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REVIEW

RESVERATROL, A NATURAL CHEMOPREVENTIVE AGENT AGAINST DEGENERATIVE DISEASES

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Resveratrol, a natural chemopreventive agent against degenerative diseases. E. IGNATOWICZ, W. BAER-DUBOWSKA. Pol. J. Pharmacol., 2001, 53, 557–569.

Resveratrol (3,5,4'-trihydroxystilbene) is a naturally occurring compound shown to modulate the risk of cardiovascular degenerative diseases (atherosclerosis) and inhibit chemical carcinogenesis in rodents. Various studies have demonstrated the effect of this phytoalexin on biological mechanisms involved in cardioprotection. These include modulation of lipid turnover, inhibition of eicosanoid production, prevention of the low-density lipoprotein oxidation and inhibition of platelet aggregation. Carcinogenesis in animal models can be divided at least into three stages: initiation, promotion and progression. Initiation occurs as result of interaction of a reactive form of carcinogen with DNA. Chemical carcinogens like polycyclic aromatic hydrocarbons are metabolized to reactive species by cytochrome P450 dependent enzymes activated through aryl hydrocarbon (Ah) receptor. The inhibition of tumor initiation by resveratrol most probably occurs through preventing the activation of Ah receptor. Resveratrol affects also several factors involved in tumor promotion and progression. Since tumor promoting agents alter the expression of genes whose products are associated with inflammation, chemoprevention of cardiovascular diseases and cancer may share the same common mechanisms. This includes principally modulation of the expression of growth factors and cytokines. Recently, chemopreventive properties of resveratrol have been associated with the inhibition of NF-κB. This transcription factor is strongly linked to inflammatory and immune responses, regulation of cell proliferation and apoptosis, thus it is important for tumor development and many other diseases including atherosclerosis. Although the mechanisms by which resveratrol interferes with the activation of NF-κB are not clear, it seems that inhibition of its degradation which is necessary for its cellular activation is the principal target. Based on the quantity and diversity of data available on the biological activity of resveratrol, it has to be considered a very promising chemoprotector and chemotherapeutic. Urgent investigations on its bioavailability and effects on *in vivo* systems, especially in humans, are necessary.

Key words: *resveratrol, cardioprotection, chemoprevention, reactive oxygen species, multistage carcinogenesis, transcription factor NF-κB, atherosclerosis, low density lipoprotein oxidation*

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Abbreviations: AGE – *advanced glycation end,* cNOS – *constitutive nitric oxide synthase,* COX – *cyclooxygenase,* DMBA – *7,12-dimethylbenz[a]anthracene,* HDL – *high density lipoproteins,* iNOS – *inducible nitric oxide synthase,* LDL – *low density lipoproteins,* LPS – *lipopolysaccharide,* MAP – *mitogen activated protein,* NF-κB – *nuclear transcription factor-kappa B,* oxyLDL – *oxidized LDL,* PAH – *polycyclic aromatic hydrocarbon,* PKC – *protein kinase C,* ROS – *reactive oxygen species,* TF – *tissue factor,* TGF – *transforming growth factor,* VLDL – *very low density lipoproteins*

Resveratrol (3,5,4'-trihydroxystilbene, see Figure 1 for structure) is a phytoalexin present in a wide variety of plant species, including mulberries, peanuts and grapes, and thus is a constituent of the human diet [71]. This compound, like other members of stilbene family, is produced in response to pathogen attack, UV-irradiation and exposure to ozone [4, 38, 103]. *Vitis vinfera*, or grapes, synthesize resveratrol in response to fungal infections; thus it is found at high concentrations in wine, particularly in red wine [47]. Resveratrol found in the powdered root of *Polygonum cuspidatum* (*Polygonaceae*) is an active ingredient of Chinese and Japanese folk medicine, and since ancient times, it has been used to cure diseases which contemporary medicine described as inflammation, allergy and hyperlipemia [64]. However, with the possible exception of peanuts, grapes and related products, such as red wines, are probably the most important foodstuff containing resveratrol. A primary impetus for research on resveratrol has come from the paradoxical observation that a low incidence of cardiovascular diseases may coexist with intake of a high fat diet, a phenomenon known as the French paradox [12, 25, 26, 98]. The exact mechanism by which resveratrol acts to mitigate a high fat diet from increasing the risk for coronary heart disease has not been totally elucidated but has been attributed to its antioxidant [34, 81, 101] and anticoagulative properties [9, 10, 101, 111]. Recently, resveratrol has been shown to act as a pleiotropic biological effector which regulates the multistage carcinogenesis process [8, 16, 60, 62]. These studies add a new dimension to the expanding role of resveratrol as a potential chemopreventive agent exhibiting anti-inflammatory, cell growth-modulatory and anticarcinogenic effects. The successful synthesis of resveratrol [88] along with the above observations resulted in the exponentially proliferating data on chemopreventive activity of resveratrol. The number of papers published on resveratrol in the years 1998–2000 reflects the growing interest in this molecule and will be discussed in this review.

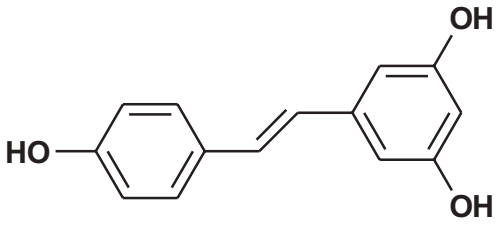

Fig. 1. Resveratrol

Chemoprevention of coronary heart disease by resveratrol

Coronary heart disease and its acute form, myocardial infarction, is a complication of atherosclerosis. Abnormalities of lipid metabolism and coagulation, as well as of other pathways which contribute to this pathology, are presented briefly below as a detailed description of atherosclerosis pathogenesis is beyond the scope of this article. A normal vascular endothelium functions depend on the interaction of key endothelial mediators: nitric oxide, prostacyclin and tissue plasminogen activator. The well-balanced cooperation between these factors provides artery wall with thromboresistance and prevents atherogenesis [48]. Several mechanisms contribute to the development of atherosclerotic lesions, and the primary one is believed to depend on the prolonged retention of lipoproteins in an artery wall and resulting local inflammation [69, 102]. The site of inflammation attracts phagocyting cells, generating reactive oxygen species (ROS) like superoxide anion, hydrogen peroxide and hydroxyl radicals [51]. In the inflammation process the release of arachidonic acid is involved as well as its metabolism to eicosanoids, prostaglandins and hydroperoxy forms of arachidonic acid [105]. Prostaglandin synthesis is regulated by cyclooxygenase (COX) gene expression. Two separate gene products, COX-1 and -2, have similar COX and peroxidase activities, although they are differentially regulated [105, 106]. Although a variety of factors can increase the mRNA levels of both COXs, the COX-2 gene generally responds in a more dramatic fashion than COX-1 gene. The oxidative

stress induced by the inflammation initiates a sequel of reactions within the artery wall, as well as in circulating blood cells and plasma components, which leads to oxidation of low density lipoproteins (LDL) [114]. The process is initiated by a hydroxyl radical, which exhibits high affinity for unsaturated fatty acids (PUFA) of an LDL molecule. This ROS is generated as a result of hydrogen peroxide reaction with transient metal ions. Transient metal cations of physiological importance (Fe^{2+}, Cu^{+}) are normally bound to specific proteins (ferritin, transferin, ceruloplasmin). The local pH changes due to the inflammation release cations from the protein binding sites [57]. LDL oxidative modification is a result of the interaction between the products of PUFA oxidation and the apolipoprotein B molecules. Under physiological conditions, LDL are protected from oxidation by the concerted action of LDL-specific antioxidants (tocopherol, coenzyme Q, carotenoids). When lipoproteins are accumulated in the artery wall over a prolonged period of time, as in the case of hypercholesterolemia, the antioxidants are depleted [115]. LDL uptake in subendothelial macrophages and/or smooth muscle cells is strictly regulated by the LDL-receptor, but oxidized LDL (oxy-LDL) is abundantly incorporated into subendothelial macrophages by an unregulated "scavenging" receptor and/or phagocytosis [7].

Cells overloaded with oxy-LDL molecules are called "foam cells" and form the basis of atheromatous plaques in the artery wall [113]. Concomitantly, oxy-LDL blocks cholesterol uptake by HDL molecules and promotes platelets' adhesion to endothelium, which initiates a complex cellular reaction leading to the development of atherosclerotic lesions [33, 98, 125]. The atherogenic modifications of lipoproteins are accompanied by the enhanced blood platelets' adhesion and aggregation, and increased expression of tissue factor (TF). TF is a glycoprotein bound to the cell surface and is a primary initiator of coagulation; however it is not expressed by monocytes-macrophages and endothelial cells. Its inappropriate appearance on these cell surfaces triggers blood clotting. TF accumulates to a great extent in elements which form human atherosclerotic plaques: macrophages, smooth muscle and endothelial cells and in cell-free, cholesterol-rich layers, and it is thought to determine plaque thrombogenicity [104].

Ischemia/reperfussion injury happens during fluid resuscitation in tissues previously deprived of the proper blood supply, e.g. in trauma and/or shock. The exhaustion of cellular ATP in the ischemic tissues induces irreversible metabolic alterations: hypoxanthine accumulation and the conversion of xanthine dehydrogenase into xanthine oxidase. Paradoxically, molecular oxygen introduced into the ischemic tissues becomes a source of ROS due to the xanthine oxidase activity [66]. However, in the human myocardium after an episode of acute coronary heart disease another mechanism of ROS-mediated injury has been found. Anoxic myocardium generates hydrogen peroxide during reperfusion, which binds to myoglobin to form a highly oxidative complex [107]. The increased oxidative stress directly impairs cardiac structure and function, causing cardiomyopathy and depression of contractile functions and organ failure [65].

Resveratrol was shown to interfere with a number of mechanisms described above, leading to the diminishment of the atherogenic changes in plasma and artery wall and improving the outcome after ischemia/reperfusion injury. Resveratrol was found to prevent lipids from peroxidative degradation [18, 37, 39, 73, 118] and to stop the uptake of oxy-LDL in the vascular wall in a concentration-dependent manner [38]. Liver parenchymal cells in culture, treated with resveratrol, showed reduced secretion of esterified cholesterol and triglycerides although the intracellular trigliceride level was unchanged. It allows the supposition that resveratrol reduces the secretion of VLDL from the liver, which is transformed into LDL in blood circulation, thus blocking hepatic lipoprotein metabolism [43, 46, 50]. Treatment of hepatoma HepG2 cells with resveratrol resulted in a decreased level of the intracellular apolipoprotein B and its secretion, which may be responsible for impaired LDL and partly VLDL synthesis [46]. Resveratrol may protect LDL molecules against peroxidation through antioxidative activity and metal chelation (its ability regarding copper chelation was described elsewhere) [6]. The common recognition of resveratrol as natural antioxidant was elucidated by Zini et al. [129] who proposed three different mechanisms through which this phytoalexin exerts its antioxidative action. Resveratrol is supposed to compete with coenzyme Q and to decrease the oxidative chain complex III, the site of ROS generation. It also scavenges superoxide radicals formed in the

mitochondria and inhibits lipid peroxidation induced by Fenton reaction products [129]. Other suggestions on antioxidative abilities of resveratrol came from Jang and Pezzuto [64]. They described the normalization of myeloperoxidase and oxidized--glutathione reductase activities upon resveratrol treatment. The inhibition of the inducible nitric oxide synthase (iNOS) by resveratrol, which may prevent cytotoxic effects of nitric oxide, was also noticed [64, 82, 122]. Resveratrol has been proved to scavenge peroxyl and hydroxyl radicals in reperfused postischemic isolated rat hearts, to limit infarct size and to reduce the formation of malondialdehyde, a non-specific marker of lipid peroxidation occurring under oxidative stress [97, 101]. Resveratrol was shown to modulate platelet coagulation through multiple mechanisms. It inhibited platelet adhesion to type I collagen which is the first step of platelet activation. This compound also reduced platelet aggregation induced by thrombin and ADP treatment and altered eicosanoid metabolism in favor of increased prostacycline and decreased tromboxane B_2 synthesis in the activated cells. The antioxidative properties of resveratrol were postulated as the mechanism underlying its diverse effects and as possible explanation of the abovementioned findings [9, 10, 38, 89, 90, 131, 132]. However, these effects were observed mainly *in vitro* in isolated platelets. In the whole blood the antiplatelet activity of resveratrol was less evident [68]. It has also been suggested that resveratrol blocks the *in vitro* aggregation due to the inhibition of mitogen activated protein (MAP) kinases in platelets [68]. The reduction of TF expression in vascular cells may also contribute to the anti-aggregatory effect of resveratrol [91].

Another explanation of anti-platelet action of this phytoalexin has been proposed by Dobrydneva et al. [29]. Resveratrol was found to inhibit Ca^{2+} influx into thrombin-stimulated platelets through interference with store-operated Ca^{2+} channels. A similar effect of resveratrol on calcium influx into cultured murine macrophages has been noticed, and this action led further to the suppression of proinflammatory interleukin-6 synthesis [128]. The studies of calcium channels in endothelial cells after exposure to resveratrol have shown the ability of this phytoalexin to control vasorelaxation mediated by nitric oxide. This effect was reversed by the constitutive nitric oxide synthase (cNOS) inhibitor, Nω-nitro-L-arginine. The non-nitric oxide--modulated pathway was also postulated in endothelium-denuded aortic tissue: the vasodilation upon resveratrol treatment was not reversed by the NOS inhibitor [20]. Moreover, resveratrol has been found to diminish the proliferation of smooth muscle cells from intima of the vessel wall and this antimitogenic activity appears to be related to a G1 $\rightarrow$ S block in the cell cycle [54, 130]. The data on the involvement of the steroid receptor on the cell membrane has been contradictory [32, 59]. The vasorelaxative activity of resveratrol depends also on direct stimulation of K^+/Ca^{2+} channels in endothelial cells [74].

In human polymorphonuclear neutrophils, resveratrol decreased the amount of 5-lipooxygenase proinflammatory products (5-hydroxyeicosatetraenoic acid, 5,12-dihydroxyeicosatetraenoic and leukotriene C4) [67], inhibited the lysosomal enzymes (lysozyme and β-glucuronidase) release upon calcium ionophore exposure, and decreased ROS generation [61, 99]. Another mechanism to account for the anti-inflammatory and cardioprotective effects of resveratrol is suppression of phospholipase A_2 and COX activities, along with inhibition of phosphodiesterase leading to an increase in the amount of cyclic nucleotide and inhibition of protein kinases involved in cell signaling [112].

Diabetes is considered a promoting factor of atherosclerosis in humans. Hyperinsulinemia following type 2 diabetes and insulin resistance are thought to be independent cardiovascular risk factors. In the tissues and blood of diabetic patients, reduced antioxidant content was found. Moreover, the formation of advanced glycation end-products (AGE) as a result of nonenzymatic glycation and oxidation of proteins has been observed [5]. AGE increase coagulation through various mechanisms involving the vascular endothelium and platelet activation [27]. The interaction of AGE with their receptor results in increased ROS production and activation of protein kinases [70]. AGE-stimulated proliferation of smooth muscle cells in the artery wall and increased DNA synthesis were inhibited by resveratrol in an animal experimental model [85].

The structural similarity of resveratrol to diethylstilbestrol, a synthetic estrogen, has led to the hypothesis that it might express a phytoestrogenic function. Endogenous estrogens have known cardioprotective properties and it seems very likely that phytoestrogens present in red wine could exert similar action [15, 44]. However, the estrogenic activity

of resveratrol may also result in the stimulation of human breast cancer cell proliferation [60, 77].

Chemoprevention of multistage carcinogenesis by resveratrol

The major stages of carcinogenesis were deduced over the past 50 years, primarily from animal model studies (particularly in mouse skin). These stages are termed: initiation, promotion and progression [1, 109]. Tumor initiation begins when DNA in a cell or population of cells is damaged by exposure to exogenous or endogenous carcinogens. If this damage is not repaired, it can lead to genetic mutations. The responsiveness of the mutated cells to their microenvironment can be altered and may give them a growth advantage over normal cells. In the classic two-stage carcinogenesis system in mouse skin, a low dose of polycyclic aromatic hydrocarbon (PAH), 7,12-dimethylbenz[a]anthracene (DMBA) causes permanent DNA damage (the initiating event) but does not give rise to tumors over the lifespan of the mouse unless a tumor promoter, such as 12-O-tetradecanoylphorbol-13-acetate (TPA), is repeatedly applied [1, 108, 109]. The tumor promotion stage is characterized by selective clonal expansion of the initiated cells, a result of TPA-induced oxidative stress, and the altered expression of genes whose products are associated with hyperproliferation, tissue remodelling and inflammation [58, 109]. During tumor progression, preneoplastic cells develop into tumors through a process of clonal expansion that is facilitated by progressive genomic instability and altered gene expression [95]. Most animal models used in carcinogenesis research were developed before the identification of the major cancer-related genes, the recognition of the importance of host susceptibility to a carcinogenic insult, or the realization that mitogenesis and apoptosis together regulate cell number. Nonetheless, these animal models have contributed significantly to our current understanding of carcinogenesis and the ways to interfere with that process. Current strategies for the prevention of cancer use mechanism-based approaches to block the carcinogenesis process at all stages along the pathway. Thus, the main targets for anti-initiation strategies are: i) modulation of carcinogen activation, ii) scavenging electrophiles and oxygen species, iii) carcinogen detoxification, iv) DNA repair processes. Targets for antipromotion and antiprogression strategies include epigenetic changes in cell signaling, inflammation, proliferation and apotosis [56].

Resveratrol has been suggested as a potential chemopreventive agent based on its striking inhibitory effects on diverse cellular events associated with tumor initiation, promotion and progression. The cancer chemopreventive potential of resveratrol was established in 1997 when it was found that this compound inhibited DMBA-induced preneoplastic lesion formation in mouse mammary organ culture and reduced the incidence and multiplicity of DMBA/TPA-induced papillomas in the two-stage mouse skin model [60]. These studies showed also that resveratrol administration decreased the number of preneoplastic lesions induced by DMBA in the mouse mammary gland organ culture model [72]. The antimutagenic activity of resveratrol was also demonstrated against foodborne heterocyclic amine, 3-amino-1,4-dimethyl-5H-pyrido[4,3-b]indole (Trp-P-1) and 2-aminofluorene in *Salmonella* bacterial tester strains [64, 123]. Most chemical carcinogens are genotoxic, causing DNA damage by reacting with DNA bases. The carcinogens form covalent adducts with DNA. DMBA like other polycyclic aromatic hydrocarbons requires metabolic conversion to DNA-reactive intermediates. The metabolic activation is catalyzed by cytochrome P450 through oxidation of the carcinogen molecule. DMBA is metabolized by multiple forms of cytochrome(s) P450 which are characterized by different regio-selectivity. CYP1A1 is the major isozyme that catalyzes the two step-oxidation of most PAH to their bay region diol-epoxide intermediates in both rodent and human tissues. Recent studies have indicated that CYP1B1 and 2C6 are also involved in DMBA metabolism [14]. The transcriptional induction of the CYP1A1 is regulated by the Ah receptor, a cytosolic protein present in a number of rodent and human tissues [52]. The binding of ligands like PAH to the Ah receptor usually results in coordinated expression of genes encoding not only CYP1A1, but also CYP1A2 and the 1B1 and II phase enzymes responsible for conjugation (detoxification) of the reactive metabolites. Studies *in vitro* showed that resveratrol affects the expression of cytochrome P450 and is one of the most selective inhibitors of human P450 1A1 [22]. Inhibition of CYP1A1 transcription may occur by preventing activation of the Ah receptor. Resveratrol promotes Ah receptor translocation to the nucleus and binding to DNA at dioxin-respon-

sive elements, but transactivation does not take place [17, 23]. Resveratrol also induces the phase II enzyme NAD(P)H, quinone oxidoreductase, which detoxifies many quinones. This enzyme is part of the gene battery activated through the Ah receptor [87]. The effect of resveratrol on the other isozymes of P450 have not been studied in detail, but the inhibition of alkoxyresorufine dealkylases, markers of CYP1A and 2B1/2 *in vitro*, was demonstrated [120]. Resveratol also decreased the levels of DMBA-DNA adduct formation in an *in vitro* system [2]. Beside P450, there are other enzyme systems involved in carcinogen activation such as peroxidases, including cyclooxygenases and certain transferases such as N-acetyltransferase and sulfotransferase [31, 49]. Each of these enzymes provides a potential target for modulating carcinogen activation. It has been reported that resveratrol decreased the activity of COXs [64, 94].

Tumor promoting agents are not mutagenic as carcinogens are, but rather alter the expression of genes whose products are associated with hyperproliferation, apoptosis, tissue remodelling and inflammation. Over the past few years it has become clear that apoptosis and mitogenesis are equally important in the homeostasis of cell number, and that the growth advantage manifested by initiated cells during promotion is usually the net effect of increased proliferation and decreased apoptosis. Thus, in addition to cell proliferation, apoptosis has emerged as a critical target for prevention [78]. Changes in gene expression as a consequence of external tumor promoter stimuli usually activate (but sometimes inactivate) specific signal transduction pathways. The major target for phorbol esters and other promoters is protein kinase C (PKC). Its activation seems to be a critical event in carcinogenesis. By activating PKC, phorbol esters and related tumor promoters appear to bypass the normal cellular mechanisms for regulating cell proliferation [56]. Resveratrol has been shown to inhibit isolated and cellular PKC in model systems. Phosphorylation of substrates that resemble protamine sulphate was particularly affected [116]. Resveratrol has been incorporated into model phospholipid membranes, altering the phospholipid phase polymorphism, and has inhibited PKC α enzymatic activity *in vitro* [42]. Several lines of evidence suggest that tumor promoters generally increase the expression of a number of growth factors and cytokines. TPA induces the transforming growth factors TGF-α, TGF-β, tumor necrosis factor-alfa, the granulocyte-macrophage stimulating factor, and interleukins 1 and 6. The profile of growth factor induction differs for promoters with various initial mechanisms of action, although most seem to induce TGF-α messenger RNA (mRNA) expression. TPA treatment also increases expression of the epidermal growth factor receptor, possibly as a consequence of activating c-Ha-*ras* [28].

In addition to inducing changes in gene expression by activating specific signaling pathways, tumor promoters can elicit the production of proinflammatory cytokines, such as tumor necrosis factor, and several interleukin and nonprotein factors, such as nitric oxide, involved in inflammation and carcinogenesis [35]. Of critical importance to the promotion process is the release of arachidonic acid and its metabolism to eicosanoids [41]. Eicosanoids are involved not only in the inflammation process, but also in the immune response, tissue repair, and cell proliferation. Suppression of prostaglandin biosynthesis through selective inhibition of COX is, hence, now regarded as an important cancer chemopreventive strategy. TPA increases significantly the COX-2 mRNA level and has been referred to as a phorbol ester-inducible immediate early gene product [106]. Resveratrol was shown to inhibit COX-1 activity in microsomes derived from sheep seminal vesicles [60]. More recently, Subbaramaiah et al. [117] have reported that resveratrol inhibits the catalytic activity of the COX-2 in cultured human mammary epithelial cells with and without TPA treatment. Likewise, human recombinant COX-2 expressed in baculovirus was inhibited by resveratrol. Moreover, resveratrol effectively suppressed the COX-2 promoter-dependent transcriptional activity in human colon cancer cells [86]. Besides inhibiting the catalytic activity of COX-2, this compound also blocked TPA-mediated induction of *cox-2* mRNA in cultured human mammary epithelial cells through repression of transcription factor-AP-1-dependent transactivation [75, 117].

The tumor promoting activity mediated by TPA has also been associated with oxidative stress, as exemplified by increased production of superoxide anion radicals, H_2O_2, reduction of superoxide dismutase activity, which is able to detoxify superoxide anion radicals, and interference with glutathione metabolism, a key intracellular component capable of protecting cellular constituents from attack of peroxide and free radicals [40, 92, 110]. Be-

side scavenging ROS, resveratrol was also found to block the production of carbon or nitrogen-centered free radicals, such as the phenylbutazone peroxyl radical and the benzidine-derived radical. Resveratrol, along with other antioxidants like vitamin E and melatonin, prevented the oxidative DNA damage induced in rat kidneys by $KBrO_3$ [13]. Topical application of resveratrol onto the dorsal skin of CD-1 mice led to profound attenuation of oxidative stress and expression of epidermal TGF-β 1 and c-*fos* induced by TPA; but the induction of *cox*-1, *cox*-2, c-*myc*, c-*jun* and TNF-α mRNAs was not affected [63].

Some anti-inflammatory chemopreventive agents have been found to suppress the growth and proliferation of transformed cells through induction of apoptosis [93, 100]. Little information is available with regard to the apoptosis-inducing capability of resveratrol in tumor cells. However, the inhibition by resveratrol of the growth of several types of human breast epithelial cells, which was independent on the estrogen receptor status of the cells by resveratrol was reported [84]. In this regard it was shown that resveratrol inhibited the growth of estrogen receptor-positive MCF-7 cells and human oral squamous carcinoma cells (SCC-25) [30]. The data on growth modulation of estrogen-dependent T47D breast carcinoma cells are contradictory. In some experiments, resveratrol suppressed their growth, in others, stimulation was observed [24, 44]. The proliferation of K-562 human erythroleukemia cells and P-815 was also suppressed by resveratrol treatment, which might be associated with the inhibition of ribonucleotide reductase [36]. The suppression of human promyelocytic leukemia (HL-60) cells by resveratrol was shown to be mediated *via* induction of apoptosis, as determined by nuclear fragmentation, chromatin condensation, time-related increase in the frequency of subdiploid (apoptotic) cells, and internucleosomal DNA fragmentation [119]. Besides suppression of proliferation, the compound induced differentiation of HL-60 cells, which appears to be associated with reversible cell cycle arrest at the S-phase check point [96, 119]. Moreover, resveratrol was found to induce apotosis in the same cells by triggering the CD95 signaling system [24]. In the mouse JB6 epidermal cell line, resveratrol induced apotosis through activation of p53 activity, however apotosis occurred only in cells expressing a wild type of p53 [55].

In summary, the presented data indicate that resveratrol promotes homoeostasis and affects the earliest and the late stages of carcinogenesis. Thus, resveratrol may be considered not only a potential chemopreventive, but also a chemotherapeutic agent to control tumor development.

Association of the chemopreventive properties of resveratrol with inhibition of activation of the nuclear factor-kappaB

Recently, much data has shown up indicating the interference of resveratrol with the nuclear factor-kappaB (NF-κB). This transcription factor is strongly linked to inflammatory and immune responses [52]. NF-κB is also important for the regulation of cell proliferation and apotosis, cell transformation and tumor development [3, 45, 79,124] and many other diseases including atheriosclerotic lesions [11]. NF-κB controls the gene expression of cytokines, chemokines, growth factors, and cell adhesion molecules as well as some acute phase proteins, including the inflammatory mediators iNOS and COX-2 [126, 127]. NF-κB was first identified as a B-cell nuclear factor and given its name on the basis of its ability to bind to an intronic enhancer of the immunoglobulin κ-light chain gene. Since then NF-κB has been identified in numerous cell types and is found to be activated by a wide range of inducers, including ultraviolet irradiation, cytokines, inhaled occupational particles, and bacterial or viral products. In resting cells, NF-κB resides in cytoplasm in an inactive form bound to an inhibitory protein known as IκB. Upon cellular activation by extracellular stimuli, IκB is phosphorylated and proteolytically degraded or processed by proteasomes and other proteases. This proteolytic process activates NF-κB, which then translocates into the nucleus. In nuclei, NF-κB, can initiate or regulate early-response gene transcription by binding to decameric motif – κB, found in the promoter or enhancer regions of specific genes [19]. Presently, five mammalian NF-κB family members have been identified and cloned. All these family members share a highly homologous domain (Rel) composed of ~300 amino acid residues that are responsible for DNA binding, dimerization, and interactions with IκB. Evidence for a potential role of NF-κB in carcinogenesis was provided by the observation that activation of NF-κB is required in oncogenic Ras-induced transformation [83]. Upon inhibition of

NF-κB activation with the superrepressor form of IκBα, oncogenic Ras transformed cells exhibited a loss of cell viability, indicating that oncogenic Ras requires the cell survival-promoting function of NF-κB to overcome the role of the death signal initiated in transformed cells. The mechanisms by which resveratrol can interfere with the activation of NF-κB are not clear. One possibility is that resveratrol can interact with ankyrin domains present in IκB because the phosphorylation of IκB is inhibited by resveratrol. Such interaction could conceivably hinder IκB phosphorylation and subsequent dissociation of NF-κB. In addition resveratrol blocked the expression of mRNA-encoding monocyte chemoatrractant protein-1, a NF-κB regulated gene. A cell treated with lipopolysaccharide (endotoxin, LPS) can generate ROS which activate protein tyrosine kinase. Furthermore, resveratrol has been found to possess potent protein kinase inhibitory activity and antioxidant activity. Protein tyrosine kinase has been implicated in NF-κB activation [76]. Therefore, resveratrol might inhibit the activation of NF-κB, the LPS-induced phosphorylation and degradation of NF-κB [80]. Since activation of NF-κB is necessary for LPS-triggered induction of iNOS, inhibition of this transcription factor may also result in a decrease in exogenous nitric oxide synthesis which is responsible for cytotoxic effects of this signal molecule [122, 126]. On the other hand, endogenous induction of nitric oxide inhibits NF-κB and interfere with several signaling pathways that lead to activation of this transcription factor [53].

Resveratrol also induced apotosis in fibroblasts after induced expression of oncogenic *H-Ras* [53]. Thus, resveratrol is likely to function by inhibiting inflammatory and oncogenic diseases, at least in part, through the inhibition of NF-κB activation by blocking IκB kinase activity. These data may also explain some aspects of the "French paradox" and provide a molecular rationale for the role of a potent chemopreventive compound in blocking the initiation of inflammation and carcinogenesis.

Final remarks

The presented chemopreventive abilities of resveratrol do not limit its activities in other fields. Some relatively new and unexplored directions of research on this molecule involve allergy and brain function. A preliminary study reported by Cheong et al. [21] showed anti-allergic properties of resveratrol, as assessed by the β-hexosoaminidase release from cells. Resveratrol induces phosphorylation of mitogen activated protein (MAP) kinases in the human neuroblastoma cells. MAP kinases are involved in signal transduction in cells. In particular, enzyme type ERK2 has been related to synaptic changes linked to learning processes [121]. As authors postulate the reduction of dementia upon moderate wine intake, this finding may enlarge our understanding of the protective role of resveratrol. Nevertheless, till now most of the data comes from *in vitro* studies. Indeed, up to the present, the evidence for resveratrol absorption and metabolism in humans is scant and the question arises if the strong biological activity of resveratrol *in vitro* can be fully reproduced *in vivo* in a comparable dose range.

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