Vascular Protection by Natural Product-Derived Polyphenols: *In Vitro* and *In Vivo* Evidence

Authors **Valérie B. Schini-Kerth, Nelly Étienne-Selloum, Thierry Chataigneau, Cyril Auger**

Affiliation Laboratoire de Biophotonique et Pharmacologie, Université de Strasbourg, Faculté de Pharmacie, Illkirch, France

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- blood vessel
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- cardiovascular diseases

Abstract

Epidemiological studies have indicated that regular intake of fruit and vegetables and beverages such as red wine and tea, which contain high levels of polyphenols, is associated with a reduced risk of cardiovascular diseases. The beneficial effect of polyphenol-rich natural products has been attributable, at least in part, to their direct effect on blood vessels, and in particular on endothelial cells. Indeed, polyphenols from tea, grapes, berries, and plants have been shown to activate endothelial cells to increase the formation of potent vasoprotective factors including nitric oxide (NO) and endothelium-derived hyperpolarizing factor. Experimental and clinical studies have also indicated that chronic intake of several polyphenol-rich natural products is able to improve endothelial dysfunction and to decrease vascular oxidative stress associated with major cardiovascular diseases such as hypertension. Altogether, these observations suggest that polyphenol-rich sources of natural products have the potential to improve the function of blood vessels and, hence, to protect the vascular system.

Introduction

Endothelial cells lining the luminal surface of all blood vessels have a key role in the control of vascular structure and function mostly via the generation of potent vasoprotective factors including nitric oxide (NO), endothelium-derived hyperpolarizing factor (EDHF), and prostacyclin (PGI_2, **Fig. 1**). NO is produced from L-arginine by the enzyme termed NO synthase in endothelial cells. NO can diffuse towards the underlying vascular smooth muscle to reduce vascular tone and to prevent smooth muscle cell proliferation and migration thereby maintaining the arterial wall in a quiescent state. NO has also been shown to prevent the expression of numerous proinflammatory and pro-atherothrombotic mediators such as monocyte chemoattractant protein-1, adhesion molecules, and tissue factor. Moreover, NO helps to maintain blood fluidity by preventing the adhesion and aggregation of platelets and the adhesion of monocytes. Although EDHF has a minor role in most types of large arteries, it contributes to inhibit vascular tone in the coronary circulation and also in arterioles by hyperpolarizing the vascular smooth muscle. PGI_2, generated from arachidonic acid by cyclooxygenases, inhibits vascular tone in some arteries and acts in synergy with NO to efficiently prevent platelet activation.

The endothelial function is impaired in major cardiovascular diseases such as hypertension and atherosclerosis as indicated by blunted endothelium-dependent relaxations and/or vasodilatations (**Fig. 1**). The endothelial dysfunction often involves a reduced bioavailability of NO and EDHF associated to an increased NADPH oxidase-dependent oxidative stress and formation of vasoconstrictor factors such as endothelin-1 and thromboxane A_2 in the arterial wall. Moreover, since the endothelial dysfunction appears before changes in the structure of the arterial wall, it has been suggested to be a key event in the initiation and development of these cardiovascular diseases.

Numerous epidemiological studies have indicated that diets rich in fruit and vegetables and beverages such as red wine and tea are associated with a reduced risk of cardiovascular diseases [1–8]. The protective effect has been attributable, at least in part, to their high content of polyphenols, which are molecules with more than one hydroxyl group on one or more phenol unit per molecule. Polyphenols can be classified into two groups, the flavonoids and the non-flavonoids.

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Correspondence
Valérie B. Schini-Kerth, PhD
UMR 7213 CNRS
Laboratoire de Biophotonique et de Pharmacologie
Faculté de Pharmacie,
Université de Strasbourg
74 route du Rhin
67401 Illkirch
France
Phone: + 33 3 68 85 41 27
Fax: + 33 3 68 85 43 13
valerie.schini-kerth@unistra.fr

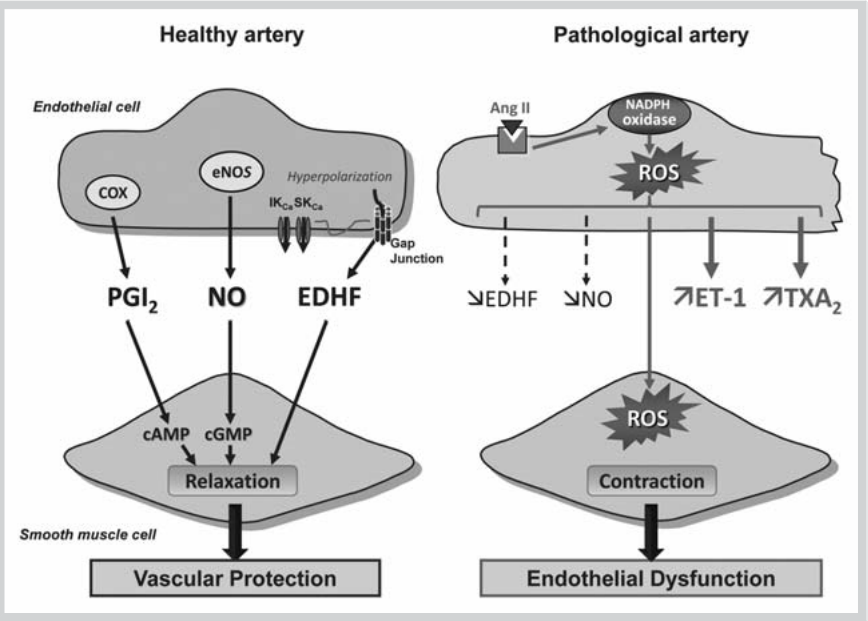

Fig. 1 Endothelium-derived vasoactive factors have a key role in the control of vascular tone in healthy and pathological arteries. In healthy blood vessels, endothelial cells promote relaxation of the arterial wall via the generation of three major vasorelaxing factors: the cyclooxygenase-derived prostacyclin (PGI_2), the endothelial NO synthase-derived nitric oxide (NO), and endothelium-derived hyperpolarizing factor (EDHF). In pathological arteries an endothelial dysfunction is observed, which often involves a reduced formation and/or bioavailability of the vasoprotective factors, an increased formation of vasocontracting factors, and an excessive oxidative stress mostly due to an increased expression of NADPH oxidase. COX, cyclooxygenase; NO, nitric oxide; EDHF, endothelium-derived hyperpolarizing factor; IK_{Ca}, intermediate conductance calcium-activated potassium channels; SK_{Ca}, small conductance calcium-activated potassium channels; ROS, reactive oxygen species; eNOS, endothelial NO synthase; Ang II, angiotensin II; ET-1, endothelin-1; TXA_2, thromboxane A2.

The largest and best-studied polyphenols are flavonoids, which include several thousand compounds, among them flavonols, flavones, flavonones, flavan-3-ols, anthocyanins, and isoflavones [9]. Polyphenols may protect the cardiovascular system by preventing oxidation of low-density lipoprotein, platelet aggregation and adhesion, and smooth muscle cell migration and proliferation. Alternatively, vascular protection may also be due to the direct action of polyphenols on the endothelial function.

Polyphenols Stimulate the Endothelial Formation of NO and EDHF in Isolated Blood Vessels

Investigations using isolated arterial rings suspended in organ chambers to determine changes in isometric tone have indicated that a great variety of natural products are able to cause pronounced relaxations of precontracted arterial rings with an intact endothelium whereas only small relaxations are observed in those without endothelium (**Table 1**) [10,11]. The kinetic of the relaxation has indicated that the decrease of vascular tone is a fast event starting within a couple of seconds and that the maximal relaxation occurs within several minutes. Endothelium-dependent relaxations have been observed in response to a great variety of grape-, tea-, berry-, and plant-derived products (**Table 1**). The endothelium-dependent relaxations to polyphenols, such as a red wine extract in rat aortic rings, is markedly reduced by inhibitors of endothelial NO synthase and guanylyl cyclase indicating the involvement of NO. Thereafter, direct evidence that red wine extracts stimulate the endothelial formation of NO in cultured cells and isolated blood vessels has been obtained using electron paramagnetic resonance [12–14]. In addition, in some types of blood vessels such as coronary arteries, endothelium-dependent relaxations to red wine extracts are only partially reduced by inhibitors of endothelial NO but abolished by the addition of inhibitors of EDHF-mediated relaxations, charybdotoxin and apamin [15–17]. Thus, these observations indicate that in some types of blood vessels such as coronary arteries, polyphenols are able to stimulate, besides NO, also EDHF-mediated relaxations (**Fig. 2**). Investigations to characterize the signal transduction pathway leading to the polyphenol-induced endothelial NO synthase activation have indicated, surprisingly, a key role of an intracellular redox-sensitive event [12,14,18]. Indeed, the red wine polyphenol-induced endothelium-dependent relaxation and formation of NO in cultured endothelial cells are markedly reduced by membrane permeant analogues of superoxide dismutase and also, to some extent, by a membrane permeant analogue of catalase whereas native superoxide dismutase and catalase, which are unable to cross membranes, did not have such effects [12,14,18]. In addition, grape-derived polyphenols caused a time-dependent formation of reactive oxygen species in cultured endothelial cells and in endothelial cells of coronary artery sections [12,15,17]. The pro-oxidant response to polyphenols triggers the Src-dependent activation of the PI3-kinase pathways resulting in the phosphorylation of Akt, which subsequently increases endothelial NO synthase activity by phosphorylating Ser1177, an activator site [12,15,17] (**Fig. 2**). Red wine polyphenols have also been shown to cause the dephosphorylation of Thr495, an inhibitor site, of endothelial NO synthase, thereby promoting further the formation of NO [12]. In addition, a role for estrogen receptors and a calcium signal have also been suggested to contribute to endothelial NO synthase activation in response to some polyphenols and in some types of blood vessels [19,20].

Polyphenols Improve Endothelial Function in Experimental Models of Cardiovascular Diseases

Several experimental *in vivo* studies have reported that ingestion of various polyphenol-rich sources prevent and/or improve the endothelium dysfunction associated with major cardiovascular diseases such as hypertension. Indeed, in angiotensin II-induced hypertension in rats, the increased systolic blood pressure is associated with an endothelial dysfunction characterized by a reduced acetylcholine-induced endothelium-dependent relaxation in aortic rings and an increased oxidative stress due to an enhanced formation of reactive oxygen species throughout the arterial wall [21]. Oral intake of a red wine polyphenol extract

Table 1 Several polyphenol-rich plants and fruits are able to induce endothelium-dependent relaxation *ex vivo*.

Plant	Model	Reported effects	References
Plants			
Açaì stone extract	rat mesenteric vascular bed	endothelium-dependent NO-mediated relaxation	[69]
Crataegus extract WS1442®	rat aortic rings and human internal mammary artery rings from coronary bypass patients	endothelium-dependent NO-mediated relaxations	[70]
Gardenia ternifolia leaf extract	porcine coronary artery rings	endothelium-dependent relaxation	[71]
Hibiscus sabdariffa extract	rat aortic rings	endothelium-dependent NO-mediated relaxation	[72]
Lysimachia clethroides extract	rat aortic rings	endothelium-dependent NO-mediated relaxation	[73]
Parkia biglobosa leaf extract	porcine coronary artery rings	endothelium-dependent relaxation	[74]
Procyanidin-rich extract of *Croton celtidifolius*	rat mesenteric vascular bed	endothelium-dependent NO-mediated relaxation	[75]
Pycnogenol®	rat aortic rings	endothelium-dependent NO-mediated relaxation	[76]
Siberian ginseng extract	dog carotid arterial rings, rat aortic and mesenteric artery rings	endothelium-dependent NO-mediated relaxation	[77]
Spondia mombin leaf extract	porcine coronary artery rings	endothelium-dependent relaxation	[71]
Grape-derived products			
Purple grape juice	porcine coronary artery rings	endothelium-dependent NO- and EDHF-mediated relaxation	[17]
Grape marc extract	rat aortic rings	endothelium-dependent relaxation	[29]
Grape procyanidin extract	rat aortic rings and mesenteric vascular bed	endothelium-dependent relaxation	[10, 78]
Grape skin extract	porcine coronary artery rings	endothelium-dependent NO-mediated relaxation	[79]
Procyanidin-rich red wine fractions	rat aortic rings	endothelium-dependent relaxation	[11]
Provinols®	rat femoral artery rings	endothelium-dependent NO-mediated relaxation and NO formation	[80]
Spanish wines	rat aortic rings	endothelium-dependent relaxation	[81]
Red wine	rat aortic rings	endothelium-dependent NO-mediated relaxation	[82]
Red wine	rat perfused mesenteric bed	endothelium-dependent NO- and EDHF-mediated relaxation	[16]
Red wine extract	rat mesenteric artery rings	endothelium-dependent NO-mediated relaxation	[83]
Teas			
Green and black tea	rat aortic rings	endothelium-dependent NO-mediated relaxation	[84, 85]
Berries			
Blackcurrant extract	rat aortic rings	endothelium-dependent NO-mediated relaxation	[86]
Chokeberry and bilberry anthocyanin extracts	porcine coronary artery rings	endothelium-dependent relaxation	[87]
Cranberry juice	rat aortic rings	endothelium-dependent NO-mediated relaxation	[88]
Raspberry extract and fractions	rabbit aortic rings	vasorelaxant effect associated with fractions enriched in lambertianin C and sanguiin H-6	[89]
Strawberry extract	rabbit aortic rings	endothelium-dependent NO-mediated relaxation	[90]

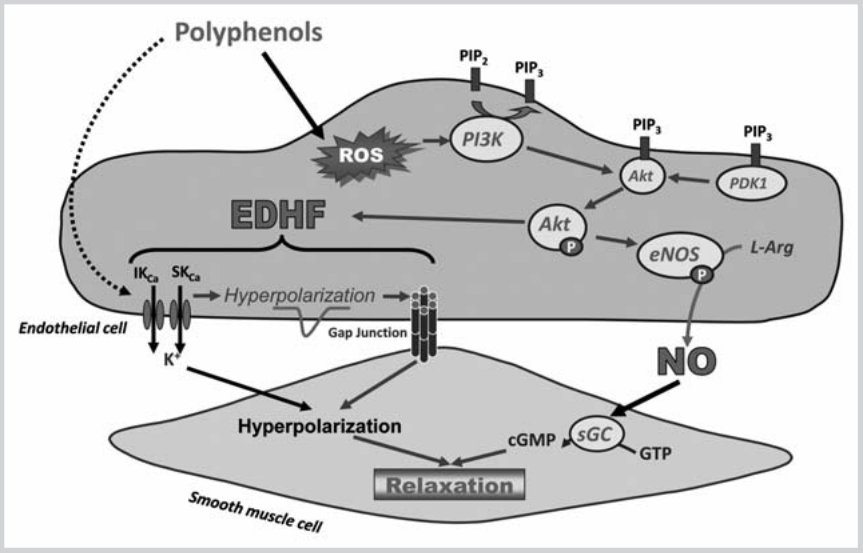

Fig. 2 Polyphenols are potent inducers of the endothelial formation of NO and EDHF via the PI3-kinase/Akt pathway. ROS, reactive oxygen species; PI3K, phosphatidylinositol 3-kinase; PDK1, phosphoinositide-dependent kinase 1; eNOS, endothelial NO synthase; L-Arg, L-arginine; NO, nitric oxide; sGC, soluble guanylyl cyclase; EDHF, endothelium-derived hyperpolarizing factor; IK_{Ca}, intermediate conductance calcium-activated potassium channels; SK_{Ca}, small conductance calcium-activated potassium channels.

(150 mg/kg/day) in the drinking water prevented the angiotensin II-induced increase in systolic blood pressure, and reduced significantly endothelial dysfunction and oxidative stress in the arterial wall [21]. Moreover, the angiotensin II-induced vascular oxidative stress is, at least in part, due to the upregulation of several NADPH oxidase subunits including nox1 and p22phox; this effect is significantly reduced by the red wine extract [21]. Besides preventing NADPH oxidase expression, polyphenols may also pre-

vent vascular oxidative stress by reducing NADPH oxidase activity and by increasing the expression of antioxidant enzymes such as catalase [22,23]. In spontaneously hypertensive rats, ingestion of grape-derived polyphenols also reduced blood pressure and vascular oxidative stress, and these effects were associated with an improved ventricular hypertrophy and cognitive function [24]. Moreover, a beneficial blood pressure lowering effect of oral intake of polyphenol-rich products has been observed in several additional experimental models of hypertension such as the two-kidney one-clip Goldblatt rats [25], the DOCA salt-induced hypertension [26], and the N^{ω}-nitro-L-arginine-induced hypertension [16,27].

Besides hypertension, polyphenol-rich natural sources have also been shown to improve the endothelial dysfunction in several other types of cardiovascular diseases such as in atherosclerosis, diabetes, and metabolic syndrome. In an experimental model of diet-induced atherosclerosis, the ingestion of grape-derived polyphenols prevented the development of fatty streak lesions in the aortic arch of Golden Syrian hamsters [28–30]. A grape-derived extract prevented also the development of hypertension, cardiac hypertrophy, and vascular oxidative stress associated with the upregulation of NADPH oxidase expression in fructose-fed rats, an experimental model of insulin resistance and metabolic syndrome [31,32]. In Zucker fatty rats, an experimental model of obesity and related metabolic syndrome, intake of a red wine phenolic extract improved endothelial dysfunction by increasing NO- and EDHF-mediated relaxations associated with a reduced NADPH oxidase expression [33]. In addition, chronic ingestion of authentic polyphenols such as genistein and epigallocatechin-gallate improved endothelium-dependent relaxations of aortic rings in streptozotocin-induced diabetic rats [34,35]. Moreover, catechin ingestion prevented the development of the endothelial dysfunction by reducing the expression and activity of NADPH oxidase in the prediabetic Otsuka Long-Evans Tokushima fatty rats [36].

Altogether, these studies indicate that chronic ingestion of polyphenols has a beneficial effect on vascular health in several experimental models of cardiovascular diseases such as hypertension, diabetes, and atherosclerosis. The beneficial effect is often associated with an improved endothelial function and vascular oxidative stress mainly subsequent to a reduced expression of NADPH oxidase (**Fig. 3**).

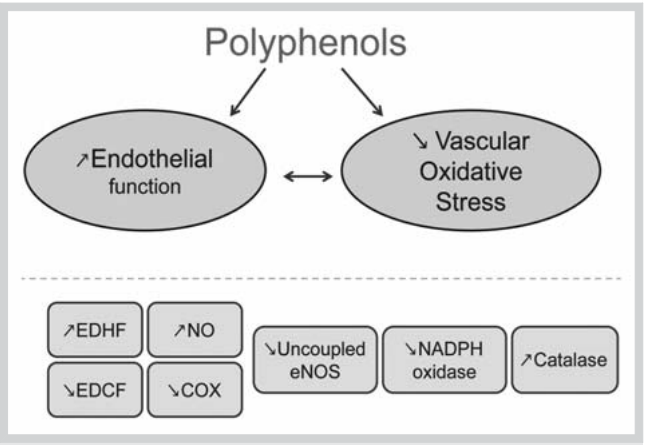

Fig. 3 Polyphenols improve vascular function in major cardiovascular diseases mostly by improving the endothelial dysfunction and reducing vascular oxidative stress. COX, cyclooxygenase; EDCF, endothelium-derived contracting factors; EDHF, endothelium-derived hyperpolarizing factor; NO, nitric oxide; eNOS, endothelial NO synthase.

Polyphenols Improve Endothelial Function in Humans

Consistent with epidemiological investigations and animal studies, clinical studies suggest that polyphenol-rich natural sources may also have a beneficial effect on vascular function in humans. The endothelial function can be assessed by flow-mediated dilatation (FMD) in humans, and its impairment (endothelial dysfunction) is an independent predictor of cardiovascular outcomes in subjects with cardiovascular risk factors or established cardiovascular diseases [37].

In healthy subjects, the basal FMD is increased after consumption of 3 mL/kg or two glasses of red wine with or without alcohol [38–40], and also, in a synergistic manner, by the intake of red wine and olive oil, major components of the Mediterranean diet [41]. Moreover, intake of red wine has been shown to counteract high fat diet-induced endothelial dysfunction in human volunteers [42]. Nonalcoholic polyphenol-rich beverages such as grape juice also increased FMD in healthy individuals [43]. A similar finding has also been observed after chronic consumption of 2 g/day of a grape seed extract [44] and a single dose of 46 g of dark chocolate [45]. Schroeter et al. [46] suggested that the effect of dark chocolate is related to its content in epicatechin. Indeed, ingestion of a low dose of purified epicatechin (1 or 2 mg/kg body weight) increased to a similar extent basal FMD. In addition, chronic consumption of a procyanidin-rich maritime pine bark extract Pycnogenol® (180 mg/day for 2 weeks) increased NO-mediated forearm blood flow in response to acetylcholine in healthy subjects [47]. Moreover, Franzini et al. indicated that diets which contain a high level of polyphenol-rich natural sources such as red wine, grapefruit, berries, and dark chocolate, improved endothelial function as assessed by FMD in healthy individuals [48].

In addition, several clinical studies suggest that polyphenol-rich sources also have the potential to improve endothelial dysfunction associated with major cardiovascular diseases. For example, Hall et al. reported that supplementation of a low-fat meal with 80 mg of soybean isoflavones increased FMD in postmenopausal women, a population with an increased risk of cardiovascular diseases [49]. In addition, intake of dark chocolate and red wine increased FMD and decreased blood pressure, respectively, in adult cigarette smokers who exhibit an increased atherogenic potential [50–52]. Endothelial dysfunction associated with metabolic disorders (hypercholesterolemia, diabetes, increased body mass index) has also been improved by acute and chronic ingestion of natural sources of polyphenols such as cocoa, red wine, and grape juice [53–56]. In patients with coronary artery disease, FMD is increased after consumption of 450 mL of black tea and after chronic daily ingestion of 900 mL of black tea for 8 weeks [57]. A similar effect has also been observed after ingestion of a single dose of 300 mg of the epigallocatechin gallate-rich green tea extract Teavigo® [58], 600 mg of a red grape extract [59], 250 mL of red wine [60,61], and after intake of 8 mL/kg/day for 2 to 8 weeks of Concord grape juice [62,63]. Chronic consumption of pomegranate juice (50 mL daily for a year) also reduced systolic blood pressure and the intima-media thickness in patients with severe carotid artery stenosis [64].

Moreover, consumption of polyphenol-rich sources has been associated with a reduced systolic blood pressure in hypertensive

patients. Indeed, Taubert et al. showed that daily ingestion of 100 g of dark chocolate for two weeks reduced systolic and diastolic blood pressure in mildly hypertensive patients [65]. Intake of purple grape juice, roughly equivalent to two glasses, for 8 weeks improved blood pressure in hypertensive patients in one study [66] but not in another [67]. In addition, Aviram et al. showed that daily consumption of 50 mL of pomegranate juice for 2 weeks by hypertensive patients reduced systolic blood pressure by 5% [68]. Altogether, these studies suggest that chronic consumption of polyphenol-rich sources may have a beneficial effect on the endothelial function both under physiological and pathophysiological conditions.

Conclusion

Both experimental and clinical studies performed during the last 15 years support the view that several natural sources of polyphenols such as grape-derived products, berries, tea, and plants are able to improve the endothelial function both *in vitro* and *in vivo* mostly by stimulating the endothelial formation of NO, a potent vasodilator and inhibitor of platelet activation.

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