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MINIREVIEW

BIOLOGICAL EFFECTS OF RESVERATROL

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Summary

Resveratrol (3, 4', 5 trihydroxystilbene) is a naturally occuring phytoalexin produced by some spermatophytes, such as grapevines, in response to injury. Given that it is present in grape berry skins but not in flesh, white wine contains very small amounts of resveratrol, compared to red wine. The concentrations in the form of trans- and cis- isomers of aglycone and glucosides are subjected to numerous variables. In red wine, the concentrations of the trans-isomer, which is the major form, generally ranges between 0.1 and 15 mg/L. As phenolic compound, resveratrol contributes to the antioxidant potential of red wine and thereby may play a role in the prevention of human cardiovascular diseases. Resveratrol has been shown to modulate the metabolism of lipids, and to inhibit the oxidation of low-density lipoproteins and the aggregation of platelets. Moreover, as phytoestrogen, resveratrol may provide cardiovascular protection. This compound also possesses anti-inflammatory and anticancer properties. However, the bioavailability and metabolic pathways must be known before drawing any conclusions on the benefits of dietary resveratrol to health.

Key Words: resveratrol, wine polyphenols, antioxidant, atherosclerosis, carcinogenesis

A variety of foods and beverages of vegetable origin contain several non-flavonoid classes of phenolic compounds synthesized by plants (mainly spermatophytes) in response to injury or fungal attack. Among them, resveratrol has been identified as the major active compound of stilbene phytoalexins and is presumed to be beneficial for human health. It is synthesized by *Polygonum cuspidatum* ("Kojo-kon" in Japanese) roots, which have long been used in traditional oriental medicine. Trans-resveratrol was first detected in grapevines (*Vitis vinifera*) in 1976 by Langcake and Pryce (1), who found that the compound was synthesized by leaf tissues in response to fungal infection (mainly *Botrytis cinerea*) or exposure to ultraviolet light.

The interest in compounds present in grapevines was stimulated when epidemiological studies showed an inverse correlation between red wine consumption and incidence of cardiovascular diseases. Results from screening of individuals show that the protection is partially due to ethanol present in wine as

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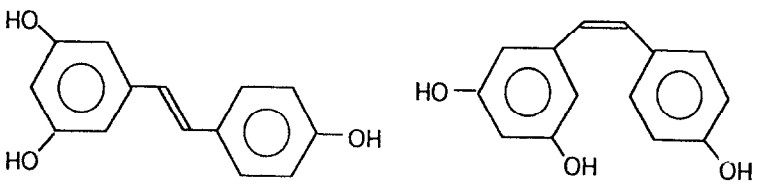
well as in other alcoholic drinks. Ethanol acts through a haemostatic mechanism and an increase in circulating high-density lipoproteins (HDL) (reviewed in ref. 2). However, it has been suggested that constituents other than alcohol could have a protective effect (3). Numerous studies provide support for the bioactivity of phenolic components, which, for a large part, have a flavonoid structure (reviewed in ref. 4). Resveratrol was first taken into consideration when its presence in wine was reported in 1992 by Siemann and Creasy (5).These authors suggested that this compound might be the biologically active ingredient of red wine. Since then, the properties of resveratrol have been extensively investigated.

Characterization and Analysis

Resveratrol (3, 4', 5 trihydroxystilbene) exists in cis- and trans- isomeric forms (Fig. 1) but the cis-isomer has never been identified in grape extract (1, 6). It is the parent molecule of a family of polymers named viniferins. Plants also synthesize glucosides (piceid = resveratrol 3-0-β glucoside). The extraction of resveratrol and related products from natural sources is time-consuming and yields low amounts of the compound. Hence, research on biological properties only really started when trans-resveratrol was obtained through organic synthesis. Jeandet et al (6) identified the product from its UV-spectral characteristics and infrared absorption peaks in the range of 2800 to 3500 cm^{-1} (OH band) and at 965 cm^{-1} (trans form of the double bond). Trans-resveratrol (M_r = 228) is now commercially available and the cis form can be obtained by UV irradiation (5). Trials conducted under various conditions showed that trans-resveratrol remained stable for several months (except in high pH buffers) when completely protected from light (7). The values for molar absorptivity were: trans-resveratrol [$UV_{\lambda max}$ (EtOH) nm (ε) 308 (30000)], cis-resveratrol [$UV_{\lambda max}$ (EtOH) nm (ε) 288 (12 600)].

Studying the biological properties of resveratrol requires the analysis of complex mixtures containing very low amounts of several stilbenes. A complete extraction, within a short time, is required to reduce the loss from denaturation and isomerization. During the last decade, several methods have been developed. They are mainly based on high-pressure liquid chromatography (HPLC) and gas chromatography (GC) coupled or not with mass spectrometric (MS) detection. Generally, HPLC methods use a C18 reverse phase column with detection at 307 and 280 nm corresponding to the trans- and cis- resveratrol absorbance maxima respectively (5). The highly sensitive fluorimetric detection of stilbenes (0.01 μg/L), which is more specific than UV detection was subsequently used to determine the resveratrol and pterostilbene (dimethoxyderivative) content in grape berries and wine (8). By using an electrochemical detector, Mc Murtrey et al (9) measured the resveratrol content of various red wines without pretreatment. The limit of detection was approximately 1 μg/L. A simple and rapid procedure that separates and quantifies the four major forms of resveratrol in wine (cis- and trans-isomers of aglycone and piceid) has been proposed by Lamuela-Raventos et al (10) for analysing Spanish red wines. It uses a HPLC system with direct injection and a diode array UV-visible detector.



trans-resveratrol cis-resveratrol

FIG. 1.

Chemical structures of trans- and cis-resveratrol (3, 4', 5 trihydroxystilbene).

For trans-resveratrol, the limit of detection is 3 μg/L and the limit of quantitation is 10 μg/L. In GC analysis, resveratrol is injected in the form of trimethylsilyl derivative (6). By combining HPLC and GC-MS, Jeandet et al (11) determined the concentrations of the two resveratrol isomers in red wine with a detection limit of 10 μg/L. By using a HPLC method and recording the ^{1}H-NMR spectra, Mattivi et al (12) found that piceid, mainly the cis- isomer, were major components of wine stilbenes at the beginning of fermentation, whereas the final wine contained higher amounts of aglycone (mainly trans).

In order to easily analyze red wines, as well as grape juices or jams within a large range of resveratrol concentrations (0.05 to 10 mg/L), Goldberg et al (13) proposed a rapid and sensitive GC-MS method involving a solid phase extraction followed by direct injection of samples without previous chemical treatment. By coupling diode array detection and fluorimetry, Jeandet et al (14) achieved separation of major stilbene phytoalexins of grapevine by HPLC. Recently, capillary electrophoresis was used to directly analyze the two resveratrol isomers in wine (15). The procedure is fast (< 15 min) but not very sensitive (300 μg/L). Given that most of the methods published are not convenient for biological samples, Blache et al (16) developed a specific GC technique measuring concentrations (detection limit, 50 μg/L) of cis- and trans- resveratrol in plasma, lipoproteins and cells after a short purification step.

Concentrations in Wines

The presence of resveratrol has been detected in numerous types of wine. As indicated in Table 1, analysis of red wine originating from various countries (USA, France, Italy, Spain, Japan) show large variations in ranges of concentrations. They depend on grape cultivar, geographical origin, wine type, *Botrytis* infection and oenological practices. The progress in methodology probably accounts for the highest values being found in the more recent analyses.

A major factor is the fermentation time in contact with grape skins because resveratrol is produced by the skin but not by the fruit flesh (6). This explains the low concentrations in white wine which has a short maceration time (5, 17). Recently, Romero-Perez et al (18) have reported values found by several authors in white wines of various origin. In general, they detected less than 0.1 mg/L resveratrol (cis + trans). The authors quantified the four monomeric forms of resveratrol. They found between 0.05 and 1.8 mg/L of total stilbenes. The mean values were: aglycone, 0.13 (trans) and 0.06 (cis) ; piceid, 0.16 (trans) and 0.12 (cis). In rosé wines, the levels of resveratrol monomers were between levels in white and red wines. The maceration time with skin but also varietal differences in the types of grapes used influence the resveratrol content of wine. Thus Jeandet et al (10) noted that Pinot noir (made with red grapes) is much richer in resveratrol than Chardonnay (made with white grapes) despite a similar maceration time with skins. The relatively thin skin of Pinot noir grapes might render them very sensitive to traumatic damage, *Botrytis* infection and UV light (19). However a high *Botrytis* infestation is not advantageous. Jeandet et al (11) found that grapes infected 10% by *Botrytis* produced a wine containing high levels of resveratrol with no effect on the quality. In contrast, wines obtained from grapes affected 40% or 80% by *Botrytis* had the lowest resveratrol levels. The authors explained this result by assuming that resveratrol formed in highly *Botrytis*-infested grapes might had undergone degradation by exocellular enzymes of the fungus. The high proportion of cis isomer (> 40% of total resveratrol) in some wine vinted with or without protection from light indicated that isomerization of the trans- isomer was not due sunlight exposure subsequent to its formation in grape berries. Goldberg et al (20) suggested that the cis- isomer might be produced during fermentation by yeast enzymes or released from viniferins. The authors analyzed more than 450 commercial wines, and found considerable variations in the trans to cis ratio. Wines high in trans were also high in cis suggesting that concentrations of both isomers are subjected to the same variables.

TABLE 1
Resveratrol Concentrations of Red Wine

Origin	Resveratrol concentrations (mg/L)	Ref no.
USA France	Chardonnay, up to 0.1[1] ; California < New York Bordeaux, 0.3–0.6	5
France (Burgundy)	Pinot noir, 0.4–2	21
USA (California)	Pinot noir, 0.2–0.7 Cabernet Sauvignon, < 0.09	17
North America, Australia, Europe	0.1–12 California, Australia, Italy < Oregon, Canada, France	13
USA (California)	< 0.02–1.7 (highest in Pinot noir, 5)	9
France	Beaujolais (Gamay), 3.2–3.6	9
France	aglycone, 0.5–5 ; glucoside, 0–14.5 Cabernet franc,Gamay, Grenache < Pinot noir, Mourvèdre, Cabernet Sauvignon	22
USA (California)	aglycone isomers, 0.3–3 (mean 1.5)	23
Spain	aglycone + piceid isomers (trans > cis) 2.5–13.8 R_t : Pinot noir, 5.1 ; Merlot, 4 ; Grenache, 2.4 ; Cabernet Sauvignon, 1.4 ; Trempanillo, 1.3	10
Europe	aglycone isomers (trans > cis), France, 3.7–7.1 Beaujolais, Midi, Rhône < Bordeaux, Burgundy Italy, 1.2 < Spain, Portugal, 2.8 < central Europe, 3.1 < Switzerland, 6.9	20
America	South America, 1.8 < California, 3 < Oregon, 6.3	20
Italy	Recioto and Amarone (area of Verona), 0.05–0.8	24
USA (Southeast)	aglycone isomers, 0.1–42 ; highest concentrations in Muscadine (trans, up to 13.4 ; cis, up to 31.9). Presence of tetrahydroxystilbene	25
Japan	total stilbenes, 0.8–13.4 (mean 4.4); aglycone (mean 1.8, trans > cis) ; piceid (mean 2.5, cis > trans) ; highest concentrations in Pinot noir and Merlot	26
USA (California)	Cabernet Sauvignon, 0.4-2 ; Pinot noir, 1.2 ; Merlot, 3.5	15

[1] Concentration of trans-resveratrol (R_t) where unspecified.

Biological Activities

The response in living organisms depends on bioavailability. The capacity of the intestine to absorb resveratrol was attested more than fifteen years ago by the health benefits obtained by orally administrated resveratrol. In rats fed on diets inducing hyperlipidemia, resveratrol and piceid inhibited the hepatic accumulation of triacylglycerol and cholesterol (27). Likewise, these stilbenes prevented liver injury in rats fed peroxidized oil (28). Recently, Bertelli et al (29) demonstrated that a fraction of resveratrol present in red wine (6.5 mg/L as cis and trans forms) was absorbed by rats. Assays with an acute dose of 26 μg or a daily intake of 13 μg resveratrol over 15 days showed that the compound quickly entered the blood stream and could be detected in significant concentrations in plasma and several organs. The same group of authors studied further the plasma kinetics and bioavailability of red wine resveratrol administrated by an intragastric tube (28 μg per rat). They described pharmacokinetics by an open one- or two-compartment model. There was a significant cardiac bioavailability and a strong affinity for the liver and kidneys (30). In another study, Bertelli et al (31) showed that, at modest dosages, resveratrol was pharmacologically active both in vitro and in vivo. The authors suggested that an average drinker of wine can particularly in the long term, absorb a sufficient quantity of resveratrol to explain the beneficial effect of red wine on human health.

However, we are still in need of studies on the metabolism and physiological effects of the different forms of resveratrol taken with food. In particular, piceid bioavailability has not yet been studied. It seems possible that resveratrol glucosides can be absorbed by human small intestine, as flavonoid glucosides are (32).

Antioxidant activity. Changes in low-density lipoproteins (LDL) properties by oxidation of polyunsaturated fatty acids (PUFA) is believed to play a major role in atherosclerosis. The oxidation affects the protein moiety (apo B) of LDL particles impairing their catabolism by the regulated apo B/E receptor system. Therefore, the protective role of foods rich in phenolic compounds has been attributed to their antioxidant properties (reviewed in ref. 33). Frankel et al (University of Davis, California) were the first to demonstrate that trans-resveratrol added to human LDL, reduced the copper-catalyzed oxidation (34). At the concentration of 10 μmol/L, the LDL peroxidation was more inhibited by resveratrol than by a polyphenolic extract of red wine. However, the stilbene was less potent than either epicatechin or quercetin. By measuring the formation of degradative products from PUFA during porcine LDL oxidation, we observed that trans-resveratrol mainly acted by chelating copper whereas flavonoids were better scavengers of free radicals (35). In contrast with Frankel et al, (34) we observed that resveratrol was more protective than flavonoids during copper-catalyzed oxidation. The kinetics of conjugated diene formation showed that the addition of resveratrol to LDL prolonged the lag-time, which preceed the onset of oxidation in a dose-dependent manner. At the concentration of 1.5 μmol/L, the lag time was twice as long with resveratrol than with flavonoids (catechin, epicatechin or quercetin) when oxidation was induced by copper. Contradictory results were

TABLE 2
Major Biological Activities of Resveratrol

- Inhibition of lipid peroxidation (LDL, membranes)
- Chelation of copper
- Free-radical scavenging
- Alteration of eicosanoid synthesis
- Inhibition of platelet aggregation
- Anti-inflammatory activity
- Vasorelaxing activity
- Modulation of lipid metabolism
- Anticancer activity
- Estrogenic activity

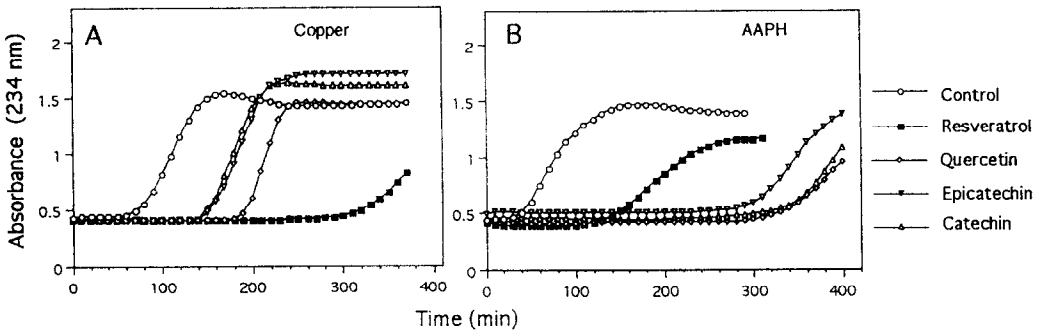


FIG. 2.

Kinetics of LDL oxidation as determined by measuring the change in absorbance at 234 nm. LDL (50 μg protein/mL) were incubated at 37°C in the presence of either 5 μM $CuSO_4$ (A) or 1 mM AAPH (B). The medium did not contain antioxidant (control) or contained 1.5 μM of a defined antioxidant as indicated.

obtained when oxidation was induced by the free radical generator AAPH (2,2'-azobis (2-amidinopropane dihydrochloride) (Fig. 2).

These findings were confirmed in other assays (36) comparing the capacity of resveratrol and flavonoids to reduce the loss of PUFA and to normalize the cellular uptake of LDL via the B/E receptor system. The high capacity of resveratrol to chelate copper is potentially useful *in vivo* since LDL are known to have a high ability to bind copper. We found that the chelating capacity of the cis-isomer was about half that of the trans-isomer, whereas both isomers were equally efficient in scavenging free radicals. This suggests that the spacial position of hydroxyl groups is of prime importance for chelation of copper. However, trans-resveratrol could not chelate ferrous ions (35). The efficiency of antioxidants probably depends on their concentration in the area of oxidation. By adding trans-resveratrol to porcine plasma we observed that it was distributed between subsequently isolated lipoproteins and was associated with lipid as well as protein moieties. This may facilitate the protection of lipoprotein PUFA (37).

Resveratrol also inhibits the peroxidation of membrane lipids. In rat liver microsomes, Blond et al (38) showed that in non-enzymatic or in NADPH-dependent peroxidation, the concentration required to produce 50% inhibition was about three times lower with resveratrol than with quercetin. Furthermore, resveratrol and piceid extracted from *Vitis vinifera* cell cultures were found to prevent metal-induced lipid peroxidation in microsomes and LDL (39). The authors compared the response of these compounds to that of other polyphenols (astringin and astringinin) and found that the presence of 4'-hydroxy in ring B and the meta-hydroxy structure in ring A are essential for the antioxidant activity of stilbenes.

By protecting cell membranes, resveratrol probably reduces the deleterious effects of oxidative stress in living cells. Sun et al (40) used PC12 cells (rat adrenal pheochromocytoma cells) to examine the effects of resveratrol during lipid oxidation induced by iron and ethanol. These cells are considered as a model for dopaminergic neuronal cells and are very sensitive to heavy metal ions and free radical attack. The authors observed that resveratrol protected cells from peroxidative stress and tissue damage. Furthermore, Chanvitayapongs et al (41) showed that a combination of trans-resveratrol and vitamin C and/or E was more effective in protecting cells than was any of these three antioxidants alone. The results indicated that resveratrol not only possessed antioxidant and antimutagen properties, but also could reduce cell death. In other studies carried out in the same laboratory, Draczynska-Lusiak et al (42) found that tissue injury and cell death was partly due to the internalization of cytotoxic oxidized VLDL (very-low density lipoproteins) and LDL. This may contribute to the

manifestation of neurodegenerative diseases. The addition of antioxidants, such as resveratrol, vitamins C or E, to the incubation medium of PC12 cells effectively prevented cell death induced by oxidized lipoproteins. The effect of resveratrol might be related to its amphipatic character, which allows the protection of cellular and subcellular components.

Resveratrol also exerts a hepatoprotective activity. It is known that the proliferation of stellate cells, which play a critical role in the development of liver fibrosis, is enhanced by oxidative stress. Therefore, the compounds that can inhibit the activation of these cells may prevent the hepatic fibrogenesis. Kawada et al (43) found that resveratrol inhibited stellate cell activation by disrupting signal transduction pathway and cell cycle protein expression. Moreover, the authors showed that resveratrol could inhibit the production of nitric oxide and tumor necrosis factor α (TNF-α) by lipopolysaccharide-stimulated Kupffer cells.

Modulation of lipid and lipoprotein metabolism. As already stated, resveratrol was found almost twenty years ago to be beneficial for rat lipid metabolism (27, 28). Recent studies carried out at the University of Toronto, used the human hepatocarcinoma cell-line HepG2 which retain most of the functions of normal liver parenchymal cells (44, 19). The authors observed a significant decrease in the intracellular concentration of apo B in response to increasing concentrations of trans-resveratrol in the medium (up to 50 μmol/L). Moreover, the secretion rate of cholesterol esters and triglycerides were lowered, suggesting that fewer VLDL and therefore fewer LDL were produced. This is potentially beneficial since LDL may be atherogenic.

Some observations have challenged the protective effects of resveratrol against atherosclerosis. In diet-induced hypercholesterolemic rabbits, dietary trans-resveratrol (0.6 mg/kg for 5 days and then 1 mg/kg from days 6 to 60) did not reduce the very high cholesterol content in plasma (about 30-fold the normal content) and did not alter the electrophoretic mobility of LDL isolated from a 12 h-fast plasma. The response of aorta was unexpected since severe atherosclerotic lesions were found in resveratrol-treated rabbits (45). In another study (46), trans-resveratrol was daily injected to female rats at high amounts (20 and 40 mg/kg). After 21 days of treatment, the lipoprotein profile remained unaltered, and there was no change in the susceptibility of lipids associated with serum proteins to copper-mediated peroxidation.

Antiplatelet aggregation. Platelet aggregation is linked to the synthesis of eicosanoids from arachidonic acid. Prostacyclin PGI_2, a potent vasodilatator and thromboxane TxA_2, a proaggregant and vasoconstrictor agent, are produced by the cyclooxygenase (COX) pathway. Furthermore, hydroxyacids (HHT, HPETE and HETE) and leukotrienes (LT) are produced by the lipoxygenase pathway. LTB_4 is a mediator of inflammation and a proaggregant agent. By using stilbenes isolated from *Polygonum* roots, Kimura et al (47) showed that at concentrations of 10^{-6} - 10^{-3} mol/L, resveratrol inhibited the formation of lipoxygenase products and TxB_2 (stable metabolite of TxA_2) by rat peritoneal polymorphonuclear leucocytes. These substances are involved in inflammatory processes, such as the formation of chemotactic substances and platelet aggregation. Moreover, at 10^{-3} mol/L, resveratrol inhibited arachidonic acid-induced platelet aggregation. More recently, the antiplatelet activity of resveratrol was evaluated in platelet-rich plasma from healthy humans (48). The collagen-induced platelet aggregation was reduced by 50.3% in the presence of 3.6 μg/L trans-resveratrol. Assays with 1000-fold diluted red wine containing 3.6 mg/L polyphenols and 1.2 μg/L natural trans-resveratrol showed an inhibition of 42%, which increased to 78% when 1.2 μg/L trans-resveratrol was added to the same wine. These findings indicate an interaction between resveratrol and red wine components. By comparing the antiaggregating effects of both resveratrol isomers, the same group of authors (49) observed that at similar concentrations, the trans-isomer was slightly less active than the cis-isomer.

Pace-Asciak et al (50) also used human platelet-rich plasma but induced aggregation by thrombin or ADP. They found that the inhibition by trans-resveratrol and quercetin was dose-dependent.

Resveratrol also inhibited the synthesis of TxB_2, HHT and, to a lesser extent, 12-HETE. However, very high concentrations were required to obtain 50% inhibition (130 and 165 μmol/L with ADP and thrombin respectively). In further studies, the same team carried out ex-vivo studies to test the effects of polyphenols in 24 healthy male subjects aged 26-45 years (51). Each consumed the following beverages for periods of 4 weeks: red wine, white wine, commercial grape juice or commercial grape juice containing 4 mg/L trans-resveratrol. The daily volumes were 375 mL for wine and 500 mL for grape juice. Assays with fasting platelet-rich plasma showed that the two wines and resveratrol-enriched juice increased the resistance of platelets to thrombin-induced aggregation and lowered TxB_2 content. The platelet ratio of TxB_2/12-HETE increased after the intake of commercial juice, whereas it decreased after the intake of resveratrol-enriched juice. The authors concluded that trans-resveratrol could be absorbed from grape juice in biologically active quantities. The failure of red wine rich in polyphenols to show any advantage suggested that the dominant anti-aggregatory component of wine was ethanol.

The activity of resveratrol 3-0-β-D-glucopyranoside on platelet aggregation has also been reported (52). The highest activity was found with collagen as inducer and the response with other inducers decreased in this order: adrenaline > arachidonic acid > ADP. The authors tested the activity of other related hydroxystilbenes and glucosides. The activity appeared to be related to the different location and orientation of the hydroxyl groups and the configuration of the double bond; glucosylation of stilbenes depressed the activity.

Vaso relaxing activity. Resveratrol extracted from *Polygonum cuspidatum and multiflorum* roots partially antagonized the contraction of trachea isolated from antigen-sensitized guinea-pigs. This suggested a resveratrol inhibitory action on arachidonic metabolism (53). In order to test the cardiovascular effects of grape products, Fitzpatrick et al (54) compared their ability to relax rat aorta rings and found no vasorelaxation in assays with trans-resveratrol. In contrast, Chen and Pace-Asciak (55) observed that both quercetin and trans-resveratrol caused a nitric-oxide (NO)-mediated relaxation of precontracted endothelium-intact rat aorta. The compounds also induced a NO-independent vasodilatation of denuded aorta.

Anticancer activity. In Japan (56), an extract of *Yucca schidigera* showed an antimutagenic effect in bacteria cells. The hydroxyl groups of the active compound, identified as resveratrol, were required for this activity. Furthermore, resveratrol obtained from *Cassia quinquangulata* (Leguminosae) had a cancer chemopreventive activity in assays representing the three major stages of carcinogenesis (57). It acted as an antioxidant and antimutagen and induced the detoxification of carcinogens. Moreover, it mediated anti-inflammatory process mainly by inhibiting cyclooxygenase-1 (COX-1) and hydroperoxidase functions. Resveratrol also inhibited the progression of cancer by inducing cell differentiation. Direct evidence of the chemopreventive activity of resveratrol was assessed by its ability to inhibit the development of preneoplastic lesions in carcinogen-treated mouse mammary glands in culture as well as tumorigenesis in a mouse skin cancer model.

Likewise, synthetic trans-resveratrol inhibited the cellular process involved in the three stages of tumor development (58). The authors compared the effect of resveratrol in three human breast epithelial cells (immortal receptor-negative and malignant receptor-negative or receptor positive). With each line, the inhibition of proliferation was dose- and time-dependent. Moreover, treatment with resveratrol reduced the number of viable cells and prevented the exponential growth. This suggested that resveratrol is a potential chemopreventive agent for both hormone-responsive and non-responsive breast cancers. Because cyclooxygenase-2 (COX-2) is important for tumorigenesis, the ability of trans-resveratrol to modulate the gene expression of this enzyme was investigated in human mammary and oral epithelial cells (59). The production of prostaglandin PGE_2 was induced by phorbol ester. Thus, synthetic trans-resveratrol suppressed the activation of COX-2 gene expression by inhibiting the protein kinase C signal transduction pathway. The compound also directly inhibited the activity of COX-2.

In human HepG2 hepatoma cells, resveratrol ws found to inhibit the expression of the enzymes which metabolizes arylhydrocarbons to genotoxic metabolites (60). Moreover , Casper et al (61) showed that resveratrol was a competitive antagonist of the arylhydrocarbon receptor. Since, the compound was effective at micromolar concentrations, both ex-vivo and in vivo, the authors suggested that it has adequate potency to reduce the inflammatory endothelial cell damage caused by environmental toxicants.

Furthermore, in murine leukemia cells, trans-resveratrol inhibited the ribonucleotide reductase which is involved in the cell proliferation (62). Resveratrol is probably less toxic than hydroxyurea and more active for scavenging the tyrosyl radical of the enzyme and inhibiting DNA synthesis. Trans-resveratrol has also a chemotherapeutic potential. Clement et al (63) showed that it induced apoptotic cell death in human leukemia cells as well as in human breast carcinoma cells. The resveratrol-mediated cell death specifically involved the CD-95 system, known as mediator of apoptosis.

Estrogenic activity. The similarity in structure between trans-resveratrol and the synthetic estrogen diethylstilbestrol, asks the question about estrogenic activity. By using estrogen positive or negative human breast adenocarcinoma cells, Gehm et al (64) showed that, at concentrations comparable to those required for its other biological effects (3-10 μmol/L), resveratrol competed with estradiol for binding to the estrogen receptor. Resveratrol increased the expression of native-regulated genes and stimulated the proliferation of estrogen-dependent breast cancer cells. The authors suggested that the contradiction with the results of Jang et al (57) could be due to the type of cancer cell lines used in the study (mouse mammary cancers are estrogen-insensitive). However, with the same type of estrogen-positive human breast cancer line (MCF-7), resveratrol at higher concentrations (20-160 μmol/L) inhibited the cell proliferation (58). As emphasized by Kopp (65), resveratrol, as a phytoestrogen, potentially contributes to the cardioprotective effects associated with red wine consumption. Yet, we must still consider whether it may produce undesirable adverse effects.

Other effects. The role of resveratrol in preventing cardiovascular diseases may also be related to its capacity to modulate the function of polymorphonuclear leukocytes (PMN). These cells may contribute to the pathogenesis of the disease. The effects of trans-resveratrol on the functional and biochemical responses of PMN upon in vitro activation were examined (66). The results indicated that resveratrol interferred with the release of inflammatory mediators by activated PMN and down-regulated adhesion-dependent thrombogenic PMN functions.

Several other properties of resveratrol have been reported. Thus, Murakami et al (67), showed that some naturally occuring hydroxystilbenes such as resveratrol, are inhibitors of gastric H^+ K^+ ATPase. This enzyme is an important target for peptic ulcer therapy. The phenolic hydroxyl groups are involved in this activity, but stilbenes with neighbouring hydroxyl groups are the most effective inhibitors.

Resveratrol and α-viniferin inhibit the L-dopa oxidase activity of tyrosinase (68). This enzyme catalyses the rate-limiting step in the biosynthesis of melanin which is involved in local hyperpigmentation diseases.

Conclusion

The studies reported in this review support a role of dietary resveratrol in the prevention of cardiovascular diseases. Moreover, resveratrol possesses anticancer properties and, as phytoestrogen, it may favorably influence several physiological processes. Given that resveratrol has a stilbene structure, the action mechanisms differ somewhat from that of flavonoids. This may be avantageous if we assume that the presence of several types of compounds at sites where they can be effective

strengthens the protection. As underlined by Soleas et al in a recent review (19), the biological efficacy of dietary resveratrol cannot be judged before testing its effects on humans. Resveratrol accounts for 1% or less of red wine polyphenols. Accordingly, acquiring knowledge on absorption, metabolism and biochemical activity in humans, is linked to the selectivity and sensitivity of analytical methods.

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