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Review

Polyphenols as potential therapeutical agents against cardiovascular diseases

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Abstract:
Increasing evidence suggests that polyphenols from fruits, vegetables and beverages such as wine and tea may exert protective effects on the cardiovascular system. Indeed, research in the field of polyphenols points out their antioxidant and free radical scavenging properties, leading to lower low-density lipoprotein (LDL) oxidation and platelet aggregation. These compounds are also able to modulate the generation of nitric oxide (NO) from vascular endothelium and to interfere with the mechanisms leading to inflammation and endothelial apoptosis, contributing to the prevention of the endothelial dysfunction, known to play a central role in the pathogenesis of cardiovascular diseases. This article reviews the potential targets of polyphenols involved in the complex pathophysiological events occurring in cardiovascular diseases, such as hypertension, atherosclerosis and stroke.

Key words:
cardiovascular diseases, polyphenols, antioxidant, endothelial function, NO

Abbreviations: ang II – angiotensin II, BAEC – bovine aortic endothelial cells, cGMP – cyclic guanosine monophosphate, COX – cyclooxygenase, EDHF – endothelium-derived hyperpolarizing factor, eNOS – endothelial nitric oxide synthase, ET-1 – endothelin-1, HUVEC – human umbilical vein endothelial cells, ICAM-1 – intercellular adhesion molecule-1, IL – interleukins, iNOS – inducible nitric oxide synthase, LDL – low-density lipoprotein, MAP – mitogen-activated protein, MCP-1 – monocyte chemoattractant protein-1, NADPH – nicotinamide adenine dinucleotide phosphate, NO – nitric oxide, PAF – platelet-activating factors, PDE – phosphodiesterase, PECAM-1 – platelet/endothelial cell molecule-1, ROS – reactive oxygen species, RWPC – red wine polyphenolic compounds, SMC – smooth muscle cells, SOD – superoxide dismutases, TNF – tumor necrosis factor, TXA_2 – thromboxane A_2, VCAM-1 – vascular cell adhesion molecule-1

Introduction

Epidemiological studies have shown an inverse correlation between polyphenols enriched diet and reduced risks of cardiovascular diseases [100]. Polyphenols are widely distributed in the human diet, mainly in plant-derived food and beverages (fruits, vegetables, nuts, seeds, herbs, spices, tea and red wine) and represent more than 8000 phenolic structures. On the one hand, flavonoids are the major constituents of this group with more than 4000 compounds. They share a common flavan core formed with 15 carbon atoms arranged in 3 rings and this class can be divided into

flavanols (catechin, epicatechin), flavonols (quercetin, myricetin, kaempferol), anthocyanidins (cyanidin, delphinidin), flavones (apigenin, diosmin), flavanones (naringenin, hesperetin) and chalcones (phloretin). On the other hand, the non-flavonoids compounds contain an aromatic ring with one or more hydroxyl group. This group includes stilben (resveratrol), phenolic acids (gallic acid) saponin (ginsenoside) and other polyphenols like curcumin and tannins. The role of polyphenols in plants may partly explain the biological properties observed *in vitro* or *in vivo*: they are involved in defense against infection and confer protective effects to the plants against stress, such as ultraviolet radiation, pathogens and physical damages [65].

There have been several studies trying to quantify the amount of polyphenols in the human diet. In the USA, Kuhnau [52] has established that the consumption of glycosilated flavonoids was about 1 g/day. In a Dutch study in 1987–88, the intake of polyphenols was estimated at 23 mg/day of mixed flavonoids in aglycone forms [45]. This value can be related to 28 mg/day obtained in Denmark, corresponding to the total amount of flavonoids daily ingested [48]. In Western countries, the total consumption of flavonols, flavanones, flavanols, and isoflavones is estimated to be 100 to 150 mg/day [58]. Due to their chemical structures polyphenols may interfere with different factors involved in the pathogenesis of cardiovascular diseases. In this review, we focus on potential vascular protecting properties of the polyphenols able to maintain or restore the endothelial function and on the mechanisms involved.

Role of vascular endothelium

The endothelium is formed by a monolayer of cells that make up the inside of blood vessels. The vascular endothelium plays a key role in the regulation of both the vascular tone and the vascular homeostasis, but also in the structural transformations occurring in pathological conditions. The endothelium modulates the balance between opposing mechanisms that are vasodilatation/vasoconstriction, pro-coagulant/antithrombotic effects, cell proliferation/apoptosis. Indeed, the endothelium has a strategic position at the blood/tissue interface and is also in direct contact with various circulating factors such as antioxidants, oxidized low-density lipoprotein (LDL), but also pro-inflammatory cytokines like the tumor necrosis factor (TNF) or interleukins (IL) [16, 17]. These factors can interfere with the vasomotricity or the production of endothelial agents such as nitric oxide (NO) [89]. They are involved in various physiological functions and are able to modulate cellular processes like apoptosis, proliferation and migration of endothelial cells [2, 29, 107]. Thus, an impairment of endothelial functions may lead to numerous deleterious processes, damaging the vascular cells but also the surrounding tissue. So, the endothelial dysfunction plays a pivotal role in the pathogenesis of cardiovascular diseases such as atherosclerosis and hypertension [63].

Regulation of vascular tone

The first role of the endothelial cells is the modulation of the vascular tone, by producing vasodilator and vasoconstrictor factors. NO is the principal vasodilator released by the endothelium and is formed from L-arginine *via* the endothelial NO synthase (eNOS) enzyme. NO can diffuse passively to the vascular smooth muscle cells (SMC), activate the guanyl cyclase, leading to the accumulation of cyclic guanosine monophosphate (cGMP), which activates the protein kinase G and induces the vasorelaxation of the endothelium. The endothelium-derived hyperpolarizing factor (EDHF) contributes to vasodilatation by acting on K^+ channels. Moreover, prostacyclin (PGI_2) is the major endothelial metabolite of arachidonic acid generated through the cyclooxygenase (COX) pathway and leads also to vasodilatation.

On the contrary, the endothelium can also produce vasoconstrictor factors such as endothelin-1 (ET-1), angiotensin II (ang II) and thromboxane A_2 (TXA_2) [34, 43, 46, 57, 66].

Regulation of vascular permeability

Endothelial cells adhere to one another through junctional structures formed by transmembrane adhesive proteins that are responsible for homophilic cell-to-cell adhesion. Adherent junctions and tight junctions are the main types of junction. Another kind of junction, the gap junction, allows cells to communicate with each other. They are composed with connexins and connexons to form channels for the intercellular passage of ions and small-molecular weight mole-

cules [8]. Transcellular and paracellular mechanisms allow the endothelial cells to modulate the passage of plasma proteins and circulating cells from blood to tissues. The transcellular pathway involves vesicular systems, whereas the paracellular pathway is regulated by opening of cell-to-cell junctions and/or by rearrangement of their architecture. However, various molecules such as inflammatory cytokines, endothelin or free oxygen radical can increase the intercellular permeability by creating gaps [14, 82]. In order to modulate the trafficking of circulating blood cells, the endothelial cells express cell surface molecules. For instance, to accelerate the migration towards sites of infection, cell-associated molecules can direct leukocytes. Activated endothelial cells express selectins (P-selectin, E-selectin and L-selectin) to mediate the leukocytes rolling [78]. Other adhesion molecules such as intercellular adhesion molecule-1 (ICAM-1), vascular cell adhesion molecule-1 (VCAM-1) or platelet/endothelial cell molecule-1 (PECAM-1) are expressed on endothelial cells and interact with surface molecules called integrin expressed on leukocytes to allow transmigration. Furthermore, endothelial cells can produce chemokines like IL-8 and monocyte chemoattractant protein-1 (MCP-1) to attract leukocytes to the inflammatory site [99].

Regulation of thrombosis and adhesion

Haemostasis is controlled by endothelial cells that can modulate both coagulation and fibrinolysis [9, 79]. The antithrombotic property of the endothelium is mediated by the production of thrombomodulin, which binds to thrombin to inactivate its procoagulant activity. Moreover, endothelial cells produce platelet-activating factors (PAF), NO and PGI_2 and express ectonucleotidases, leading to the downregulation of platelet activation. As far as the fibrinolysis concerned, endothelial cells produce proteases like the tissue plasminogen activator, which allow the synthesis of plasmin that can degrade fibrin [9, 79, 81, 99].

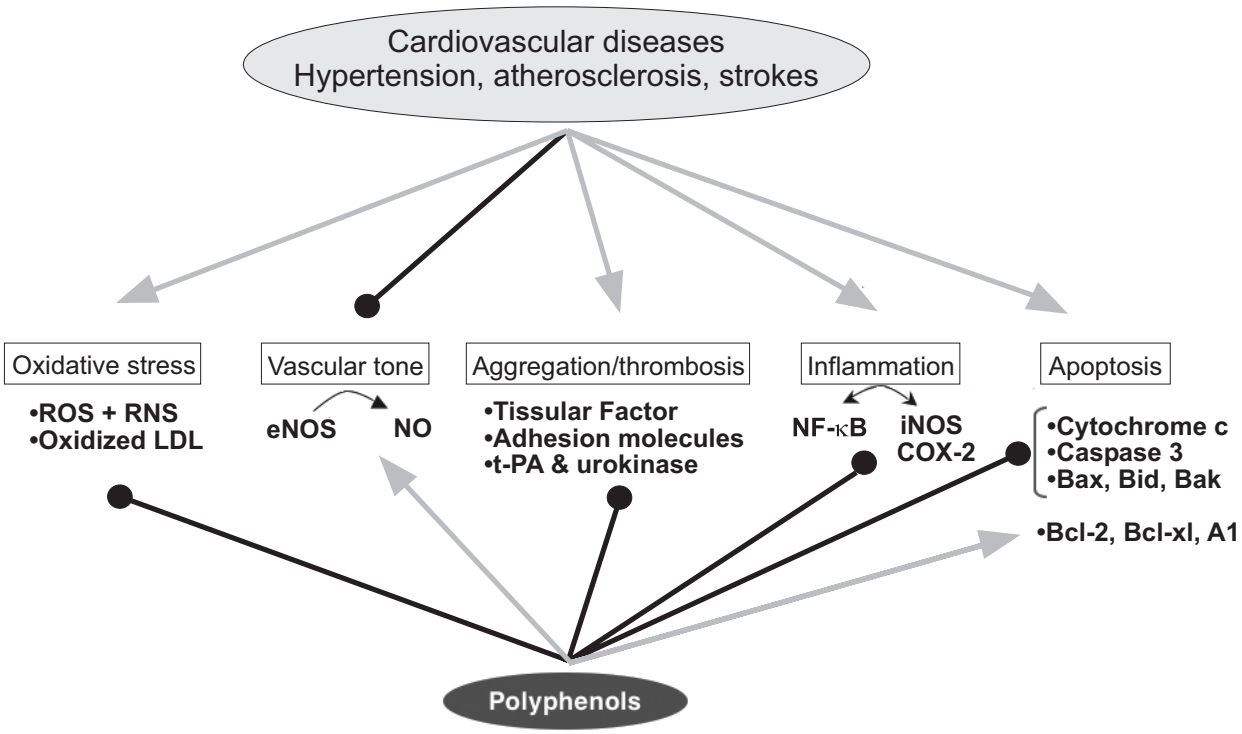

Fig. 1. Cellular and molecular targets of polyphenols against cardiovascular diseases. ROS – reactive oxygen species, RNS – reactive nitrogen species, LDL – low-density lipoprotein, eNOS – endothelial nitric oxide synthase, NO – nitric oxide, t-PA – tissue plasminogen activator, NF-κB – nuclear factor-κB, iNOS – inducible nitric oxide synthase, COX-2 – cyclooxygenase-2. Grey arrows represent a stimulatory effect; black lines represent an inhibitory effect

Beneficial effects of the polyphenols on cardiovascular diseases

Polyphenols as antioxidants

Polyphenols are the most abundant antioxidant in the diet. Their intake is 10 times higher than vitamin C and 100 times higher than vitamin E or carotenoids. As reviewed by Middleton [65], polyphenols exert antioxidant activities (Fig. 1). Polyphenols like catechin or quercetin can directly scavenge reactive oxygen species (ROS), such as superoxide (O_2^-), hydrogen peroxide (H_2O_2) [76], or hypochlorus acid (HOCl) [13], which can be very deleterious by damaging lipids, proteins and DNA. The phenolic core can act as a buffer and capture electrons from ROS to render them less reactive [19]. Furthermore, polyphenols, quercetin in particular, can chelate metals like iron involved in free radicals formation [50, 70]. Indirectly, polyphenols can interfere with the cellular detoxification systems, such as superoxide dismutases (SOD), catalase or glutathione peroxidases [51, 93]. Besides, polyphenols can inhibit enzymes generating ROS as xanthine oxidase and nicotinamide adenine dinucleotide phosphate (NADPH) oxidase [70, 73]. Polyphenols antioxidant potential has been reviewed by Rice-Evans and Miller [87]: among all the polyphenols, quercetin and myricetin exert the most potent free radical scavenger activities, followed by kaempferol. Catechin seems to be the less efficient scavenger.

In vitro, the investigation of the antioxidant potential of tea flavonoids, mainly flavonols and flavonol glycosides, shows that they are strong antioxidants, up to 5 times more effective than vitamin C or E [7, 30]. More recently, Raza et al. [84] observed that epigallocatechin gallate from tea can modulate the oxidative stress by affecting the production of ROS, glutathione, and cytochrome P450 2E1 activity.

Red wines are also rich in polyphenols, mainly phenolic acids, resveratrol, flavonols, flavanols, procyanidins, and anthocyanins. Red wines exhibit a stronger antioxidant capacity than white wines due to the phenolic content [42, 88].

Regarding to their antioxidant properties, polyphenols may reduce the LDL-peroxidation as discussed below.

Polyphenols and lipidemia

Another significant property of the polyphenolic compounds is their ability to reduce lipid sensitivity to oxidation. *In vitro*, resveratrol, a polyphenolic compound in red wine, protects against oxidized LDL-induced cytotoxicity in endothelial cells [74]. In animals, Vinson et al. [102] reported that polyphenols from red wine or grape juice reduce the plasmatic concentration of lipids in hamsters. This is consistent with studies in humans. Short-term ingestion of purple grape juice has been shown to reduce LDL susceptibility to oxidation in patients with coronary artery disease [96]. This has to be related to the work of Frankel et al., who observed an inhibition of LDL oxidation in humans by resveratrol [39]. This is also in harmony with the recent study in hypercholesterolemic postmenopausal women with red wine complementation [67].

However, in several studies, the LDL oxidation was not modified by polyphenols intake. De Rijke et al. [25] did not observe any modification of the LDL oxidizability in healthy volunteers after red wine consumption (550 ml during 4 weeks). O'Reilly et al. [72] observed that the intake of flavonoids from onions and green tea does not modify the level of LDL oxidation in human. Moreover, a study in postmenopausal women has recently determined that the intake of lyophilized grape powder (rich in flavans, anthocyanins, quercetin, myricetin, kaempferol, and resveratrol) during 4 weeks does not reduces the LDL oxidation, even if plasma triglycerides, plasma LDL-cholesterol and apolipoproteins B and E concentrations, are lowered by the treatment [106]. Therefore, the effect of polyphenols on LDL oxidation may vary depending on their structure, the type of natural diet they were originated from and the dose used.

Polyphenols and vascular tone

Hypertension is a high risk factor in the development of cardiovascular diseases. In fact, the modifications of the vascular walls constitute year after year an aggravating factor of the haemodynamic trouble, causing a vicious circle and therefore hypertensive cardiopathies and cardiac failures.

In humans, 30 min after the consumption of red wine or polyphenols (1 g/kg body weight), circulating NO concentration increases to 30 and 40 nM, respectively. Furthermore, a reduction of the blood pressure (11 mmHg) and an increase of heart rate are observed [62]. In hypertensive patients, the use of olive oil can reduce the blood pressure [37].

In animals, several studies performed on isolated vessels like the aorta or the mesenteric artery of rats show that red wine polyphenolic compounds (RWPC) are able to induce an endothelium-dependent relaxation of the vessels [3, 38]. The polyphenols effect on the endothelium is mainly due to NO production [3, 31, 105]. Polyphenols from red wine, grape skin or an isolated polyphenol (quercetin) possess an anti-hypotensive effect. We provide evidence that short-term oral administration of RWPC produces a decrease in blood pressure in normotensive rats. This haemodynamic effect was associated with an enhanced endothelium-dependent relaxation and an induction of gene expression (of inducible NO synthase and COX-2) within the arterial wall, which together maintain unchanged agonist-induced contractility [28]. We provide also evidence that RWPC can accelerate the regression of blood pressure and improves structural and functional cardiovascular changes produced by chronic inhibition of NO synthesis [12]. Recently, we reported that RWPC prevents L-nitro-arginine-methyl ester-induced hypertension, cardiovascular remodeling and vascular dysfunction *via* the increase of NO-synthase activity and prevention of oxidative stress. Thus, the beneficial effects of plant polyphenols in prevention of hypertension may result from their complex influence on the NO balance in the cardiovascular system [80]. The mechanism of endothelial NO release elicited by polyphenols has been investigated. RWPC can modulate the production of NO through an extracellular Ca^{2+}-dependent mechanism in endothelial cells [4]. Resveratrol and quercetin have been shown to induce an increase of the intracellular concentration of Ca^{2+} ($[Ca^{2+}]_i$), by activation K^+ channels or inhibition of Ca^{2+}-ATPases of the endoplasmic reticulum in endothelial cells [55, 64]. Delphinidin, an anthocyanin contained in natural diet including red wine, can stimulate endothelial cells and induce an increase of both $[Ca^{2+}]_i$ and tyrosin phosphorylation of intracellular proteins, leading to the regulation of eNOS. Phospholipase C and tyrosine kinases are both implicated in this Ca^{2+} signaling [59]. Another study has shown that RWPC may also promote the release of endothelial NO through the redox sensitive PI3/Akt pathway [69]. Other *in vivo* studies have confirmed the activation of the NO-synthase by different isolated polyphenolic compounds, such as resveratrol [103] or RWPC [54, 80, 83]. Finally, polyphenols can also modulate the level of NO by acting on the phosphodiesterases (PDE) PDE-2 and PDE-4 in endothelial cells [10, 11, 56]. Recently, anthocyanins have been found to inhibit the human cGMP-specific PDE-5 *in vitro* [26].

Atherosclerosis

Atherosclerosis is characterized by a progessive obstruction of an artery. The initiation of such a disease is the accumulation on the arterial wall of lipids, which progress through the endothelium, where they are oxidized by endothelial cells, SMC and macrophages [5, 40, 77]. LDL oxidation can be amplified by ROS and reactive nitrogen species (RNS) generation. This leads to an accumulation of macrophages at this place, whose role is to clear the oxidized LDL and become "foam cells". Then, an inflammatory process takes place with the recruitment of inflammatory cells and this results in SMC proliferation and migration. Also an increase of extracellular matrix deposit around the site of inflammation occurs and this leads to plaque formation, which occludes more or less the vessel [1, 40]. This phenomenon is related to the loss of the natural ability of the blood vessel to relax.

The beneficial effects of polyphenols on atherosclerosis have been studied [18, 41, 96] and RWPC are able to limit the initiation and the progression of atherosclerosis *via* their antioxidant, anti-LDL oxidation, anti-platelet aggregation, but also with an increase of HDL concentration and an inhibition of SMC proliferation. Finally, they may maintain "healthy blood vessel" by the generation of NO, which plays a pivotal role in the vascular tone.

In addition, administration of red wine in the hamster reduces the neointimal hyperplasia associated with a decrease of a protein implicated in the monocytes recruitment in the vascular wall, which is one of the mechanism observed in the restenosis [35]. Very recently, we reported that oral administration of RWPC reduces in-stent neointimal growth, lipid deposition in association with its anti-inflammatory property in iliac arteries from hypercholesterolemic rabbits [32].

Platelet aggregation plays a role in the development of athero-thrombotic process. Indeed, decreased platelet aggregation is frequently associated with low incidence and prevalence of cardiovascular diseases. The effect of red and white wine and grape juice on platelet activity has been compared in an *in vivo* dog model [27]. Red wine and grape juice, but not white wine, exert an anti-platelet activity. This observation has to be related to the study *in vivo* in human using

dealcoholized red wine [86, 90], which contain procyanidins and catechins. A recent work with anthocyanins confirms the inhibition of the platelet function by polyphenols [85]. The major mechanism involved in the anti-platelet aggregant polyphenols effect is related to their ability to inhibit enzymes implicated in the production of TXA_2 (COX) and as well as to inhibit lipoxygenase [23].

Inflammation

In cardiovascular diseases, an important inflammatory process takes place with the mobilization of leukocytes. Both COX and lipoxygenase are involved in the release of arachidonic acid, which leads to the release by inflammatory cells such as neutrophils of cytokines like IL-1β and chemokines as IL-8. Polyphenols, quercetin in particular, have been shown to inhibit COX and lipoxygenase [36, 49, 53]. Furthermore, resveratrol is also considered to be a molecule with anti-inflammatory action, by inhibiting PG biosynthesis [61].

In humans, Badia et al. [6] have observed that moderate red wine consumption was able to reduce the adhesion of monocytes to endothelial cells through down-regulation of adhesion molecules on the monocytes surface. Moreover, curcumin blocked cytokine-mediated NF-κB activation and pro-inflammatory gene expression by inhibiting inhibitory factor I-κB kinase activity [47].

However, resveratrol may have pro-inflammatory effects at low concentrations (up to 11 μM) whereas it has anti-inflammatory properties at high concentration (43 μM) [33].

Apoptosis

Apoptosis of endothelial cells highly affects endothelium permeability and thus facilitates the development of various pathologies. Polyphenols have been shown to exert protective effects *in vitro* against apoptosis mediated by oxidized LDL and hydrogen peroxide in bovine aortic endothelial cells (BAEC) and fibroblasts [101]. Nevertheless, resveratrol can induce apoptosis in human umbilical vein endothelial cells (HUVEC) [97]. Polyphenols can modulate caspases activity. Indeed, polyphenols from oolong tea, theasinensin A, can induce apoptosis of tumor cells by activating caspases 3 and 9 [75], whereas epicatechin is able to inhibit the activation of caspase 3 in human fibroblasts [95].

Furthermore, polyphenols can modulate the level of expression of protective proteins (Bcl-2, Bcl-xL, A1) or pro-apoptotic factors (Bax, Bid, Bak) [68, 98]. Thus, resveratrol induces apoptosis by increasing the cellular level of Bax, with the inhibition of its degradation by the proteasome or inhibition of the expression of Bcl-2. The inhibition by polyphenols of apoptosis induced by oxidized LDL in BAEC is also related to the modulation of the calcium homeostasis. Recently, we demonstrate that delphinidin is able to protect endothelial cells against apoptosis [60]. Of particular interest is the finding that the anti-apoptotic effect of delphinidin results from increased eNOS expression *via* mitogen-activated protein (MAP) kinase inhibitor-sensitive pathway. The effect of delphinidin also involves the NO and guanylyl cyclase-dependent pathway and is associated with the maintenance of endothelial $[Ca_2^+]_i$ level in a physiological range and the decrease of cytochrome *c* release from the mitochondria.

Ischemia

There is an inverse correlation in clinical studies between the tea and flavonoids intake and incident myocardial infarction [44]. Moderate red wine consumption is also associated with a reduction of the complications risk after a cardiac ischemia [24].

Experimental studies in animal models have shown that grape juice, red wine or isolated polyphenols like flavon, resveratrol and quercetin reduce the contractile dysfunctions of the heart and protect against cellular lesion induced by a cardiac ischemia [15, 20, 71, 91]. These effects may be observed following an oral intake of these substances or after their perfusion in an isolated heart before the induction of an ischemia. Using an *ex vivo* rat model of cardiac ischemia/reperfusion, we have recently published that short-term RWPC treatment protects against post-ischemic infarction *via* decreased oxidative stress and implies an involvement of NO-dependent pathway [83].

Cerebral ischemia is caused by an alteration in the cerebral blood flow, which leads to complex disorders and many factors contribute to the final outcome. The severe reduction of the blood flow initiates a series of interconnected pathophysiological mechanisms, such as impairment in energy metabolism, loss of ionic homeostasis, excessive release of excitatory amino acids (mainly aspartate and glutamate) and increase of the oxidative stress. All these processes lead to brain tissue damage and cell death. Polyphenols can interact

with the pathophysiological events that take place during cerebral ischemia. As protective agents, polyphenols can prevent the endothelial dysfunction. In fact, their properties in lowering blood pressure and reducing the lipid oxidation potentially prevent the initiation of atherosclerotic plaque development. Polyphenols can also have beneficial effects during stroke. Indeed, in different experimental models of stroke, resveratrol allows to reduce the infarct volume (for review, see Simonyi et al. [94]). The mechanisms involved in the neuroprotection may be inhibition of the lipid oxidation processes. Resveratrol can also activate peroxisome proliferators-activated receptor α. Furthermore, it can scavenge ROS such as O_2^- as the reperfusion occurs [92]. Moreover, polyphenols can interfere with the cell death mechanisms induced during stroke: Wang et al. have observed a decrease of the neuronal death with grape polyphenols [104].

We have investigated the potential neuroprotective effects of RWPC in a rat model of transient cerebral ischemia [21, 22]. RWPC was given orally during one week before the induction of the stroke. Microdialysis analysis has shown that polyphenols do not modify the energy metabolism and the oxidative stress, but are able to reduce the excitotoxicity by inhibiting the massive release of glutamate and aspartate. The cerebral blood flow has also been monitored and polyphenols can improve the residual blood flow both during occlusion and early reperfusion. Furthermore, polyphenols may induce a vascular remodeling, with an increase of the internal diameter of the brain vessels. As far as the brain infarct is concerned, the volume of the infarct is reduced by 60% with the RWPC treatment. To try to better understand the mechanisms involved in these experiments in terms of neuroprotection, proteomic studies have been performed on the rat brain submitted to transient cerebral ischemia. We have observed a modification of the expression level of proteins involved in the maintenance of neuronal caliber and axon formation, in the protection of oxidative stress and energy metabolism.

Conclusion

Due to the pleiotropic properties of polyphenols and the potential synergy of action on vascular endothelium when they are combined to each other, they may be good candidates in prevention and treatment of cardiovascular diseases. However, many clinical studies have used a mixture of several polyphenols, and the active molecule(s) is (are) not known and further studies needs to be done. Besides, a better knowledge of the bioavailability of polyphenols is needed; this may partly explain the apparent contradicting results obtained in the literature. Nevertheless, polyphenols may give hopeful picture of the future and may represent a new class of medicinal products against cardiovascular diseases.

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