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Review

Resveratrol: Preventing properties against vascular alterations and ageing

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Cardiovascular diseases are the leading cause of death in developed countries where the common pathological substrate underlying this process is atherosclerosis. Several new concepts have emerged in relation to mechanisms that contribute to the regulation of the vascular diseases and associated inflammatory effects. Recently, potential antioxidants (vitamin E, polyphenols) have received much attention as potential anti-atherosclerotic agents. Among the polyphenols with health benefic properties, resveratrol, a phytoalexin of grape, seem to be a good candidate protecting the vascular walls from oxidation, inflammation, platelet aggregation, and thrombus formation. In this review, we focus on the mechanism of resveratrol cardiovascular benefic effects. We analyze, in relation with the different steps of atherosclerotic process, the resveratrol properties at multiple levels, such as cellular signaling, enzymatic pathways, apoptosis, and gene expression. We show and discuss the relationship with reactive oxygen species, regulation of pro-inflammatory genes including cycloxygenases and cytokines in molecular inflammatory and aging processes, and how the regulation of these activites by resveratrol can lead to a prevention of vascular diseases.

Keywords: Aging / Antioxidant / Atherosclerosis / Inflammation / Review

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Abbreviations: Ang II, angiotensin II, **apoE**, apolipoprotein E; **CHD**, coronary heart disease; **COX-2**, cyclooxygenase 2; **DL**, lipoprotein; **EC**, endothelial cell; **ERK**, extracellular signal-regulated kinase; **fMLP**, formyl methionyl leucyl phenylalanine; **ICAM**, intracellular adhesion molecule; **IL-1**, interleukin-1; **LPS**, lipopolysaccharide; **MAPK**, mitogen-activated protein kinase; **MPO**, myeloperoxidase; **oxLDL**, oxidized LDL; **PDGF**, platelet-derived growth factor; **PKB**, protein kinase B; **PKC**, protein kinase C; **ROS**, reactive oxygen species; **SMC**, smooth muscle cell; **TNFα**, tumor necrosis factor α; **VEGF**, vascular endothelial growth factor; **VSMC**, vascular smooth muscle cell

1 Introduction

Vascular diseases including coronary heart disease (CHD), cerebrovascular and peripheral vascular diseases are the largest cause of mortality and morbidity in industrialized countries. For many decades, various investigations have searched to identify the risk factors in cardiovascular diseases, such as genetic factors, hypertension, and age. Some factors depend on our lifestyle, such as smoking and diets. Indeed, diet high in fat and/or calories can lead to hypertriglyceridemia, a potent atherogenic risk factor. Besides a high-energy diets, certain unsatured fatty acids may be pro-atherogenic and pro-inflammatory, while some nutrients may protect against vascular diseases and associated inflammatory effects. A protective effect may be obtained with a diet rich in vitamin E [1], β-carotene [2], and in polyphenolic compounds present in fruits, vegetables, and beverages. For example, in France, as compared with other western countries with a fat-containing diet, the strinkingly

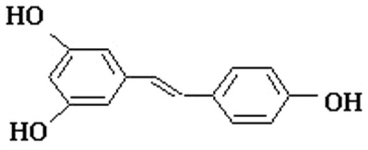

Figure 1. Chemical structure of resveratrol (3,5,4′-trihydroxystilbene in classical nomenclature).

low incidences of CHD have been partly attributed to the consumption of red wine, which contains high levels of polyphenols [3]. Similarly, benefic effects may be attributed to the flavonoids of green tea. Indeed, several cohort studies demonstrate a significant inverse association between flavonoid consumption and cardiovascular risk [4]. The benefic effects of these compounds seem to be due their antioxidant/antiradical activities protecting the vascular walls from oxidation, inflammation, platelet aggregation, and thrombus formation. Vascular wall stiffening is also age-dependent, due to in part to an enhancement of oxidative stress. Among the polyphenols with benefic properties, resveratrol, a phytoalexin of grape, reproduces the effect of a caloric restriction against the aging phenomena [5, 6]. Many studies evaluate resveratrol (Fig. 1) as a protective factor of degenerative diseases. Resveratrol possesses a myriad of cardiovascular benefic effects and can act at multiple levels, such as cellular signaling, enzymatic pathways, apoptosis, and gene expression.

2 Resveratrol and atherosclerosis

The main cause of the coronary damages and particularly ischemic vascular diseases is atherosclerosis. Briefly, the atherosclerotic process is the result of disruption of normal reactions between blood (plasmatic proteins, lipoproteins, growth factors, lymphocytes, platelets) and normal cellular elements of the arterial wall. So, various compounds can act at different cellular levels to brake the atherosclerotic lesion formation. These new anti-atherogenic components are present in the diet. Indeed, various antioxidant compounds presents in food, such as vitamin E, flavonoids, and polyphenols, could be good candidates against atherosclerosis. Among these polyphenols, resveratrol could be a good agent acting at different stages of atherogenesis (lipid accumulation and low-density lipoproteins (LDLs) oxidation; monocyte and lymphocyte infiltration; cellular smooth muscle proliferation and migration, platelet aggregation).

2.1 Resveratrol and lipoproteins

Target disruption of the apolipoprotein E (apoE) or LDL receptor (LDLR) genes, as well as overexpression of the human apolipoprotein B (apoB) gene in mice, results in marked increases in very-low-density lipoprotein (VLDL) and/or LDL levels and subsequently contributes to atherosclerosis promotion. In hypercholesterolemic mice (apoE-/-/LDLR-/-), resveratrol decreases the plasma lipid concentrations (total cholesterol and triacylglycerols) and reduces platelet aggregates [7]. The plasmatic concentration of lipids can also be reduced by the action of other apolipoproteins, such as apoB or apolipoprotein I/II (apo I/II). So, resveratrol is able to reduce apoB content and secretion (which may be responsible for impaired LDL and VLDL synthesis) as well as the intracellular content and the rate of secretion of cholesteryl esters from hepatoblastoma cells [8, 9]. The rate of secretion of triglycerides (TGs) is also reduced by resveratrol, but the intracellular TGs content is unaffected. Taken together, these changes would tend to decrease the level of VLDLs which are rich in TGs and possess potential atherogenic properties (direct supply of cholesterol to fibroblasts; alterations of endothelial functions; transformation of monocytes-macrophages in foam cells). These events are found also *in vivo* in rats where resveratrol treatment decreases serum TGs, VLDL+LDL-cholesterol levels [10]. By its estrogen-like structure, resveratrol could act on apoII. Indeed, the hepatic expression of apoII is in part modulated by the estrogen-mediated stabilization of its mRNA which is due to the estrogen-regulated mRNA stabilizing factor (E-RmRNASF). E-RmRNASF protects the RNA from target endonucleolytic degradation and its hepatic expression is modulated by estrogenic xenobiotics. Resveratrol seems to act as a phyto-estrogen and it acts as an agonistic compound stimulating the E-RmRNASF expression [11]. These results suggest that resveratrol would have the capacity to modulate and block certain aspects of hepatic lipoprotein metabolism which predispose to atherosclerosis.

2.2 Resveratrol and oxidative stress

The second important event in the lesion formation is LDL oxidation in the intima [12, 13]. Lipid peroxidation is a chain reaction process which can be induced by different free-radical sources (ionizing irradiation, UV light). Several groups have reported that oxidized-LDL (ox-LDL) can stimulate platelet aggregation [14] or promote procoagulant activity in the surface of human monocytes/macrophages by an increase in tissue thromboplastin activity [15] or by stimulating the expression and secretion of the tissue factor (TF) by monocytes or aortic endothelial cells [16].

Frankel *et al.* [17] were the first to demonstrate that resveratrol reduced the oxidation of human LDL induced by their incubation with metal ions, such as copper. This effect should be assigned to the chelation of copper because metals act as pro-oxidants by electron transfer, releasing

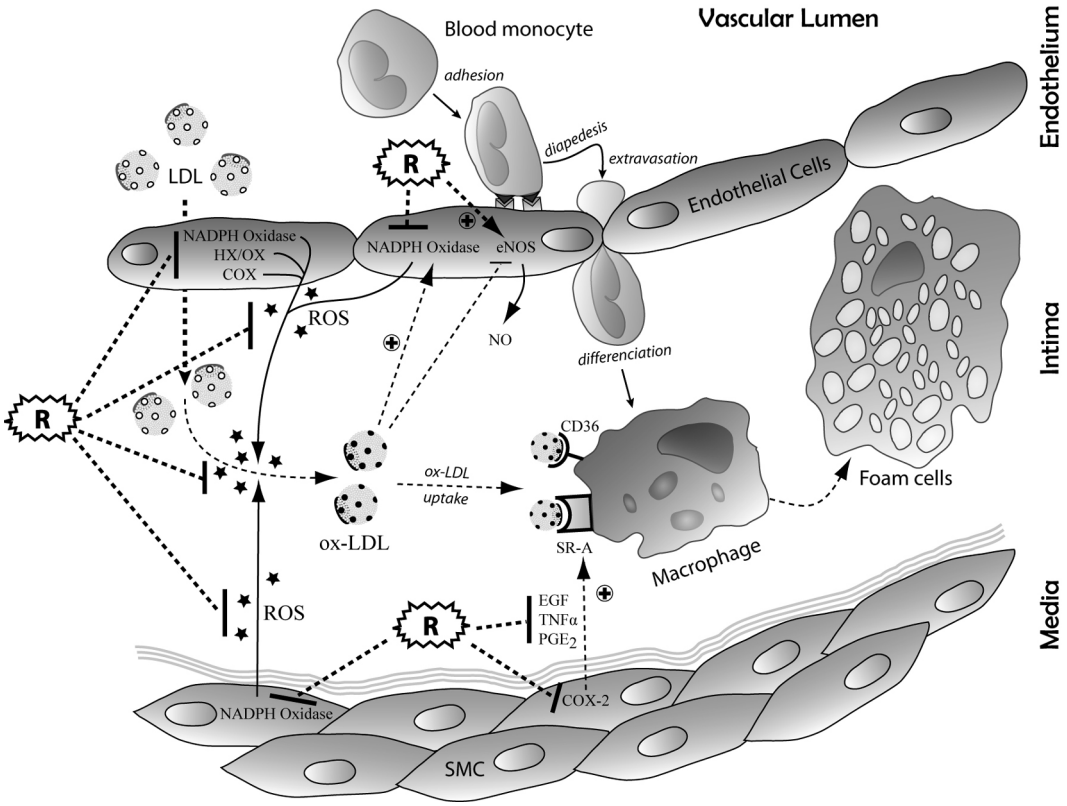

Figure 2. Resveratrol effects on initial events of atherosclerosis. Resveratrol (R) prevents the initial events by scavenging the ROS (★), by inhibition of the enzymatic systems producing ROS (NADPH oxidase, hypoxanthine/xanthine oxidase (HX/XO), cyclooxygenase (COX)), by downregulation of scavenger receptor (SR-A) stimulated by several factors, by induction of eNOS involving a vasorelaxation.

free radicals from polyunsaturated fatty acids and hydroperoxides. It has been demonstrated that resveratrol suppresses lipid peroxidation both by chelation of copper [18 – 20] and by scavenging of the free radicals [18, 19, 21]. The efficiency and action mechanism of *trans*-resveratrol have been demonstrated in the radical liposome oxidation where it appeared that the *para*-hydroxyl groups show a greater radical-scavenging activity than the *meta*-hydroxyl groups of *trans*-resveratrol [22]. Moreover, the spatial position of hydroxyl groups is likely more propitious to the chelation of copper in the *trans*-isomer than in the *cis*-isomer [19]. Due to its hydroxylated structure, resveratrol can form a radical derivative stabilized by the delocalization of two electrons between the two aromatic cycles and the methylene bridge joining these two cycles. In addition to metal ion-induced oxidation of LDLs, various enzymatic systems present in endothelial cells (ECs) or macrophages are implicated in the oxidation of LDL (Fig. 2). Resveratrol can act on these systems including nicotinamide adenine dinucleotide (NADPH)-dependent oxidases [23], hypoxanthine/xanthine oxidase (HX/XO) [24], 15-lipoxygenase (15-LO), myeloperoxidase (MPO), and nitric oxide synthases (NOSs). The inhibitory action of resveratrol on different systems is summarized in Table 1. The actions on these enzymes (Fig. 2) contribute to reduce the intracellular reactive oxygen species (ROS) formation in ECs [25] and to inhibit leukocyte adhesion [26 – 28].

Resveratrol is able to induce cellular antioxidants and phase 2 enzymes (see Table 1) [26, 28 – 31]. These modifications contribute to increase the resistance to cardiac cell injury elicited by ROS. Resveratrol reduced the generation of H_2O_2, and normalized the levels of oxidized glutathione reductase and MPO activities (Fig. 3) [32 – 34]. By normalization of the ROS levels, resveratrol limits the oxidative stress which inhibits NO synthesis by eNOS necessary for vasorelaxation (Fig. 3).

Oxidation induced by endothelial cells or by macrophages depends on lipoperoxides generated intracellularly and then transferred to the LDL. Cellular lipoxygenases, especially 15-LO, appear to be involved. Various studies demonstrated that resveratrol inhibits lipoxygenases, in particular in human neutrophils where resveratrol strongly inhibits the

Table 1. Resveratrol effects on enzymatic systems implicated in the oxidation of LDLs

Enzyme	Cell systems	Resveratrol actions	Resveratrol concentration	Ref.
NAD(P)H oxidase	Macrophage homogenates	Activity ↘	Res: 1 – 100 μM[a)]	[27]
	Rat aortic homogenates	Activity ↘	Res: 1 – 10 μM	[26]
	Endothelial cells	Prevents the strain-increased NADPH oxidase activity	Res: 0.1 – 100 μM	[23]
HX/XO oxidase	Rat cardiac cells	Inhibits XO/xanthine-mediated cytotoxicity	Res: 100 μM	[24]
	Isolated rat leukocytes	Prevents leukocytes recruitment	Res: 0.7 mg/kg	[28]
Myeloperoxidase	Human neutrophils	Activity ↘	Res: 10^{-6} – 10^{-2} mg/mL	[34]
	Mouse skin extracts	Normalizes the levels of activity	Res: 1-25 μM	[32, 33]
Superoxide dismutase	Chinese hamster lung fibroblasts	Activity ↗	Res: 100 μg/mL	[30]
	Rat cardiac cells	Activity ↗	Res: 25 – 100 μM	[24]
Catalase	Chinese hamster lung fibroblasts	Activity ↗	Res: 100 μg/mL	[30]
	Rat cardiac cells	Activity ↗	Res: 25 – 100 μM	[24]
	Cardiac tissue	Activity ↗	Res: 14 mg/kg/day	[29]
Glutathione peroxidase	Chinese hamster lung fibroblasts	Activity ↗	Res: 100 μg/mL	[30]
	Human lymphocytes	Activity ↗	Res: 10 – 100 μM	[31]
Glutathione reductase	Rat cardiac cells	Activity ↗	Res: 25 – 100 μM	[24]
	Human lymphocytes	Activity ↗	Res: 10 – 100 μM	[31]
Glutathione-*S*-transferase	Rat cardiac cells	Activity ↗	Res: 25 – 100 μM	[24]
	Human lymphocytes	Activity ↗	Res: 10 – 100 μM	[31]
NQO1	Rat cardiac cells	Activity ↗	Res: 25 – 100 μM	[24]

a) Res: *cis*-resveratrol; HX/XO, hypoxanthine/xanthine oxidase; NQO1, NAD(P)H, quinone oxidoreductase 1; ↗ increases the activity of the enzyme cited in the first column; ↘ decreases the activity of the enzyme cited in the first column

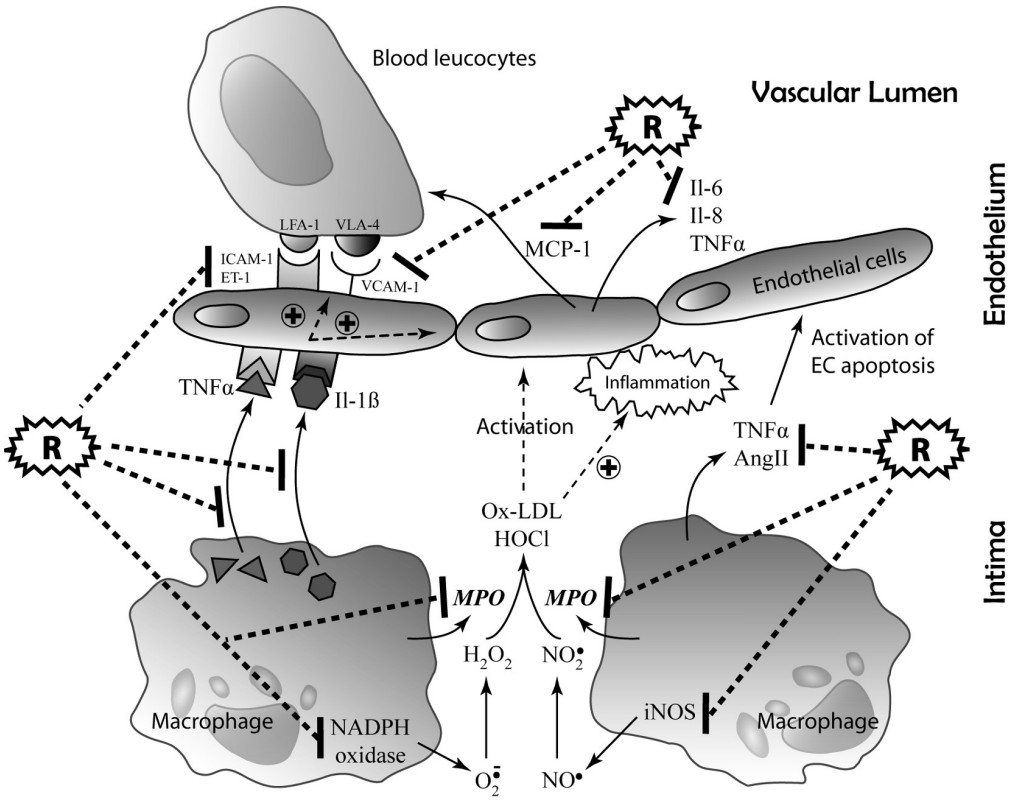

Figure 3. Resveratrol effects on chemokine production. Resveratrol (R) decreases significantly the expression of ICAM-1 and VCAM-1 induced on endothelial cells by TNF-α or lipopolysaccharide LPS, as well as neutrophile and monocyte endothelial adhesion. Resveratrol can also inhibit iNOS as well as MPO secreted from macrophages and so reduces the endothelial activation and inflammation mediated by oxLDLs. The polyphenol can reduce the apoptosis of ECs induced by TNFα and AngII.

5-LO and 15-LO producing various proinflammatory products in the arachidonate metabolism [35 – 38].

In addition to metal ions and ROS, ferrylmyoglobin and peroxynitrite are also potent oxidants implicated in oxidation of LDLs. Resveratrol was able to decrease the accumulation of hydroperoxides in LDL promoted by ferromyoglobin by reduction of the oxoferryl complex to metmyoglobin. Moreover, this polyphenol inhibits LDL apoprotein modifications induced by peroxynitrite [39]. ROS production by polymorphonuclear leukocytes stimulated by formyl methionyl leucyl phenyalanine (fMLP) can be also strongly inhibited by resveratrol [40]. Moreover, resveratrol could act on targets in blood cells and in lipoproteins. Indeed, resveratrol was incorporated into blood cells and lipoproteins after *in vitro* incubation with plasma, lipoproteins, and cells [41]. In fact, due to its lipophilic character, resveratrol is able to bind the lipoprotein particles suggesting that this event improved its antioxidant activity [42]. In lipoprotein particles, resveratrol is predominantly associated with their lipid moiety, but can also be associated with the protein moiety. Among plasma proteins, serum albumin could be involved [43]. This binding could explain that resveratrol reduces the oxidative alterations of lipid and protein moieties of LDL [18]. By protecting apoB domains involved in the receptor activity of cells, resveratrol could reduce the nonspecific uptake of oxLDL by macrophages.

2.3 Resveratrol and macrophages

Under normal conditions, the monocytes enter, through diapedesis, the subendothelial space, where they differentiate into macrophages (Fig. 2). Under endothelial dysfunction, circulating monocytes adhere to the arterial endothelium, migrate to the subendothelial space, and differenciate into resident macrophages within the subendothelial matrix. OxLDLs stimulate the expression of scavenger receptors CD36 and the class A scavenger receptor (SR-A) within monocytes, macrophages, and smooth muscle cells (SMCs) (which normally do not express this receptor). These receptors internalize the oxLDL in a specific manner, leading to a massive accumulation of cholesterol esters until foam cells are formed. These macrophage-derived foam cells make up the fatty streak that precedes more advanced sclerotic lesions (Fig. 2).

Oxidative stress caused by phorbol esters or ROS upregulates the SR-A in human SMCs, which normally do not express this receptor [44]. Resveratrol inhibits the activity and the expression of SMC cyclooxygenase-2 (COX-2) which normally produced prostaglandin E_2 (PGE_2) which upregulates SR-A expression [44]. Various growth factors, such as interleukin-1 (IL-1), tumor necrosis factor α (TNFα), epidermal growth factor (EGF), platelet-derived growth factor (PDGF), and transforming growth factor β (TGFβ) increase SMC SR-A activity [45]. Resveratrol could be able to decrease SMC SR-A activity through the action of these factors, such as the decrease of EGF [46] (Fig. 2). So, by the reduction of the interaction between oxLDL and macrophage scavenger receptors (playing an atherogenic role), resveratrol contributes to prevent an early step in atherogenesis. At a molecular level, the acute formation of oxLDL induced by ROS leads to the activation of mitogen-activated protein kinase (MAPK) pathways, which might be important for mitogenic signaling of oxLDL in vascular SMCs (VSMCs) (see below Fig. 5). Resveratrol inhibits oxLDL-induced mitogenesis of VSMCs through the blocking of the ROS generation and the activation of the extracellular signal-regulated kinase (ERK) pathway [47].

2.4 Resveratrol and foam cell formation

We have previously mentioned that oxLDLs favor the transformation of macrophages into foam cells. The development of foam cells that contain massive amounts of cholesterol ester is a hallmark of both early and late atherosclerotic lesions. OxLDL-derived cholesterol brought into the macrophage *via* scavenger receptors consists of free cholesterol as well as of cholesterol esters that are hydrolyzed in lysosomes. In addition, oxLDLs stimulate ECs to produce chemokines, granulocyte and macrophage colony-stimulating factors and they have direct chemotactic activity for monocytes to endothelium. Resveratrol contributes to reduce the production of chemokines which may be responsible for the chemotaxis and accumulation of macrophages in fatty streaks (Fig. 3). Resveratrol is able to inhibit interleukin-6 (IL-6) release by stimulated peritoneal macrophages in mice [48, 49], and in cortical mixed glial cells [50]. This action could result from a blocking of calcium ion influx by resveratrol (see Section 2.8). Moreover, resveratrol contributes to reduce inflammatory response in atherosclerosis when macrophages (or SMCs, ECs) appear to be activated and produce numerous inflammatory products, such as TNFα, IL-6, monocyte chemoattractant protein-1 (MCP-1) (Fig. 3). Lesion progression is influenced by interactions between monocyte/macrophage and T cells. Lesional T cells appear to be activated, expressing both Th1 and Th2 cytokines [51] (Fig. 4). Resveratrol was able to inhibit the release of Th1-derived cytokines, such as interferon γ (INFγ) which stimulates macrophage production of pro-inflammatory cytokines, IL-2 production by splenic lymphocytes, and TNF-α and IL-12 production by peritoneal macrophage [52 – 54] (Fig. 4). The expression of mRNA encoding MCP-1 was also blocked by resveratrol [55]. Resveratrol was also able to inhibit Th2-derived cytokines, such as IL-4, which exerts antagonistic effects on INFγ activity in macrophages and inhibition of Th1 cell function. Resveratrol inhibits the lipopolysaccharide

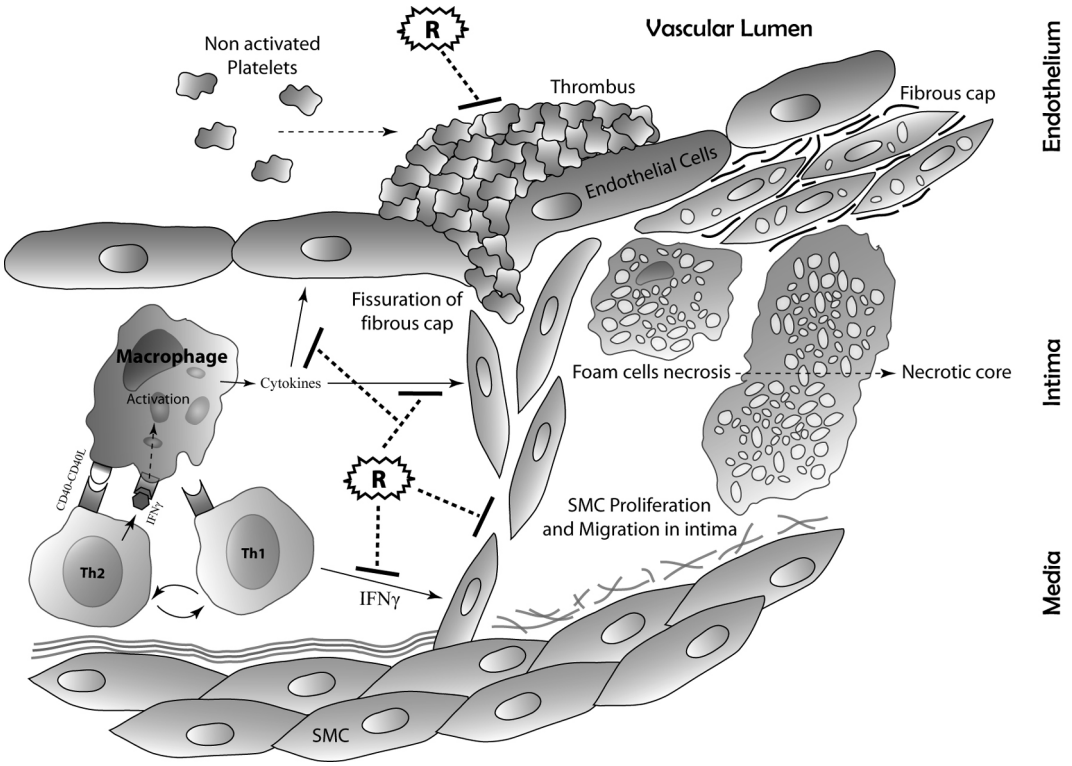

Figure 4. Resveratrol effects on advanced atherosclerotic lesion formation. Resveratrol (R) blocks the cytokine production and reduces the SMC proliferation and migration. Furthermore, resveratrol inhibits paletelet aggregation as well as pro-aggregants/pro-inflammatory agents (eicosanoids, leukotrienes) and subsequently inhibits the formation of a thrombus.

(LPS)-induced expression of IL-1mRNA in monocytes and ECs [56]. Concerning IL-8, the gene transcription as well as the protein production are inhibited by resveratrol [57].

This inhibition of cytokine production by resveratrol is important for the regulation of adhesion molecule expression. Indeed, activated T lymphocytes and macrophages generate and release several cytokines with a number of biological effects on neighbouring cells [58]. So, various proinflammatory stimuli (*e.g.*, interleukins, INFγ, TNFα, LPS) induce the expression of vascular adhesion molecule-1 (VCAM-1) and intracellular adhesion molecule-1 (ICAM-1). These molecules mediate the firm adhesion of monocytes to the vascular endothelium in early atherosclerosis stages (Figs. 2 and 3). Like others compounds of the tyrphostine family which possess tyrosine kinase inhibitory activity [59, 60], resveratrol inhibits both the stimulated expression of VCAM-1 and monocyte adhesion to human vascular endothelial cells [61, 62]. These effects also affect E-selectin and ICAM-1. Indeed, resveratrol decreased significantly the expression of ICAM-1 and VCAM-1 induced on endothelial cells by TNF-α or LPS [63], as well as neutrophil and monocyte endothelial adhesion [64, 65]. This inhibition of adhesion molecule expression occurs in rat at the same resveratrol plasmatic concentrations ranging from 100 nmol/L to 1 μmol/L [61, 66]. It has been suggested that resveratrol may act as a rapid molecular signal interfering in the mechanism of VCAM-1 and ICAM-1 expression [67]. Vascular ECs can also be activated by proteolytic enzymes, such as elastase, which cause detachment or lysis of ECs and degradation of subendothelial matrices [68] and stimulate EC secretion of growth factors for SMCs [69]. Resveratrol inhibits the release of lastase and by polymorphonuclear leukocytes stimulated by fMLP and C5a and also inhibits their secretion [40]. So this modification of adhesion may support the use of resveratrol as an immunomodulating compound.

2.5 Resveratrol and VSMCs

VSMCs contribute to the pathogenesis of atherosclerotic lesions, since their proliferation and migration are critical events for progressive intima thickening and development of arterial wall sclerosis. OxLDLs can also promote the proliferation of the smooth muscle cells (SMCs) which are in part of resident intimal cells that preceded the lesions and in part their progeny that arose as a response to various stimuli (*e.g.*, lipid accumulation, disruption of intimal structure). Intima SMCs accumulate large amounts of cholesterol

esters and become foam cells (Fig. 4). Inhibition of VSMC proliferation may have a beneficial effect in retarding the development of atherosclerotic disease.

Resveratrol could delay atherogenesis by inhibition of VSMC proliferation [70, 71]. Indeed, resveratrol is able to reduce SMC proliferation induced by diverse mitogens, such as serum, endothelin, and PGDF. The antimitogenic effects of resveratrol are not mediated by the induction of apoptosis, but appear to be related to blocking G1 → S transition of cell cycle [72, 73] and DNA synthesis [71]. In fact, resveratrol leads to a reversible arrest in the early S phase of the VSMC cycle. However, the molecular mechanism is so far controversed: Haider *et al.* [73] have shown that the VSMC cycle arrest was accompanied by an accumulation of an hyperphosphorylated retinoblastoma protein, a decrease of cellular levels of the cyclin-dependent kinase inhibitors p21(Cip1), p27(Kip1), and an enhancement of phosphorylation of the p53 protein. On the contrary, Mnjoyan and Fujise [71] have shown that p21 and p53 are increased but this effect depends on the resveratrol concentration. Indeed, at lower concentration (6.25 – 12.5 μM), resveratrol inhibits VSMC proliferation without apoptosis, but at higher concentration (25 μM) resveratrol induces apoptosis in serum-stimulated VSMCs but not in quiescent VSMCs. These results suggest that resveratrol may be able to selectively eliminate abnormally proliferating VSMCs of the arterial walls *in vivo*. Resveratrol can also inhibit VSMC proliferation induced by advanced glycation end-product (AGEs) of plasma proteins and/or matrix proteins which are mediators implicated in various vascular complications [74]. AGEs increase coagulation through various mechanisms involving the vascular endothelium and platelet activation [75]. AGEs also increase DNA synthesis and propyl hydroxylase activity, a marker of collagen synthesis in rats VSMCs. These phenomena are inhibited by resveratrol in an animal experimental model [76]. In this same perspective of fighting against atherosclerosis process, it has been shown that the inhibition of pulmonary artery endothelial cell proliferation by resveratrol is correlated with the suppression of cell progression through S and G2 phases of the cell cycle [77, 78].

2.6 Resveratrol and vasorelaxation

Resveratrol is able to inhibit the production of endogenous vasoconstrictors and thereby regulates vasomotion which is impaired in atherosclerosis. The key regulators of the vasomotor function are the vasodilatator NO and the vasoconstrictor endothelin-1 (ET-1). In VSMCs, oxidative stress increases ET-1, which is involved in endothelial dysfunction, generation, and autocrine ET-1 activity. Resveratrol inhibits strain-induced ET-1 secretion [23, 79], ET-1 mRNA level, and ET-1 promoter activity [23]. This inhibition of strain-induced ET-1 gene expression was partially due to resveratrol attenuation of activator protein 1 (AP-1)-binding activity and resveratrol interference in the ERK1/2 pathway through attenuation of ROS formation [23] (Fig. 5). Resveratrol inhibits ET-1 surproduction and cytosolic phospholipase A_2 (PLA_2) activity stimulated by oxidative stress [79]. ET-1 expression can be induced by several substances such as angiotensin II (Ang II), thrombin, PDGF-A, and TNFα [80]. So, resveratrol can reduce ET-1 expression by its action on the latter factors. Indeed, resveratrol can act on Ang II. Ang II-induced hypertrophy of vascular VSMCs is a pivotal step in the development of CHDs. Resveratrol could fight Ang II-induced VSMC hypertrophy by interfering with the phosphatidylinositol 3-protein (PI3K)/Akt and p70 ribosomal protein S6 kinase (p70(S6K)) [73, 81]. Indeed, resveratrol is able to attenuate the phosphorylation of p70(S6K) as well as the phosphorylation of Akt/protein kinase B (PKB) and ERK1/2, both essentially involved in Ang II-mediated hypertrophy (Fig. 5). This resveratrol action on Ang II can protect from cardiac fibrosis which results of a prolonged activation of cardiac fibroblasts (CFs) leading to a reduction of myocardial contractile function. Resveratrol inhibits Ang II-induced ERK1/2 and ERK kinase activation in CFs [82]. Moreover, pretreatment of CFs with resveratrol reduced both Ang II- and TGFβ-induced CF differentiation to the myofibroblast phenotype, indicated by a reduction in α-smooth muscle actin expression and stress fiber organization in CFs. So, resveratrol appears to act as an antifibrotic agent in the myocardium. Furthermore, the reduction of Ang II concentrations would reduce the increase of NADPH oxidase-derived ROS. ET-1 activates specific receptors, designated as ET_A and ET_B [83]. So, resveratrol by its action on PLA_2 and other signalling pathways appears to protect against VSMC contraction mediated by the ET_A-receptor.

The inhibition of strain or the induction of vasorelaxation can also be dependent on NO production, Na^+ concentration, or cGMP pathways. For NO production, it has been clearly documented that resveratrol can modulate the NO level by its action on both eNOS and iNOS. Under normal conditions, ECs produce NO at a low level to control vessel dilatation. However, in atherosclerosis, a high level of NO has been found within early lesions and advanced atheroma even though expression of eNOS is reduced. On the contrary, the inductible Ca^{2+}-independent NOS, also known as iNOS, is increased. It has been shown that resveratrol can cause NO-mediated relaxation of precontracted endothelium-intact rat aorta through an increase of NO *via* eNOS [84 – 88]. At the molecular level, resveratrol enhances eNOS expression and inhibits iNOS expression at the promoter level (see below resveratrol and nuclear targets). Various studies have reported the similarity between the resveratrol structure and diethylstilbestrol, a synthetic phytoestrogen. It seems that resveratrol could be a nonflavo-

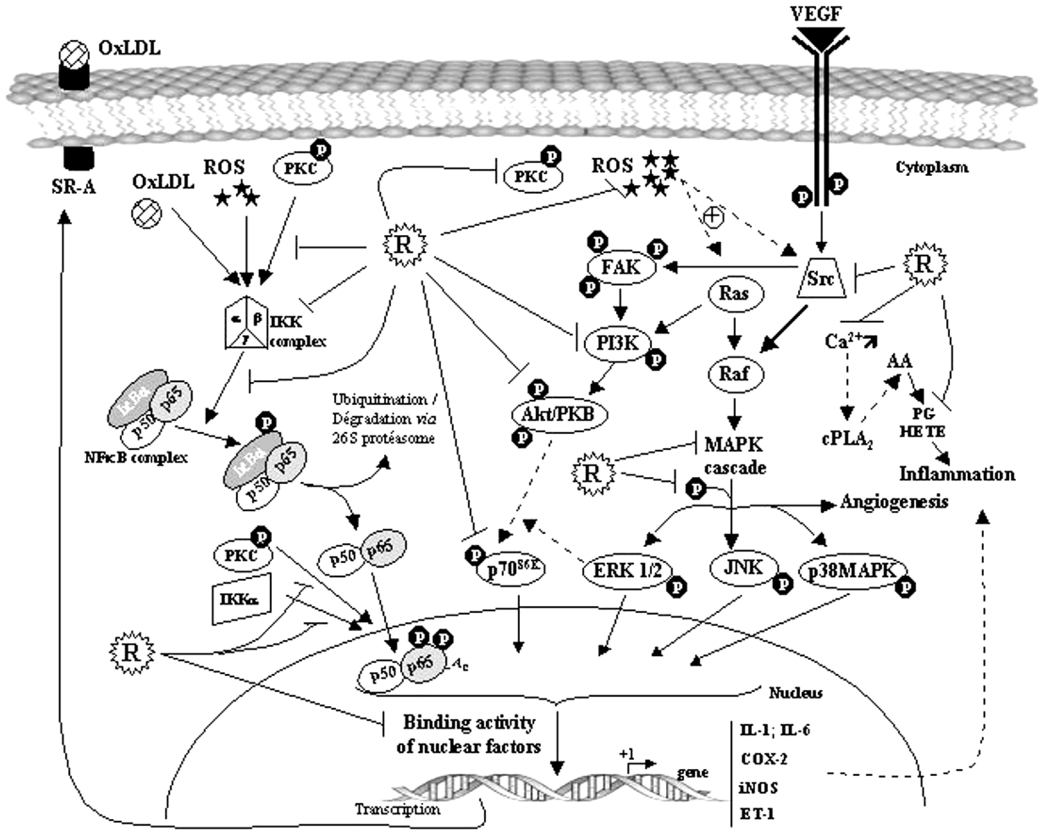

Figure 5. Resveratrol effects on signaling pathways. Resveratrol inhibits VEGF-induced angiogenesis by disruption of ROS-dependent Src kinase activation. By this action on Src and MAPK cascade, resveratrol inhibits angiogenesis and the translocation of nuclear factors into the nucleus from the cytoplasm. Moreover, resveratrol inhibits exogenous oxidants, calcium flux, and synthesis of pro-inflammatory compounds. The polyphenol can also inhibit stimuli involved in the activation of NFκB pathway, such as PKCα signal transduction, IκB phosphorylation, IKK activity, and p50/p65 nuclear translocation. These effects on the signaling pathways lead to a downregulation of many gene transcription (COX-2, iNOS, VACM, SR-A, *etc.*) involved in atherosclerotic process and inflammation.

noid phytoestrogen, and act as an estrogen receptor agonist [89–91]. As well as estrogens which can upregulate eNOS by increasing promoter activity and enhancing the binding activity of the transcription factor Sp1, resveratrol increases the activity of the eNOS promoter, eNOS mRNA stabilization, as well as eNOS protein and activity [92]. This upregulation induced by resveratrol is not prevented by a receptor agonist. This upregulation of eNOS could involve a transcriptional mechanism by the binding of transcription factors (*e.g.*, Sp1, GATA, PEA3, YY1) to the proximal portion of the eNOS promoter. But Wallerah *et al.* [93] showed that resveratrol did not change protein-DNA binding, suggesting that the transcriptional activation induced by resveratrol could involve a multifactoriel process or another transcription factor not tested. On the contrary to the eNOS expression, resveratrol inhibits iNOS expression by blocking transcription of its gene through downregulation of NFκB-binding activity. The vasorelaxation mediated by the polyphenol was reversed by the constitutive Ca^{2+}-dependent NOS (cNOS). The compound also induces a NO-independent vasodilatation on the denuded aorta, and the vasorelaxative activity of resveratrol depends also on the direct stimulation of K^+/Ca^{2+} channels in ECs [93]. So, it seems that the ability of resveratrol to modulate calcium channels in ECs could contribute to control the vasorelaxation mediated by NO (see Section 2.8).

Concerning the cGMP pathway, resveratrol increases cGMP in coronary arteries, mostly by activation of pGC [94]. Resveratrol activates membrane-bound guanylate cyclase GC-A, the receptor for atrial natriuretic factor (ANF) [95]. At molecular level, the cGMP/kinase-G is an antiproliferative signaling in SMCs and it dilates blood vessels through the reduction of intracellular calcium. The cytotastic actions of cGMP in SMCs involve apoptosis, inhibition of PI3K and MAPKs interfering with the cell-cycle machinery [96]. So the activation of pGC by resveratrol triggers vasorelaxant responses that remain effective in endothelium-disrupted arteries.

Resveratrol could also influence the vasorelaxation through an action on the activity of BK(Ca) channels which are functionally expressed in vascular ECs; it controls the K^+ efflux and affects intracellular Ca^{2+} concentration. In fact, resveratrol opens large and small conductance Ca^{2+}-activated K^+ (BKCa) channels, but not ATP-sensitive K^+ channels [97] and increases the activity of large conductance BKCa channel in ECs [93, 98]. The resveratrol-stimulated increase in the channel activity is independent of internal Ca^{2+}. So, the increase in K^+ efflux through resveratrol-induced stimulation of KCa channels in ECs may contribute to produce vasorelaxation.

2.7 Resveratrol and angiogenesis

Angiogenesis is important in atherosclerosis where EC migration and proliferation are essential events in this process. The vascular endothelial growth factor (VEGF) co-localizes with endothelial cells, macrophages, and SMCs in atherosclerotic plaques. Resveratrol inhibits VEGF-induced angiogenesis by abrogating VEGF-mediated tyrosine phosphorylation of vascular-cadherin and its complex partner, β-catenin [99]. The inhibition of VEGF-induced angiogenesis is mediated by the disruption of ROS-dependent Src kinase activation and the subsequent VE-cadherin tyrosine phosphorylation. Resveratrol can also reduce VEGF by its action on NADPH oxidase [26, 27] which regulates the induction of VEGF expression [100] and the VEGF-induced angiogenesis [101]. VEGF expression can be also regulated by pro-apoptotic factors such as Ang II, which may be accumulated in response to EC damage. Consequently to its effect on Ang II, resveratrol can inhibit Ang II-mediated VEGF expression [81]. Furthermore, it inhibits both the fibroblast growth factor (FGF) receptor- and the VEGF receptor-mediated EC responses [102]. Furthermore, other factors are involved in angiogenesis, such as protein kinase C (PKC) and cyclooxygenase. Similar to several PKC inhibitors, resveratrol, which inhibits various isoforms of PKC (see Section 2.9), could prevent angiogenesis through kinase blockage. Likewise, recent studies reported that cyclooxygenase-1 regulates angiogenesis in vascular ECs [103]. So, by this inhibitory action on cyclooxygenase gene expression (see Section 2.9) resveratrol could inhibit angiogenesis. Unlike single angiogenic factor antagonists, the therapeutic value of resveratrol (at very low concentrations of 1 – 2.5 μM) is that it blocks a common angiogenic pathway triggered by several angiogenic factors.

2.8 Resveratrol and platelet aggregation/ thrombosis

Platelets contribute to the rate of development of atherosclerosis and CHD through several mechanisms. It has been shown that resveratrol reduces platelet aggregation in human platelet-rich plasma in particular after induction by thrombin and adenosine-5′-diphosphate (ADP) treatment [104 – 106]. These *in vitro* results were found again *in vivo* [107]. In fact, thrombin downregulates endothelial ectonucleotidase activity resulting in high levels of ADP and ATP which lead to platelet and endothelial activation. Resveratrol inhibits thrombin-induced ADP and ATP secretion from platelets, decreases the neutrophil function, and restores the CD39/ATPDase (ATP diphosphohydrolase) activity in response to thrombin [108]. Furthermore, when activated by thrombin, platelets produce ROS. This free radical generation can be reduced by resveratrol in blood platelets [109, 110]. In addition to thrombin and ADP, the platelet-activating factor (PAF) has been reported to be also involved in atheromatosis generation. Resveratrol was able to inhibit PAF-induced platelet aggregation [111] and its pro-inflammatory effects [28]. The PAF-induced platelet aggregation is accompanied by the release of thromboxane A_2 (TxA_2), a pro-aggregant and vasoconstrictor agent. Moreover, PAF stimulates polymorphonuclear leukocytes to aggregate, to release leukotrienes, and to generate superoxides. Similarly, PAF promotes aggregation of monocytes. So, resveratrol inhibiting PAF effects can reduce the effects of pro-aggregants/pro-inflammatory agents, such as eicosanoids and leukotrienes.

The synthesis of eicosanoids and leukotrienes from arachidonic acid is also linked to the platelet aggregation. The synthesis of products from arachidonic acid in human platelets occurs according to several pathways, such as the lipoxygenase pathways, the cyclooxygenase (COX) and the prostaglandin H synthase (PHS) pathways. Resveratrol is able to act on the lipoxygenase family. Towards the substrate (linoleic acid), resveratrol inhibits both 5-lipoxygenase and 15-lipoxygenase as a competitive inhibitor [37, 38]. Resveratrol prolongs the lag phase of both enzymes, indicating a possible reduction of Fe(III) to Fe(II) at the catalytic site [112]. Pinto *et al.* [37, 113] have shown that resveratrol inhibits the dioxygenase activity of lipoxygenase and is simultaneously oxidized by the peroxidase activity of lipoxygenase. The oxidized form of resveratrol is a lipoxygenase inhibitor as efficient as the reduced form. This lipoxygenase inhibition by resveratrol prevents the release of pro-inflammatory substances (see Table 2) [35, 40, 114] and consequently blocks the synthesis of hepoxilins, mediators of calcium mobilization, vascular permeability, and neutrophil activation [115, 116].

Resveratrol is also a competitive inhibitor of COX and peroxidase activity of PHS [38, 117]. As far as PHS is concerned, both COX and peroxidase activities depend on ferriprotoporphyrin IX [118, 119]. Again, the prolonged lag phase of the COX reaction was indicative of a reduction of Fe(III) to Fe(II) [119, 120]. COX inhibition by resveratrol

Table 2. Effects of resveratrol on arachidonic acid metabolism

Arachidonic acid metabolism	Resveratrol actions	Resveratrol concentrations	Cell systems	Ref.
Enzymes of arachidonic acid metabolism				
Cyclooxygenase	Inhibits activity	Res: 5 – 100 μM	Human erythroleukemia cells	[38, 117]
Prostaglandine H synthase	Inhibits the cyclooxygenase and peroxidase activities	Res: 5 – 100 μM	Human erythroleukemia cells	[38]
5-Lipoxygenase	Inhibits activity	Res: 1 – 10 μM	Human erythroleukemia cells	[38]
	Inhibits the dioxygenase activity	Res: 13 μM		[37]
15-Lipoxygenase	Inhibits activity	Res: 1 – 10 μM	Human erythroleukemia cells	[38]
12-Lipoxygenase	Inhibits activity	–	–	[117]
Products of arachidonic acid metabolism				
5-HETE, 5,12-diHETE, 12-HETE, LTB4, 6-*trans*-LTB4, 6-*trans*-*epi*-LTB4, LTC4, TxB4, TxB2	Prevents the release or the production of the products which are pro-inflammatory, pro-aggregant, and vasoconstrictor agents	Res: 48 μM	Polymorphonuclear leucocytes	[40]
		Res: 1 – 100 μM	Platelets	[116]
		Res: 0.6 – 10 μM	Polymorphonuclear leukocytes	[35, 114]
		Res: 30 μM	Human erythroleukemia cells	[38]
		–	3T6 fibroblast	[122]
		–	Murine resident peritoneal macrophages	[121]
		–	Human erythroleukemia cells	[117]

HHT, hydroxyheptadecatrienoate; 12-HETE, 12-hydroxyeicosatetraenoate; 5-HETE, 5-hydroxy-6,8,11,14-eicosatetraenoic acid; 5,12-diHETE, 5,12-dihydroxy-6,8,10,14-eicosatetraenoic acid; LTC4, leukotriene C4; LTB4, leukotriene B4; TxB4, thromboxane B4; TxB2, thromboxane B2

prevents the release of COX products, such as prostaglandins and thromboxanes (see Table 2) [35, 40, 114, 116, 117, 121, 122]. By inhibition of PLA_2, resveratrol decreases the release of arachidonate from cell lipids and thus the synthesis of metabolites by COX and lipoxygenase pathways [122]. Moreover, the polyphenol could act on the Ca^{2+} influx, subsequently reducing the activation of PLA_2 and the aggregation process. Indeed, an increase in intracellular free Ca^{2+} is an essential component of the aggregation process in platelets. Ca^{2+} must enter the cell from the external media through specific and tightly regulated Ca^{2+} channels in the plasma membrane. It appears that resveratrol is an inhibitor of store-operated Ca^{2+} channels and calcium influx in thrombin-stimulated human platelets [123 – 125]. Moreover, the blocking of calcium ion influx into cultured murine macrophages by resveratrol is one of the possible mechanisms of the pro-inflammatory IL-6 biosynthesis inhibitory action of resveratrol [48]. Nevertheless, Slater *et al.* [126] found that the resveratrol-dependent inhibition of PKCα activity is competitive with respect to phorbol ester but not competitive with respect to Ca^{2+} and phosphatidylserine suggesting that resveratrol competes for phorbol ester binding site to the C1 domains.

Ca^{2+} regulates various pathways and is a major second messenger implicated in signal transduction pathways regulating cell cycle, proliferation, and apoptosis. Several pro-atherogenic stimuli induce EC apoptosis through Ca^{2+}-dependent pathways and contribute to the development of vascular lesions. OxLDL-mediated endothelial cell apoptosis is dependent on an increase in intracellular Ca^{2+}. Thus, alterations in intracellular Ca^{2+} in ECs may cause EC dysfunction in response to oxLDL and may influence the EC response to oxLDL and inflammatory cytokines, particularly TNFα. Resveratrol blocking of the Ca^{2+} influx could prevent EC apoptosis. Several matrix elements play also an important role in platelet aggregation, such as collagen and fibrinogen. Resveratrol was shown to block the first step of platelet activation by inhibiting platelet adhesion to type I collagen and to decrease collagen-induced platelet aggregation [127, 128] (Fig. 4). Moreover, resveratrol inhibited the messenger RNA (mRNA) expression of type I collagen [129]. At the cellular level, in the platelets resveratrol can inhibit MAPK activation induced by collagen, thrombin, and ADP [130] (Fig. 5). So, resveratrol could block receptor-mediated signaling events in platelets.

Concerning the blood platelet adhesion to fibrinogen, another initial step of platelet activation, resveratrol inhibits adhesion of both thrombin- and ADP-activated platelets to fibrinogen [131] or after activation by LPS or LPS with thrombin [128, 132]. Moreover, resveratrol could protect against atherosclerosis by promoting fibrinolysis. Indeed the polyphenol such as others compounds (catechin, epicatechin) is able to upregulate the gene transcription of both tissue-type plasminogen activator (t-PA) and urikinase-type PA (u-PA), which are fibrinolytic proteins [133]. A few studies have demonstrated only quantitative differences in the activity of the two forms; for example, the *cis*-isomer induces a greater decrease in collagen-induced platelet aggregation than the *trans*-isomer [127], and in the cyclooxygenase-1 assay, *trans*-resveratrol appears to be more

active than *cis*-resveratrol [134]. In fact, it appeared that the two isomers exert a protective effect against the oxidation and the production of proinflammatory substances. As well as *trans*-resveratrol, *cis*-resveratrol protects against oxidation by inhibiting ROS production, decreasing NAD(P)H oxidase activity [27]. On the signaling targets, it seemed that both *trans*- and *cis*-isomers of resveratrol possess comparable protein-tyrosine kinase inhibitory activities [135]. On the nuclear targets, *cis*-resveratrol, as *trans*-resveratrol, modulates the NF-κB signaling pathway and consequently blocks the expression of genes related to this pathway. So, *cis*-resveratrol downregulates the ICAM-1 gene, synthesis, NOS and COX-2 mRNA synthesis and their protein, the transcription of various chemokines, such as Scya2 (chemokine MCP-1), and inhibits proinflammatory mediator production, such as nitric oxide and prostaglandins [27, 136]. So, by the important antioxidant properties of *cis*-resveratrol, a consumption of food containing resveratrol could lead to cardioprotective effects by the combinated effects of the two isomers.

Thrombosis plays a critical role in the development, progression, and clinical after-effects of atherosclerosis. The primary initiators of thrombus (mostly based on platelet aggregates) are the cell surface receptor for factor VII (a), and the tissue factor (TF). The reduction of TF expression in vascular cells, ECs, and monocytes may also contribute to the anti-aggregatory effects of resveratrol [56, 137] (Fig. 4). In fact, resveratrol reduced TF activity and TFmRNA level by inhibition of nuclear factor kappa B (NFκB)/c-Rel-dependent transcription and by impairing the transactivation potential of p65 [137, 138]. The diminution of c-Rel/p65 activity was dependent on inhibition of degradation of the c-Rel/p65 inhibitory IκBα (inhibitor of κB) [138] (Fig. 5). The antithrombotic properties of resveratrol have been also shown *in vivo*. Indeed, resveratrol orally administrated with a high-fat diet in genetically hypercholesterolemic mice (apoE-/-/LDLR-/-) suppressed the formation of atheroma in the aortae and reduced the laser-induced thrombosis in their carotid arteries [7].

2.9 Resveratrol and signaling/nuclear targets (Table 3)

Specific nonantioxidant effects of resveratrol in cellular signaling and regulation of gene expression have been studied and have an important impact on atherosclerosis development. Resveratrol was able to act on the MAPK cascade. Downstream targets for the action of MAPKs include mitogenic/pro-inflammatory enzymes and nuclear transcription factors (Fig. 5). Resveratrol is able to act at different levels. Indeed, resveratrol is able to act on an upstream pathway by inhibiting the phosphorylation and the activity of PKC [126, 135, 139]. Resveratrol inhibits PKC-catalyzed phosphorylation of arginin-rich protein substrate in a noncompetitive manner [140]. The potency of resveratrol depends on the nature of the substrate and cofactors [140]. As diacylglycerol, resveratrol interacts with the C1 domains and induces the association of PKCα with membrane vesicles. Resveratrol can also inhibit other kinases, such as Src, which activates MAPK cascade [99]. Resveratrol also inhibits the PI3K phosphorylation and prevents the Akt/PKB phosphorylation. Consistent with this action, resveratrol attenuates the phosphorylation of $p70^{S6K}$ which was shown in VSMCs to require both the Akt/PKB and the ERK signaling cascades [81]. Consequently, resveratrol disturbs the protein synthesis because $p70^{S6K}$ plays a critical role in regulating the translation of mRNAs. Resveratrol downregulates the MAPK cascade by inhibiting the tyrosine phosphorylation of ERK1/2/JNK/p38 and the translocation into the nucleus in the vascular cells [23, 47, 141]. This inhibition of phosphorylation and translocation into the nucleus from the cytoplasm reduces the expression of various genes implicated in vasoconstriction, angiogenesis, proliferation, and differenciation. In addition to its action on the MAPK cascade, the polyphenol affects nuclear factors and consequently the gene expression. Its affects NFκB which activates the transcription of several target genes implicated in initiation and progression of pathogenesis in atherosclerosis, in inflammation, as well as in cancer [142]. Many stimuli, such as oxLDL, ROS, and PKC, have the potency to activate the NFκB pathway. NFκB is located in the cytoplasm as an inactive complex when associated with the inhibitor of κB (IκB). In response to the stimuli, the catalytic subunits of IκB kinase (IKK) complex phosphorylate IκBα at two conserved serines. This phosphorylation event triggers the ubiquitin-dependent degradation of IκB by the 26S proteasome. Active p50/p65 complex is subsequently activated by phosphorylation of IKK α and PKC resulting in nuclear translocation of p50/p65 heterodimers (Fig. 5). Then the nuclear NFκB binds to specific κB DNA motifs and modulates the transcription of target genes (*e.g.*, COX, iNOS, cytokines, *etc*.).

The first study on the effect of resveratrol on NFκB showed that treatment with oxLDL and VLDL activates the NFκB binding activity and that resveratrol attenuates the activation of NFκB in PC-12 cells [143]. Furthermore, due to its properties of ROS scavenger and PKC inhibitor, resveratrol blocks stimuli-mediated phosphorylation and degradation of IκBα as well as the activation of IKKα (Fig. 5) [55, 144, 145]. Resveratrol inhibits the phosphorylation of p65 and its transactivation [137] by inhibiting kinases, such as IKKα [144], PKC [126], and the intrinsic kinase of PKCδ [146]. A recent study shows that a long treatment with resveratrol in human umbilical vein endothelial cells increases tyrosine phosphorylation of IκBα, p50-NFκB, and p65-NFκB, suggesting the involvement of such alterations in the modulation of NFκB transcription activity [147]. It has been also

Table 3. Effects of resveratrol on signaling and nuclear targets

Cell systems	Targets	Resveratrol actions	Resveratrol concentrations	Ref.
In vitro	PKC	Inhibits PKC phosphorylation Inhibits PKC activity	Res: 30 μM	[126, 135, 139]
HeLa cells	PKCδ PKD	Inhibits the activity Blocks activation loop phosphorylation and activity Blocks the translocation of PKD to the IKK complex	Res: 100 μM	[146]
Human umbilical endothelial cells	Cadherin β-catenin Src	Abrogates tyrosine phosphorylation Inhibits Src kinase activation	Res: 1 – 2.5 μM	[99]
	ERK-1/-2	Inhibits phosphorylation	Res: 1 – 100 μM	[23]
VSMC	PI3K Akt/PKB $P70^{S6K}$ ERK-1/-2	Inhibits phosphorylation Inhibits phosphorylation Attenuates phosphorylation Inhibits activation	Res: 1 – 50 μM	[81]
Porcine coronary arteries	ERK-1/-2 JNK-1 p38MAPK	Reduces the activities and the phosphorylation at active sites Inhibits MAPK nuclear translocation	IC_{50}: 37 μM	[141]
Bovine aortic smooth muscle cells	ERK-1/-2	Inhibits phosphorylation	Res: 3 – 30 μM	[47]
HeLa cells	NFκB	Blocks NFκB induction	Res : 100 μM	[146]
Monocytic cell		Blocks the phosphorylation of p65 and its transactivation	–	[137]
Myeloid, lymphoid, and epithelial cells		Inhibits nuclear translocation	Res: 1 – 25 μM	[145, 147]
Human keratinocytes	IKKα IκBα	Inhibits IKKα Blocks stimuli-mediated phosphorylation and degradation Inhibits kinase activity	Res: 5 – 25 μM	[55, 144]

reported that resveratrol is a potent inhibitor of NFκB nuclear translocation and IκB degradation [55]. Resveratrol blocks the translocation of the p65 subunit of NFκB in inflammatory agents (TNFα, phorbol 12-myristate-13-acetate (PMA), LPS, H_2O_2)-stimulated cells resulting in reduced transcriptional activity [145]. Transcription factors (GATA and AP-1) are also affected by resveratrol. Indeed, the suppression of NFκB by resveratrol coincides with the inhibition of activator protein-1 (AP-1) [148]. In fact, resveratrol inhibits stimuli-induced AP-1-mediated activity [145, 149, 150] through the inhibition of c-Src nonreceptor tyrosine kinase [151] and MAPK pathways, such as MEKK1 and JNK [145, 151], which can activate both AP-1 and NFκB pathways [152, 153]. Moreover, resveratrol reduces the DNA binding activity and transcriptional activities of AP-1 [23, 57, 154]. The disturbing of the nuclear factors (*e.g.*, NFκB, AP-1, GATA, *etc*.) by resveratrol affects the gene expression. In particular two genes, iNOS and COX-2, are involved in the coronary heart disease (CHD) process. Concerning the iNOS gene, its expression is controlled in part by NFκB [155]. So, resveratrol is able to inhibit iNOS expression in various cell types [156 – 161], in particular in macrophages regulating blood pressure where resveratrol inhibits iNOS and downregulates NFκB [160]. Concerning COX-2, various reports demonstrate the presence of COX-2 expression by SMCs in human atherosclerotic lesions [162, 163], and its expression is also regulated by various nuclear factors, such as NFκB, AP-1, and c-Jun [164]. Many studies demonstrated that resveratrol inhibits COX expression *via* an action on the nuclear factor, such as AP-1, and c-Jun [149, 150, 165, 166]. Moreover, docking studies on both COX-1 and COX-2 protein structures also revealed that hydroxylated but not methoxylated resveratrol analogues are able to bind to the previously identified binding sites of the enzymes [167]. This downregulation of COX-1/2 gene expression by resveratrol is correlated with a decrease of inflammation [121]. Indeed, the inflammatory aspect of atherosclerosis include the COX-dependent prostaglandin cascade, and so resveratrol decreases the level of prostaglandin by a reduction of COX-2 activity. Resveratrol can also act on COX-1/2 *via* the peroxisome proliferator-activated receptor (PPAR). In human VSMC a PPARα agonist has been shown to decrease NFκB activity [168]. Decreased NFκB activity reduces COX-2, so PPARα can depress the COX-1/2 induction in human. Activation of this PPAR may contribute to the anti-inflammatory activity of the pharmacological ligands that influence the development of atherosclerosis [169]. Resveratrol is able to activate PPARα in vascular ECs of mice treated with 20 mg/kg for 3 days and reduces the infarct volume by 36% at 24 h after middle cerebral artery occlusion [170]. In fact, resveratrol would be a dual activator of PPARα and PPARγ [170]. So, through its PPAR activation, resveratrol could contribute to the lipid metabolism modulation and to the

prevention of the SMC inflammatory activation. Moreover, PPARα shifts the human liver fatty-acid oxidation/glycerolipid esterification balance towards the catabolic route, thereby reducing TG supply for VLDL synthesis and contributing to the antihypertriglyceridaemic action of resveratrol. By attenuation of nuclear factors binding activity (*e.g.*, NFκB, AP-1, GATA), resveratrol perturbs the control of the expression of various genes (*e.g.*, ET-1, MCP-1, VCAM-1, ICAM-1, SR-A, IL-1, IL-6) involved in atherosclerosis and inflammatory response [23, 55, 62, 171].

3 Resveratrol prevents from ageing by mimicking caloric restriction

Ageing is an unescapable life process of all organisms. Meanwhile, the longevity is considerably different according to the life world. Life time can undergo from minutes in bacteria to centuries, or near (trees, birds, *etc*., even some human beings). So far, biochemical mechanisms involved in lifespan are still poorly understood. One of the major aspect of life span is the gene inheritage. However, environmental factors, including nutritional elements are now considered to have strong effects on gene expression/and or alteration. By the way, two well-known social examples emphasize the role of these epigenetic factors; *i.e.*, in Okinawa island, Japanese members or some families have exceptional long lifespans (age-old). However, when such families emigrated to Brasil they progressively changed their nutritional habits by switching from a fish/vegetable-rich diet to a +30% higher calorie diet containing fatty meat-enriched food). Consequently, the lineage showed a shorter lifespan (Fig. 6A).

These observations strenghtened the concept of caloric restriction (CR) benefits based on partially food deprivation. Caloric restriction reduces atherosclerosis [172], inflammation [173], effects of aging [174], insulin resistance of adipose tissue [175], and lysosomal autophagy [175]. This phenomenon is characterized by gene silencing, decreased expression of metabolic genes, such as those encoding for the growth hormone-IGF1 [176]. Under caloric restriction, the altered oxygen consumption modifyes the NAD^+/NADH ratio and leads to an NAD^+-dependent activation of sirtuin, an evolutionary conserved enzyme family which chemically modifies proteins, especially p53, the tumor suppressor involved in the longevity. For instance, the deacetylase activity of sirtuin promotes cell survival by negatively regulating the p53 tumor suppressor [177].

Interestingly, resveratrol and related compounds have been recently shown to mimic caloric restriction by lowering the Michaelis constant of sirtuin for both the NAD^+ and the acetylated substrates leading to a sirtuin-dependent deacetylation of p53 both in yeast and in human cell cultures. In addition, resveratrol increases the DNA stability as shown by the a strong decrease of the rDNA recombinaison frequency. In the mean time, resveratrol increases the lifespan of *Saccharomyces cerevisiae* by 70% (Fig. 6B) [177]. More recently, this property has been also found in higher eukaryotic organisms, *Caenorhabditis elegans* and *Drosophila melanogaster*, where the longevity is increased up to 14 and 29%, respectively, without decrease in fecondity [6]. Ageing is also linked to protein alteration, lipid oxidation, and DNA damage. Resveratrol as a potent antioxidant prevents such events. Moreover, resveratrol attenuates weight loss and total protein degradation in murine myotubes induced by proteolysis-inducing factors [178].

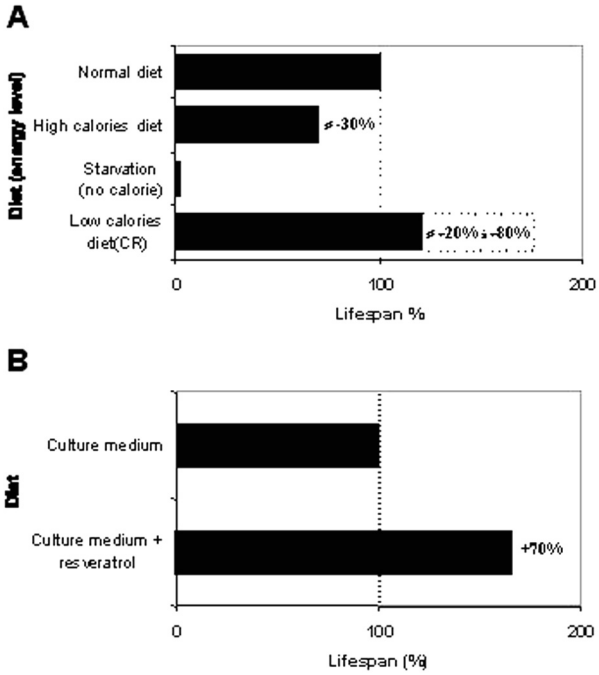

Figure 6. Effect of caloric restriction on the lifespan of living organisms and the mimicking role of resveratrol. (A) caloric restriction or "eat less to live longer"; (B) caloric restriction-mimicking role of resveratrol in *Saccharomyces cerevisiae* (adapted from data published by Howitz *et al.* [177]).

4 Conclusions

There are compelling evidences that resveratrol can act on the atherosclerotic process by affecting the major participants in the atherosclerotic disease process including an active vascular endothelium, smooth muscle cells, monocytes, and circulating lipoproteins. In these cells, the polyphenol can act on multiple therapeutic targets influencing the activity of several enzymes and modulating the expression of genes involved in atherosclerosis. Subsequently to

its action, pro-inflammatory and prothrombotic responses in atherosclerotic plaques were downregulated. In addition, resveratrol reproduces the beneficial effect of a caloric restriction on the aging phenomena which is involved, in part, in atherosclerosis and associated chronic inflammation. Although the studies carried out with cell cultures and animal models prove the promising anti-atherosclerotic effects of resveratrol, the data on the *in vivo* effect on human beings are far to be demonstrated. Indeed, the concentrations usually used in cell systems are high in relation to the concentrations found in wine or grapes. Nevertheless, several studies show that low resveratrol concentrations can act on platelet aggregation [179]. This pharmacological effect can be compatible with the resveratrol concentrations obtained after oral administration in animals, where an analysis of tissue concentrations showed that there was a significant cardiac bioavailability of resveratrol [180]. Furthermore, a recent study has shown a high absorption but a very low bioavailability of resveratrol after oral administration in human beings [181]. Localized accumulation of resveratrol in epithelial cells along the aerodigestive tract and potentially active resveratrol metabolites may still produce cardiovascular effects. So, a long-term consumption of low concentration of polyphenol, such as resveratrol, or a synergic effect with other phenolic compounds or other micronutrients intake of the Mediterranean diet could be sufficient to cause beneficial effects against vascular alterations and could constitute a potential arm for new therapeutic strategies.

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