicatechin, but not epigallocatechin gallate, was suggested on the basis of indirect evidence, that is, plasma and urinary markers of endothelial function.[28]

Effects of Flavanol-Rich Foods and Epicatechin in Animal Models. The effects of flavanol-rich food or supplements, especially those of red wine polyphenols, on endothelial function have been also analyzed in animal models of cardiovascular disease including hypertension, metabolic syndrome, stress, and aging.[29] The studies on hypertensive animals are reviewed in another section. In Zucker rats, an experimental model of obesity and related metabolic syndrome, red wine polyphenols improved endothelial dysfunction by increasing NO- and EDHF-mediated relaxations associated with a reduced $O_2^{\bullet-}$ release via decreased expression of the NADPH oxidase membrane subunit Nox1.[30] Red wine polyphenols also improved the impaired endothelial dysfunction, the oxidative stress, and the overexpression of angiotensin receptors AT1 and AT2 associated with aging and also improved the aging-related decline in physical exercise.[31] Paradoxically, in chronically stressed rats, which show increased NO compared to controls, red wine polyphenols prevented the elevated release of NO.[32] As in human studies, information about the effects of pure flavanols on endothelial function in animal models, besides the hypertensive animals reviewed below, is limited. Pure epicatechin has been reported to improve some markers of endothelial function in ApoE-knockout mice,[33] a model of atherosclerosis.

Mechanisms. The mechanisms by which flavanols mediate their vascular effects are not fully known. However, the majority of available data support the evidence that flavanol-rich foods are able to improve NO bioavailability and, thereby, increase endothelium-dependent vasorelaxation in healthy subjects as well as in various pathological conditions.[11,34] Chronic consumption of a high-flavanol diet is associated with a high urinary excretion of NO metabolites nitrite/nitrate (NOx), consistent with an augmented NO bioavalability. Besides direct effects on endothelial NO synthase (eNOS), inhibitory effects on pathways that may negatively affect NO including NADPH oxidase,[35] angiotensin-converting enzyme (ACE),[36] asymmetrical dimethylarginine,[37] and endothelin-1 (ET-1)[35] have been proposed to be affected by flavanols in the low micromolar range. A recent study has also found reduced activation of NOX2 (a NADPH oxidase isoform) by acute dark chocolate in smokers.[38] Pure epicatechin also elevated the levels of circulating NO species, for example, plasma *S*-nitrosothiols, plasma nitrite, and urinary nitrate, again consistent with increased bioavalability,[11,28] as well as reduced plasma concentrations of endothelin-1.[28]

The vasorelaxant effect of epicatechin was first investigated in isolated rat aortic rings[39] and mesenteric arteries.[40] Extracts from multiple natural products containing flavanols also produce relaxations of isolated arterial rings with an intact endothelium and increase the endothelium-dependent relaxation induced by acetylcholine in vitro.[29,41−43] The endothelium-dependent relaxations induced by flavanol extracts require relatively large concentrations (>10 μM) and are markedly reduced by inhibitors of NOS, indicating the involvement of NO. Extracts from green and black tea and dark chocolate also activate eNOS in cultured endothelial cells.[44−47] The endothelium-dependent effect of epicatechin is triggered by increased intracellular Ca^{2+} levels in the endothelium, which activate eNOS. Paradoxically, the flavanol-induced eNOS activation seems to involve a pro-oxidant effect because it is prevented by permeant analogues of superoxide dismutase.[29,48,49] The signaling pathway is mediated by Src-dependent activation of the phosphatidylinositol 3-kinase (PI3K), phosphorylation of Akt, and the subsequent phosphorylation of eNOS on the activator site Ser1177. eNOS phosphorylation may be also mediated via the Ca^{2+}/calmodulin-dependent kinase II pathway. In addition, the activation of estrogen receptors and a calcium signal have also been suggested to contribute to eNOS activation in response to some polyphenols and in some types of blood vessels.[50] In coronary arteries, endothelium-dependent relaxations of red wine polyphenols are partially resistant to NO inhibitors and are blunted by the inhibitors of endothelium-derived hyperpolarizing factor (EDHF), charybdotoxin and apamin,[48,51] indicating that flavanol-rich foods also stimulate the EDHF pathway for vascular smooth muscle relaxation.

Endothelin-1 (ET-1) is a peptide produced by endothelial cells in response to tissue injury and plays a major role in vascular dysfunction.[52] It is the most potent vasoconstrictor produced in the blood vessel wall and also augments vascular $O_2^{\bullet-}$ production, at least in part, via the ET_A/NADPH oxidase pathway leading to endothelial dysfunction and hypertension. In an in vitro study,[35] we showed that ET-1-induced endothelial dysfunction can be prevented by epicatechin, independently of its possible interference with ET-1 production. Moreover, epicatechin prevented the ET-1-induced increase in vascular $O_2^{\bullet-}$ production from both NADPH oxidase and uncoupled eNOS. These protective effects seem to be independent of both ERα and PPARγ activation but related to ERK1/2 inhibition. Furthermore, it has been described that flavanols also decrease ET-1 synthesis in cultured bovine and human aortic endothelial cells by suppressing transcription of the ET-1 gene.[53] Oral application of epicatechin lowered ET-1 levels along with increasing NO species in healthy human subjects.[28]

EFFECTS ON HYPERTENSION

High blood pressure is one of the major risk factors for developing cardiovascular disease, including coronary disease, stroke, peripheral artery disease, renal disease, and heart failure.[54,55] Efforts to reduce the prevalence of hypertension have focused on nonpharmacologic approaches that lower blood pressure. Lifestyle measures are recommended for all patients, including those with high-normal blood pressure and those who have a higher risk and require drug treatment. The lifestyle measures that are widely agreed to lower blood pressure or cardiovascular risk associated with hypertension include smoking cessation, weight reduction, physical exercise, reduction of excessive alcohol intake (particularly binge drinking), and dietary measures such as reduction of sodium, increase of potassium, and decrease of saturated and total fat intake.[54] An increase in fruit and vegetable intake has also been included in recent years in the guidelines for the management of arterial hypertension.[54] It should be emphasized that small reductions in blood pressure may have a large impact on

cardiovascular events in the population;[55] that is, a 2−3% reduction in risk is expected for each mmHg. Moreover, risk reduction is mostly independent of the antihypertensive regimen, and treatment with any commonly used antihypertensive drugs produces a similar reduction of total major cardiovascular events.

Epidemiologic Evidence and Clinical Trials. First evidence of an effect of cocoa on blood pressure was obtained in Kuna Indians.[56] This native population living on an island off the coast of Panana is protected against the age-dependent increase in blood pressure. The factors involved are clearly environmental because this protection is lost on migration to Panama City. Interestingly, the Kuna Indians consume enormous amounts of cocoa daily, whereas Panama City immigrants have replaced cocoa by other foods with lower flavanol contents. In a recent large prospective epidemiological study, Cassidy et al.[57] observed that anthocyanins and some flavones (apigenin) and flavanols may contribute to prevent hypertension. In participants ≤60 years old, a 6% reduction in risk was observed for catechin consumption when the highest and lowest quintiles were compared.

Several clinical trials have demonstrated a reduction in blood pressure after the administration of flavonoid-containing foods, such as cocoa or fruit juices, in mild to moderate hypertensive patients.[58,59] Results from a recent meta-analysis of these trials provided evidence that some flavanol-rich foods are associated with a significant reduction in blood pressure.[18] For example, short-term interventions (i.e., 1−18 weeks) with cocoa flavanols significantly reduced systolic blood pressure (SBP) by a mean of 5.9 mmHg and diastolic blood pressure by a mean of 3.3 mmHg.[18,60] In strong contrast, meta-analysis of acute black tea consumption show increased systolic (5.69 mmHg) and diastolic (2.56 mmHg) blood pressure[18] or no significant effect.[60] This may be due to the caffeine content of tea. In fact, in a recent study, chronic black tea consumption reduced blood pressure when compared to a caffeine-containing drink.[61] These differences highlight the need to study the individual purified flavanols. In addition, one of the most important limitations of studies that analyze the effects of flavanol-enriched food on blood pressure to draw an integrative conclusion is the lack of standardization (qualitative and/or quantitative) of the food components through the different studies.

In contrast to flavanol-enriched food, only one study has been performed on human subjects using purified catechin.[62] Results of this study showed that consumption of 75 mg of catechin/day for 24 weeks was effective in reducing systolic blood pressure. These studies with isolated flavanols are crucial to assess these compounds as responsible for the antihypertensive effects of flavanol-containing foods. Unfortunately, there are no studies using epicatechin in hypertensive subjects.

Effects of Flavanol-Rich Foods and Epicatechin in Animal Models of Hypertension. Animal studies using flavanol-rich foods and purified epicatechin are a valid alternative to advance the comprehension of the mechanisms underlying their blood pressure lowering effects. Several polyphenolic extracts containing mainly flavanols (e.g., red wine polyphenols, grape skin extract, cocoa extract, and black or green tea) reduced blood pressure in several rat models of experimental hypertension.[63−66] This effect was related to a combination of vasodilator and antioxidant actions. Other proposed mechanisms for the antihypertensive effects of flavanols include the ability to lower the activity of arginase-2, which is an enzyme that competes with eNOS for L-arginine,[67] and inhibitory activity on angiotensin converting enzyme (ACE) in vitro.[36]

Only a few studies have analyzed the effects of the purified flavanols in experimental models of hypertension. In two recent studies we analyzed the effects of pure epicatechin in the DOCA-salt[68] and in the L-NAME models[69] of hypertension in rats. To our knowledge, whether it exerts antihypertensive effects in genetic hypertension has never been studied. The DOCA-salt hypertensive rat is a model with a markedly depressed plasma renin activity because of sodium retention. Patients with low renin (i.e., salt-sensitive hypertension) represent approximately the 30% of the essential hypertensives and show a poor therapeutic response to angiotensin-converting enzyme inhibitors and angiotensin receptor blockers. Rats were treated for 5 weeks with epicatechin at 2 or 10 mg/kg/day. The higher dose of epicatechin prevented the increase in SBP induced by DOCA-salt.[68]

ET-1 has been shown to contribute to the pathogenesis of salt-sensitive hypertension in animals and humans, secondary to a low-renin state.[52] ET-1 is involved in the development of oxidative stress and hypertension in DOCA-salt rats, because ET_A receptor blockade reduced arterial $O_2^{\bullet-}$ levels with a concomitant decrease in SBP. Recently, it has been described that epicatechin reduced plasma ET-1 levels in healthy men,[28] in the apolipoprotein E (ApoE)(−/−) gene-knockout mouse,[33] and in DOCA-salt hypertensive rats.[68] This inhibitory effect might be mediated via Akt-regulation of the ET-1 promoter, as previously suggested in in vitro experiments.[70]

The alleviation of oxidative stress by epicatechin[68] diminished ROS-mediated NO inactivation and raised the bioavailability of NO, leading to an enhancement of the NO-mediated vasodilatory tone, which could account for the observed amelioration of hypertension. In fact, when the effects of epicatechin were studied in rats after chronic inhibition of NO synthesis with N^G-nitro-L-arginine methyl ester (L-NAME), chronic epicatechin treatment did not modify the development of hypertension,[69] despite the reduction of systemic markers of ROS, demonstrating the key role of NO in the antihypertensive effects of this flavanol.

END-ORGAN DAMAGE ASSOCIATED WITH HYPERTENSION

Sustained high blood pressure is one of the most powerful determinants of the development of cardiac, vascular, and renal diseases. Current therapeutic options for hypertensive patients must consider not only the reduction of blood pressure but also its long-term effects, that is, the preventive effects on end-organ damage. It should also be noted that most of the benefits of antihypertensive treatment are a result of lowered blood pressure per se and are largely independent of the drugs or class of drugs employed.[71]

Renal Injury. Renal damage, characteristic of mineralocorticoid-induced hypertension, has been shown to be partly independent of blood pressure.[72] Long-term administration of epicatechin at 10 mg/kg to DOCA-salt rats significantly decreased the systemic markers of oxidative stress (plasma MDA and urinary iso-PGF2α), and this was associated with reduced blood pressure and proteinuria, without increasing kidney hypertrophy. However, epicatechin at 2 mg/kg, which was unable to reduce systemic oxidative stress markers, was also unable to prevent the development of hypertension or renal hypertrophy or to reduce protein excretion,[68] showing the

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relationship of systemic oxidative stress, blood pressure, and renal damage.

Renal injury has also been consistently reported after chronic inhibition of NO synthesis. In L-NAME-induced hypertension, rats presented moderate/severe kidney injury, especially in the vasculature. They also showed low tubular casts and mild tubular atrophy. The main and most intense vascular lesion in L-NAME rats was hyaline arteriopathy and thickening of vascular wall (proliferative arteriopathy) with moderate decrease of lumen, which was observed almost always in medium-sized vessels.[69] These histological findings were associated with proteinuria, indicating functional impairment of the glomerular wall barrier. Epicatechin, at 10 mg/kg, partially prevented renal parenchyma and vascular lesions and proteinuria, indicating that epicatechin protects, at least partially, against L-NAME-induced renal injury, despite the lack of antihypertensive effect.[69]

Effects on the Heart. Left ventricular hypertrophy is found in most animal models of hypertension. Accordingly, DOCA-salt hypertensive rats showed increased cardiac and left ventricular indices as compared to normotensive control rats. However, epicatechin did not reduce significantly any of these indices, despite its preventive effect on the development of hypertension.[68] The dissociation between high blood pressure and cardiac hypertrophy in this model suggests the involvement of other trophic stimuli that are unaffected by drugs with antioxidant properties, such as epicatechin, red wine polyphenols, or apocynin.[73] A modest left ventricular hypertrophy have also been found in L-NAME-treated rats and was significantly prevented by 10 mg/kg epicatechin.[69] Moreover, chronic epicatechin (1 mg/kg) treatment confers cardioprotection in the setting of a severe form of myocardial ischemic injury in rats after permanent coronary occlusion.[74] This protection is sustained over time and preserves left ventricular structure and function.

Vascular Injury. Endothelial dysfunction is usually associated with hypertension. Endothelium-dependent vasodilator response induced by acetylcholine is altered in conductance vessels in DOCA-salt hypertension.[68,73] This endothelial dysfunction was unrelated to changes in the guanylate cyclase-cGMP pathway or to changes in eNOS expression, because responses to the NO donor sodium nitroprusside and eNOS protein were unchanged, but it was associated with an increased vascular $O_2^{\bullet-}$ content and decreased phosphorylated eNOS levels in aortic rings.[68,73,75] Chronic epicatechin at 2 and 10 mg/kg restored the impaired endothelial function. This can be attributed to increased eNOS phosphorylation at the activation site (Ser-1177) and also to a reduction in $O_2^{\bullet-}$ content at the vascular wall.[68] It should be noted that improvement of vascular oxidative stress and aortic endothelial function is not necessarily accompanied by reduced blood pressure (e.g., NADPH oxidase inhibition attenuates vascular oxidative stress but not hypertension produced by chronic ET-1).[76] Accordingly, the 2 mg/kg epicatechin treatment affected the former parameters but not the latter.[68]

NO Pathway. The improvement of endothelium-dependent relaxation to acetylcholine in DOCA-salt rats induced by epicatechin at 2 mg/kg[68] was abolished in rats with chronic inhibition of NO synthesis,[69] showing the key role of NO in the protective effect of this flavanol in hypertensive endothelial dysfunction. Interestingly, an increased eNOS phosphorylation of Ser-1177, associated with an increased vascular AKT phosphorylation in both groups of rats treated with epicatechin, was found. These in vivo results are consistent with previous in vitro observations showing epicatechin-induced eNOS activation via PI3K/AKT-mediated phosphorylation in human endothelial cells.[77] Interestingly, changes in this pathway are not dependent on the presence of epicatechin in plasma, because they were obtained after 48 h of epicatechin deprivation, suggesting the involvement of in vivo mechanisms that maintain phosphorylation. Moreover, most of the current research implies actions of epicatechin that depend on its actual presence. This discrepancy indicates a new potential mechanism (or mechanisms) for epicatechin action.

Endothelin Pathway. ET-1 is involved in the development of oxidative stress and endothelial dysfunction in DOCA-salt rats. However, 2 and 10 mg/kg of epicatechin abolished the increase in aortic intracellular $O_2^{\bullet-}$ content induced by DOCA-salt, but only the higher dose reduced plasma ET-1 levels,[68] suggesting that other mechanisms could be involved in the protective effects of low doses of epicatechin in vascular oxidative stress, such as (i) interfering with the signaling pathway of ET-1-driven $O_2^{\bullet-}$ generation and/or (ii) increasing vascular antioxidant systems. It is well established that ET-1 activates NADPH oxidase to produce vascular $O_2^{\bullet-}$ generation in DOCA-salt hypertensive rats via up-regulation of the NADPH oxidase subunits $p22^{phox}$ and $p47^{phox}$.[73] In vitro, epicatechin reduced NADPH oxidase-driven $O_2^{\bullet-}$ production stimulated by ET-1 in aortic rings through ERK1/2 inhibition.[35] Chronic epicatechin at 10 mg/kg prevented the up-regulation of $p22^{phox}$ and $p47^{phox}$ and the increased NADPH oxidase activity and the vascular $O_2^{\bullet-}$ content in DOCA-salt rats. Similar results in the vascular wall were also described using a higher dose (30 mg/kg) of its stereoisomer catechin in prediabetic OLETF rats.[78]

Nrf2 Pathway. Nuclear factor-E2-related factor-2 (Nrf2) is a transcription factor that regulates the expression of numerous ROS detoxifying and antioxidant genes. Under basal non-activated conditions, Nrf2 interacts with Kelch-like erythroid cell-derived protein 1 (Keap-1), a cytosolic repressor protein. Upon activation, the Keap-1−Nrf2 complex is dissociated, and Nrf2 translocates to the nucleus, where it binds to ARE, triggering the transcription of phase II and antioxidant defense enzymes, including NADPH:quinone oxidoreductase 1 (NQO1), heme oxygenase-1 (HMOX1), and γ-glutamylcysteine synthetase (GCLC), the rate-limiting enzyme for glutathione (GSH) synthesis. Nrf2 is activated by oxidative stress, which seems to be a countervailing mechanism to protect tissue from oxidative injury. Up-regulation of Nrf2, increased Nrf2 protein in the nucleus, and increases in mRNA of NQO1, HMOX1, and GCLC were found in the DOCA-salt rats, consistent with increased oxidative stress in this model.[68] Recently, epicatechin (10 μM) increased nuclear translocation of Nrf2 and nuclear content of phosphorylated Nrf2 in HepG2 cells.[79] We showed for the first time that chronic epicatechin increased the expression of Nrf2 and Nrf2 target genes (NQO1, GCLC, and HMOX) in the vascular wall.[68] In aortas from normotensive control rats treated with epicatechin at 10 mg/kg, the increased Nrf2/ARE pathway seems to be unrelated to changes in ROS production. Additionally, it should be mentioned that increased PI3K/AKT activity has been linked to the activation of Nrf2 induced by epicatechin at nanomolar concentrations in astrocytes[80] and HepG2 cells[79] and might be involved in the Nrf2 up-regulation in the aorta. Moreover, epicatechin at a dose of 2 mg/kg, which was unable to reduce vascular NADPH oxidase-derived $O_2^{\bullet-}$ production, increased

the Nrf2 pathway in DOCA-salt rats, leading to reduced $O_2^{\bullet-}$ content in the vessel. These results suggest that epicatechin is a more potent stimulator of Nrf2/ARE pathway than an inhibitor of NADPH oxidase-driven $O_2^{\bullet-}$ production.[68]

EDCF Pathway. In addition, increased endothelium-dependent vasoconstrictor factor (EDCF) induced by acetylcholine in the presence of L-NAME in the organ chamber was also observed in the aorta from L-NAME-treated rats.[69] These contractions have been attributed to increased endothelial release of cyclooxygenase (COX)-derived vasoconstrictor prostanoids (such as prostaglandin H_2, thromboxane A_2, or prostacyclin).[81] Epicatechin at 10 mg/kg reduced acetylcholine-induced vasoconstriction in L-NAME-treated rats and prevented the increase in COX-2, suggesting that epicatechin inhibits the release of COX-derived metabolites by down-regulating COX-2.[69]

Inflammatory Cells and Cytokines. Endothelial monolayers are intimately linked with inflammation, because they constitute a barrier between the peripheral bloodstream and inflamed tissues. The endothelium regulates recruitment and transmigration of immunologically active blood cells such as polymorphonuclear leukocytes and T lymphocytes to the site of an inflammation.[82] Monocyte recruitment is one of the early steps in hypertension-induced arteriosclerosis and perivascular fibrosis.[83] Chronic NO suppression induced an increase in ex vivo monocyte endoluminal adhesion and in in vivo perivascular macrophage accumulation, in concert with increases in oxidative stress and inflammatory cytokines in the arterial wall.[84] IL-1β, ICAM-1, and TNFα mRNA levels were increased in the vascular wall of the L-NAME-treated rats, suggesting that pro-inflammatory signals come from the arterial wall.[69] The expression of adhesion molecules and pro-inflammatory cytokines is mainly the product of inducible genes that are usually controlled, at least in part, by the redox-sensitive NF-κB pathway. The increase in oxidative stress in the vascular wall of L-NAME-treated rats probably activates the NF-κB system, which, in turn, induces the expression of pro-inflammatory cytokines.[85] These results further support this hypothesis, because epicatechin, which reduced aortic superoxide levels, also inhibited the vascular expression of these pro-inflammatory and proatherogenic markers.

CONCLUSIONS AND PERSPECTIVES

Several epidemiological investigations and dietary interventions in humans using flavanol-containing foods indicate an inverse relationship between flavanol intake and the risk of cardiovascular disease. A very wide range of biological actions of flavanol-rich foods support these potential cardiovascular protective effects, including the improvement of endothelial function and a reduction in blood pressure. Epicatechin, a major flavanol in food, induced endothelium-dependent relaxation, mainly by eNOS activation, and improved endothelial dysfunction by reducing ROS levels in both animals and humans. Unfortunately, there are no intervention studies with epicatechin in hypertensive subjects. Animal studies using purified epicatechin are a valid alternative to advance the comprehension of the mechanisms underlying its blood pressure lowering effects. These studies showed that chronic treatment with epicatechin, at doses in the range of what can be achieved in the human diet, prevents the progressive increase in SBP, the proteinuria, and the endothelial dysfunction in uninephrectomized rats subjected to chronic administration of DOCA-salt. The effects on aortic endothelial function were associated with an attenuation of vascular $O_2^{\bullet-}$ content and increased phosphorylation of Akt and eNOS. The reductions in blood pressure and proteinuria were accompanied by reduced systemic markers of oxidative stress and endothelin-1. NO plays a key role in both antihypertensive and protective effects in endothelial dysfunction because both effects were almost abolished after chronic inhibition of NO synthesis. However, no studies in genetic models of hypertension, such as SHR, which is considered to resemble human essential hypertension, have been carried out with epicatechin. The data obtained with epicatechin are consistent with favorable effects of flavanol-containing food on cardiovascular risk factors.

AUTHOR INFORMATION

Corresponding Author

*Postal address: Department of Pharmacology, School of Medicine, Universidad Complutense, 28040 Madrid, Spain. Phone: +34 913 941477. Fax: +34 913 941464. E-mail: fperez@med.ucm.es.

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Notes

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