Natural Sources of Resveratrol and Mechanisms of Action with Emphasis on Cardiovascular Disease: A Brief Review

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Abstract

Coronary artery disease (CAD) is the major cause of mortality and morbidity in many developed countries. Atherosclerosis is caused by a multistep process: injury to the endothelial lining of the arteries, infiltration of arterial intima with plasma lipoproteins; migration of smooth muscle cells (SMCs) from the media into the intima; proliferation of SMCs; synthesis of connective tissue components; artery influx of monocytes which become fat-filled foam cells through the uptake of low-density lipoproteins (LDL). Pathologic mechanisms operating in the atherosclerotic lesion are largely chronic inflammatory responses. Epidemiological studies indicate that the consumption of red wine reduces the mortality and morbidity from CAD. Polyphenols are ubiquitous groups of plant metabolites and are important ingredients of diets consumed by humans and animals. Muscadine grapes contain high levels of antioxidants, including resveratrol, a polyphenol with multiple cardioprotective effects. These antioxidants are thought to be the effective agents behind the so-called 'French Paradox,' the low mortality rate from CAD amongst the French population despite their high-fat diet and smoking. Muscadine grapes (*Vitis rotundifolia*) contain the highest level of antioxidants and resveratrol of any natural product and higher levels than in *Vitis vinifera* (the common bunch grape). Resveratrol has been shown to inhibit lipid peroxidation of LDL, prevent the cytotoxicity of oxidized LDL, protect cells against lipid peroxidation, reduce platelet aggregation, and inhibit vascular SMC proliferation. The cardioprotective effect of polyphenols has also been attributed to an increase in the plasma level of high density lipoprotein cholesterol and a decrease in the prostanoid synthesis from arachidonate. These polyphenolic compounds in muscadine grapes also reduce platelet adhesiveness and inhibit inflammatory mediators. Polyphenols provide cardioprotection

by increasing the production of the vasodilating factor nitric oxide, endothelium-derived hyperpolarizing factor and prostacycline and inhibiting the synthesis of vasoconstrictor endothelin-1 in endothelial cells. They also inhibit the expression of two major pro-angiogenic factors, vascular endothelial growth factor and matrix metalloproteinase-2 in SMCs. Thus polyphenols may provide anti-atherogenic, vasoprotective, anti-angiogenic, vasorelaxant, and anti-hypertensive effects. Resveratrol is reported to slow aging and has been suggested as a potential calorie restriction mimetic. It has been shown to stimulate Sirtuins and this property has been suggested to account for its anti-aging effects. Grape polyphenols interact with each other and this synergistic action may responsible for the enhanced health benefits. Our current studies involve the evaluation of a special natural fruit powder derived from Muscadine grapes containing the total mixture of polyphenols including resveratrol in concentrated and unadulterated form (www.nutragonllc.com). The powder is available for scientific study from Muscadine Naturals, Lewisville, North Carolina. www.muscadinenaturals.com

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Introduction

Atherosclerosis is a complex degenerative disease, primarily of the aorta and large arteries, which leads to serious cardiovascular complications such as stroke, coronary heart disease, aneurysm, and peripheral arterial disease. It starts in childhood and progresses with advancing age. Atherosclerosis may lead to coronary heart disease (CAD), the major cause of death in the United States. Cardiovascular diseases (CVD) cause over 12 million deaths in the world each year. They cause 50% of all deaths in several developed countries, and are one of the leading causes of death in many developing countries. CVD were responsible for approximately 931,108 deaths in the United States in 2001. CVD accounted for 38.5% of all deaths in the United States in 2001. The development of atherosclerosis is a complex, multi-step process: injury to the endothelial lining of the arteries, infiltration of the arterial intima with plasma lipoproteins, migration of smooth muscle cells (SMCs) from the media into the intima, proliferation of SMCs, and synthesis of connective tissue components. Additionally, monocytes in the blood become fat-filled foam cells through the uptake of plasma lipoproteins, especially

low density lipoprotein (LDL).

The major components of the atherosclerotic lesion are lipids (especially cholesterol) and proliferated SMCs. It is proposed that the multiplication of SMCs occurs because individual clones of cells are transformed by chemicals, toxins, viruses, or other factors (Benditt and Benditt 1973). Blood platelets may also adhere to the endothelial lining, resulting in fibrosis, thickening of the arterial intima, and marked stenosis of the lumen (Duguid 1949). Many studies have revealed that there are a large number of factors involved in atherogenesis: increased concentrations of LDL, high intake of dietary cholesterol, obesity, hypertension, familial history of myocardial infarction, diabetes, cigarette smoking, high concentration of lipoprotein (a) [or, Lp (a)], male sex, age, and fibrinogen. High LDL cholesterol concentration ranks as the number one risk factor for atherosclerosis and cardiovascular disease (CVD).

Several epidemiological studies have indicated cardioprotective effects of moderate alcohol and wine intake. A large Health Professionals Follow-Up Study in 50,000 male individuals indicated the relative risk of developing coronary heart disease was reduced by 25% following wine alcohol intake of 5-30g/day (3-05). It has been proposed that the low incidence of CHD in France as compared to other western countries – the "French Paradox"-has been attributed partly to the consumption of red wine in French population. This has led to a decreased morbidity and mortality from coronary heart disease in French population despite the consumption of high-fat diet and smoking. Red wine contains higher levels of polyphenols as compared to white wine and other alcohol beverages. Compounds such as resveratrol, quercetin, catechin, and proanthocyanidins are abundant in grape skin and seeds (6-27). These compounds can inhibit platelet aggregation and protect low-density lipoproteins (LDL) from oxidation (6-28 to 30). The polyphenols may also serve as antioxidants and are probably responsible for the so-called 'French Paradox,' the low mortality rate from CAD amongst the French population despite their high-fat diet and smoking (5-1 to3).

The polyphenol, resveratrol ((*trans*-3, 5, 4'-trihydroxystilbene), is abundant in red wine, compared to white wine, beer, or spirits. Wine, especially red wine contains many polyphenols that have desirable biological properties. There is some evidence indicating that wine confers

beneficial effects on the heart because of the presence of polyphenols. Resveratrol has been shown to have a wide range of biological effects including cardioprotective roles due to a reduction in myocardial damage, modulation of vascular cell functions, inhibition of LDL oxidation, and suppression of platelet aggregation.

The objective of this paper is to present a brief review of recent developments on the effects of resveratrol and the mechanisms by which resveratrol acts as a potential cardioprotective agent.

Chemistry

Resveratrol is a polyphenolic stilbene that has a C6-C2-C6 structure. Stilbenes are ethylene derivatives substituted by two phenyl rings. Ring A usually has two hydroxyl groups in *m*-position, while ring B carries a hydroxyl and methoxy groups in the *o*-, *m*-, and *p*-position. Stilbenes and stilbene glycosides occur naturally in liverworts and higher plants. These compounds constitute the bulk of the phenolic antifungal phytoalexins, which are usually synthesized only in response to infection or injury. A wide array of compounds are formed based on the number and location of hydroxyl groups; the extent to which these groups become substituted with sugars, methyl, methoxy, and other residues; the steric configuration of chemically identical molecules; and their ability to enter into reactions forming dimmers, trimers, or large polymers. The most extensively studied stilbene is *trans*-resveratrol (3, 5, 4'-trihydroxystilbene). The production of *trans*-resveratrol in grapes is accomplished by the condensation of p-coumaroyl CoA with three molecules of malonyl CoA through the activity of the stilbene synthase (1 M). There are two enzymes involved in the synthesis of resveratrol. Chalcone synthase is constitutively expressed in plants for making polyphenols. The other enzyme, stilbene synthase is made by the gene that is induced by light, pests, mold etc. This enzyme is used to make resveratrol which is used to produce two antifungal agents, α-vineferin and ε-viniferin. These two enzymes share 75% to 90% amino acid sequence identity and generate the same tetraketide, but stibene synthase enzyme cyclize this tetraketide via an intramolecular C2 to C7 aldol condensation to make stilbene, whereas the chalcone synthase cyclize via C2 to C6 condensation to make chalcone (19-05M). Chalcone-derived natural chemicals play important roles in plant fertility, disease resistance and flower color. However,

the production of resveratrol and other rare antifungal stilbenes occurs in only in a few plant species, such as grapes, peanuts, blueberries and some pine trees.

Resveratrol is important in plant biology because it serves as parent molecule for a family of polymers known as viniferin. These compounds are able to inhibit the progress of fungal infections in plants. The major source of resveratrol in the human diet is grapes and peanuts. In the folk medicine, humans have been exposed to resveratrol derived from the use of medicinal plants, such as the roots and rhizomes of *Polygonum cuspidatum*, *Veratrum formosanum*, that are commonly used in the traditional medicine for the treatment of several ailments (2). The production of the polyphenol, *trans*-resveratrol is positively correlated with the resistance of the wine to cryptogamic, and it is a good marker for gray mold resistance. In grapevine, this compound is synthesized in leaves, roots, and grape skins. It is also synthesized in response to microbial infection or stress. In grape products, in addition to the aglycone, *trans*-resveratrol, *trans*-piceid is also found. In grape berries, these compounds are mainly located in the skin cells and are absent or low in the fresh fruit. They occur in two isomeric forms, cis and trans; however the *cis*-resveratrol is a by-product of fermentation and it is rarely found in grapes.

Historical Background

The potential health benefits of wine were reported by Paracelsus, Plinius, and Galenus. Hippocrates of Kos (459-377 AD) recommended the application of wine as a tranquilizer, analgesic, and diuretic. Caesar recommended wine with meals for the protections of his soldiers from gastrointestinal infections. Galenus of Pergamon (130-200 BC) used heavy red wine to protect from gastrointestinal infection and tannin-rich red wine to protect from gastrointestinal bleeding. Hildegard of Bigen (Germany, 1098-1179) applied a special wine recipe in order to treat cardiovascular disease. Darakchasava, an ayurvedic medicine, consisting mainly of *Vitis vinifera* was used as cardiotonic in India dating back to over 1,000 years ago (4-103). The major component of this preparation is resveratrol. Resveratrol was originally identified as the active ingredient of an Oriental herb (Kojo-kan) used for the treatment of a wide variety of diseases as dermatitis, gonorrhea, fever, hyperlipidemia, atherosclerosis, and inflammation (6-38).

One of the primary uses of grapes is in the preparation of wine. Some researchers place the discovery of wine making in the southern Caucasus. The domestication of the wine grape, *Vitis vinefera*, initially occurred in this area. Grape growing and wine making then spread to Palestine, Syria, Egypt, Masopotamia, and Miditerranean countries. Wine was used for sacramental purposes in Egypt during the first part of the third millennium BC, although it was not produced there for general consumption for another 2,000 years. Modern production of better wine probably began in the seventeenth century and this might have contributed to increase their lifespan. North Americans are relatively latecomers to viticulture. Franciscan missionaries were believed to have planted the first large-scale vineyards in California some 2000 years ago.

Platelet Effects

Platelet aggregation plays an important role in the pathogenesis of acute coronary syndromes, and there is considerable evidence that antiplatelet therapy reduces CVD risk (7-05). The most widely accepted mechanism of resveratrol-mediated cardioprotection is its ability to inhibit platelet aggregation. Resveratrol and red wine were shown inhibit platelet aggregation in healthy volunteers (4-05-11). It was noted that an increase in cytosolic Ca^{2+} level serves as a stimulus for platelet aggregation ((4-05-107). Resveratrol inhibits Ca^{2+} influx in thrombin-stimulated platelets. From this study, it is clear that resveratrol operates through store-operated calcium channels, because thapsigargin, whose stimulation of Ca^{2+} influx was inhibited by resveratrol, operates through store-operated calcium channels. *Trans*-resveratrol was shown to block ADP, collagen, and thrombin induced platelet aggregation (2-34, 35).

Resveratrol also has the ability to inhibit arachidonate metabolism (2-34, 35. It can specifically inhibit thromboxane A2 formation, which could moderate thrombotic events and anti-inflammatory process. Quercetin and to a lesser extent resveratrol, inhibits the 12-lipoxygenase pathway of arachidonic metabolism, blocking the synthesis of hepoxillins which are mediators of calcium mobilization, vascular permeability, and neutrophil activation (8-05-35). Specifically, the production of 12- hydroxyeicosatetraenoate (12-HETE), which was thought to be proatherogenic (because it impairs endothelial function and prostacyclin production (8-05-36)

was markedly reduced. Resveratrol blocked the synthesis of thromboxane A2 from arachidonate, as measured by the production of its stable metabolite thromboxane B2, hydroxyheptadecatrienoate (HHT), and to a lesser extent 12-HETE from arachidonate in a dose-dependent manner. Quercetin inhibited the only 12-HETE synthesis. Because resveratrol inhibits thromboxane A2 production and quercetin inhibits the synthesis of hepoxillins, these two phenolic compounds from red wine can virtually shut down eicosanoid synthesis of human platelets *in vitro* (9-05-10).

Resveratrol mediates the anti-inflammatory process mainly by inhibiting cyclooxygenase- and hydroperoxidase functions. *Cis*-resveratrol inhibits protein-tyrosine kinase and platelet aggregation (2-3, 36). Currently, there is great interest in the importance of systemic inflammation as a pathogenic mechanism of CVD risk. There is much evidence that polyphenolic compounds may have anti-inflammatory effects. For example, resveratrol inhibits adhesion molecule expression and monocyte adhesion *in vitro (7-65)*. Additional studies are needed concerning the anti-inflammatory effect of polyphenols.

Endothelial Cell Effects

The vascular endothelium plays an important role in the regulation of vascular homeostasis, and increasing evidence suggests that changes in endothelial function contribute to the pathogenesis and clinical expression of CVD (7-05-23). Vascular homeostasis is controlled by several endothelium-derived relaxing factors such as NO, prostacycline and endothelium-derived hyperpolarizing factor (EDHF) (8-05 M). EDHF is involved in the regulation of vascular tone and this participation increases as the vessel size decreases (8-05-302). Endothelial cells regulate vascular homeostasis by synthesizing factors that act locally in the vessel wall and lumen, and the key endothelial product is nitric oxide (NO). It was originally thought that NO is an endothelium-derived vasodilator, but it is now clear that NO regulates other aspects of vascular homeostasis (7-05-25). It is now evident that NO prevents adherence of leukocytes to the endothelial surface and inhibits expression of leukocyte adhesion molecules at the endothelial surface. Thus, NO prevents platelet adhesion and platelet aggregation. NO also inhibits SMC proliferation and alters the expression of noncellular components that constitute the matrix of the

vascular wall, thus implicating NO in lesion formation, hypertrophy of the vessel wall, and vascular compliance. Therefore, NO has vasodilator, anti-inflammatory, antithrombotic, and growth-suppressing properties that are relevant to all stages of atherosclerosis.

Polyphenols have been shown to increase the formation of NO by endothelial NO synthase. Plant phenols produced endothelium-dependent vasorelaxation that was generally associated with increased cyclic GMP formation by inhibitors of NO synthase, indicating that it was mediated by the NO-cyclic GMP pathway. The relaxant effect was strongly correlated with the concentration of polyphenols in wine (8-05, Burns).

Naderali et al. (4-05-97) showed that resveratrol induces vasorelaxation in mesenteric and uterine arteries isolated from guinea pigs, irrespective of the stage of the estrous cycle. This effect was not inhibited by indomethacin, indicating that the vasorelaxation was not prostanoid-mediated and may be NO-mediated. Chen and Pace-Asciak (4-05-26) showed that pretreatment of isolated rat aorta with NO synthase inhibitor antagonized the vasorelaxation caused by resveratrol. They suggested that other mechanisms may play a role in vasorelaxation. For example, resveratrol has a stimulatory effect on Ca^{2+}-activated $Ca2^{+}$-activated K^{+} (BK_{Ca}) channels found in human vascular endothelial cells (4-05-81). It is possible that increased K^{+} efflux following the activation of BK_{Ca} channels by resveratrol may increase K^{+} concentration in the myoendothelial space followed by hyperpolarization of vascular myocytes, leading to dilation of blood vessels. Additionally, a steroidal pathway for resveratrol-induced relaxation of porcine coronary arteries has been suggested (4-05-65). Resveratrol resulted in a dose-dependent inhibition of contractions induced by histamine, in the presence of F^{-} ion, at physiological concentration, whereas higher doses were required to inhibit ouabain and tetraehtylammonium-induced contractions. Based on the estrogenic effects of resveratrol (4-05-52), it was hypothesized that the relaxation induced by resveratrol could be mediated through activation of steroid-like membrane receptors that may impact inducible NO synthase (iNOS) leading to the production of NO.

Thus, endothelium-derived factors play a major role in the control of vascular tone and are involved in other important aspects of vascular biology. In particular, endothelial NO exerts

vasoprotective effects. Its deficiency favors the development of atherosclerosis and is associated with increased cardiovascular risk in pathological situations such as diabetes, hypertension. Restoration of the equilibrium between endothelium-derived factors might be involved in vasoprotective effects of polyphenols in red wine. Even though, an increase in Ca^{2+} in endothelial cells may constitute an important mechanism leading to eNOS activation by polyphenols, it does not preclude a possible implication of the PI3-kinase/Akt pathway. There is evidence to indicate that red wine polyphenols induced the activation of the PI3-kinase/Akt pathway in porcine coronary artery endothelial cells, which in turn, caused an increased phosphorylation of eNOS resulting in an increase in the production of NO (8-05-Ndiaye).

Hung et al. (12-05M) examined the cardioprotective effect of resveratrol, an antioxidant present in red wines, in the rats after ischemia and ischemia-reperfusion. They concluded that preinfusion of resveratrol is effective to prevent reperfusion-induced arrhythmias and mortality. This protective effect on arrhythmias and cardiac cell damage by resveratrol may be associated with its antioxidant activity, free radicals scavenging activity, and increased NO release during the reperfusion period.

Long-term incubation of endothelial cells with red wine increases eNOS expression (8-05-Wallerath). This stimulatory effect was attributed to polyphenols because ethanol alone had no effect on eNOS expression, whereas as increase was observed in response to red wine extract without alcohol (8-05-Leikert). Resveratrol also stimulated eNOS expression in concentration ranging from 10 to 100 um (8-05-Wallerath 2002). The upregulation of eNOS mRNA was associated to an increase in the expression in eNOS protein and enzymatic activity. Resveratrol increased the activity of the eNOS promoter (transcriptional effect) and stabilized eNOS mRNA (posttranslational effect (8-05-Wallerath).

Red wine extracts strongly inhibit endothelin-1 release and transcription of the prepro-endothelin-1 gene in bovine aortic endothelial cells. This effect may be due to the modifications of tyrosine kinase signaling (8-05-Khan). Resveratrol also strongly inhibits strain-induced endothelin-1 secretion, endothelin-1 mRNA level, and endothelin-1promoter activity in human umbilical vein endothelial cells, partially by interfering with the ERK1/2 pathway through

attenuation of reactive oxygen species formation (8-05-Liu). In addition, pretreatment of human umbilical endothelial cells with quercetin reduces thrombin-induced endothelin-1 release (8-05-Zhao). Thus, inhibition of endothelin-1 synthesis may represent one of the mechanisms by which plant polyphenols may restore the balance between endothelium-derived vasoconstrictor and vasodilating factors when pathologically impaired, and prevent the development of hypertension or heart failure.

Smooth muscle cell effects

The proliferation of SMCs in the arterial intima is an important step in the formation of atherosclerotic plaques (16-05-2, 3). Inhibition of vascular SMC proliferation may have a beneficial effect in retarding the development of atherosclerosis. Ariam et al. (16-05M) found that red wine and red wine polyphenol extract inhibit SMC proliferation in a dose-dependent manner. Zou et al. (16-05-10) showed that the number of SMCs was significantly reduced in rabbits fed a high resveratrol diet. Several studies have shown that resveratrol has an antiproliferative effect on many cancer cell lines, including human breast cancer, oral squamous cell carcinoma, and different human leukemias (16-05-12, 14). Studies by Zou et al. (16-05-15) suggested that red wine contains other polyphenols in addition to resveratrol, which may have an antiproliferative effect. Many studies indicate that resveratrol arrests cell cycle progression at the S (DNA synthesis) phase of mitosis (16-05-16, 17). Other studies showed that resveratrol is capable of causing DNA strand breakage in the presence of copper ions, (16-05-18). Preliminary studies by Arian et al. (16-05-M) indicate that cell cycle arrest at S/G2 phase transition may also occur in SMCs exposed to resveratrol. In addition to the cell cycle arrest mechanism, investigators have proposed that resveratrol may inhibit cell proliferation by causing apoptosis. Resveratrol exposure also causes increased apoptoptic cell death and a decreased expression of the anti-apoptotic protein Bcl-2 in human leukemia cells (16-05-20). Thus, it is possible that resveratrol may inhibit cell proliferation by more than one pathway.

Iijama et al. (18-05M) stated that the down regulation of cyclin A gene expression may contribute to the antiproliferative effect of red wine polyphenols on cultured rat aortic SMCs through the inhibition of transcription factor expression. Resveratrol is an agonist for the

estrogen receptor and it is similar in structure to synthetic estrogen-like compounds. Estrogen, which is a ligand for the estrogen receptor expressed in vascular SMCs, has been shown to have antiatherogenic effect by inhibiting the proliferation vascular SMCs (18-05-26 to 37). This estrogen-like agonistic function of resveratrol is one possible mechanism for the antiproliferative effects of red wine polyphenols on vascular SMCs.

At the site of the atherosclerotic lesion, the most potent mitogenic and chemotactic agent for vascular SMCs is the platelet-derived growth factor (PDGF) released by platelets, endothelial and vascular SMCs. PDGF exerts its biological effects via activation of two subtypes of transmembrane receptor tyrosine kinases, termed α and β PDGF receptor. Ligand binding to the β receptor promotes the activation of signalizing enzymes which are important for cell migration and proliferation. Activation of phosphatidylinositor 3'-kinase (PI13K) and mitogen-activated protein kinase (MAPK) pathways as response to PDGF is implicated in vascular SMCs motility (17-05-56 to58). Red wine phenols (1-100 ug/ml) inhibited rat aortic SMCs proliferation and DNA synthesis. Vascular endothelial growth factor (VEGF) released from vascular SMCs serves as a powerful endothelial mitogen and stimulates the expression of adhesion molecules and monocyte chemotactic protein-1 (17-05-64). Red wine given to cholesterol-fed rabbits inhibited the expression of monocyte chemotactic protein-1, but this effect was found also in animals receiving white wine (17-05-65). The authors concluded that the inhibition of VEGF expression by red wine phenols involves their antioxidant properties.

Macrophage Effects

Atherosclerosis, previously believed to be a bland lipid storage disease, actually involves an ongoing inflammatory response. Recent advances in basic science have established a fundamental role for inflammation in mediating all stages of atherosclerosis including initiation, progression and thrombotic complications. In addition, low-grade chronic inflammation, as indicated by levels of the inflammatory marker C-reactive protein (CRP), prospectively defines the risk of atherosclerotic complications and provides prognostic information (15-05M). Population-based studies have shown that baseline CRP levels predict future cardiovascular

events (15-05M). In a variety of animal models of atherosclerosis, signs of inflammation occur concurrently with lipid accumulation in the artery wall.

Blood leukocytes, mediators of host defenses and inflammation, localize in the earliest lesions of atherosclerosis in animals and humans. The normal endothelium does not generally support binding of white blood cells. However, early after initiation of an atherogenic diet, patches of arterial endothelial cells begin to express on their surface selective adhesion molecules bind to various classes of leukocytes. In particular, vascular cell adhesion molecule-1 (VCAM-1) binds precisely the types of leukocytes found in early human and experimental atheroma, the monocyte and T-lymphocyte. In the intima, monocytes differentiate into macrophages under the influence of cytokines such as macrophage colony-stimulating factor. Such molecules stimulate the expression of the scavenger receptors that allow macrophages to ingest oxidized lipids and become foam cells. Using an *in vitro* model, researchers have shown that macrophages treated with red wine showed a significant inhibition of LDL oxidation (15-05-22).

Lipoprotein Effects

Considerable evidence suggests that oxidation of low-density lipoprotein (LDL) is a crucial step in the pathogenesis of atherosclerosis. Dietary antioxidants that can inhibit LDL oxidation are receiving considerable attention for their role in the prevention of coronary heart disease. LDL contains phospholipids, free and esterified cholesterol, triacylglycerols, and amino acids, which form apolipoprotein B. The proteins and the polyunsaturated fatty acids components of the LDL are susceptible to free radical-mediated oxidation, particularly if the antioxidant content of the LDL is low. When LDL is oxidized *in vitro*, there is a loss of polyunsaturated fatty acids yielding a range of fragments including hydroperoxides, aldehydes and ketones, which conjugate with other LDL-bound lipids and the apolipoprotein B. This minimally modified LDL has a number of properties that could increase its atherogenecity.

At least three types of scavenger receptors in macrophages internalize the oxidized LDL and become foam cells. The foam cells are regarded as precursors to the development of atherosclerotic plaque. *In vitro* studies indicated that LDL oxidation by endothelial cells,

macrophages and Cu^{2+} can be inhibited by polyphenols (10-05-M). These effects may be due to direct scavenging of the oxidizing species by the polyphenols or may result from the regeneration of vitamin E in the LDL molecule and/or its ability to bind LDL protein (10-05-Wang). Animal and human studies indicate that the ingestion of polyphenols increases the resistance of LDL to oxidation (10-05-Fuhrman). Consumption of red wine, quercetin, and catechin decreased atherosclerotic lesion areas by 31 to 52% in apolipoprotein E-deficient mice (10-05-Hayek). Resveratrol was assumed to protect against atherosclerosis by reducing the peroxidative degradation of LDL. The *in vitro* effectiveness of resveratrol was thought to be mainly due to its capacity to chelate copper, although it also scavenges free radicals (11-05M). *In vitro* observations by Belguen douz et al. (11-05M) support the hypothesis that resveratrol may be efficient at different sites: in the protein and lipid moieties of LDL and in their aqueous environment. Resveratrol is more effective than butylhydroxytoluene (BHT), quercetin or tocopherol on lipid peroxidation in liposomes and in rat liver (2-05-24). Resveratrol can inhibit LDL oxidation and it mainly acts by reducing the copper-catalyzed oxidation, while flavonoids are better scavengers of free radicals.

Resveratrol produces an increase in high density lipoprotein (HDL) cholesterol by preventing LDL cholesterol oxidation. Frankel et al. (13-05M) studied the antioxidant potency of synthetic *trans*-resveratrol and found that it protects human LDL against copper-catalyzed oxidation. Peroxidation, measured by hexanal formation, was inhibited by 81% and 70% in LDL from two healthy adult volunteers by the addition of 10 umol/L of resveratrol. By comparison, the addition of extracts of a red wine (California Petit Syrah), diluted 1000-fold with water, and containing 10 umol/L of total phenols, inhibited LDL oxidation by 61% and 48%. Epicatechin and quercetin, also found in wine, had about twice the inhibiting potency of resveratrol. In contrast, 10 umol/L of α-tocopherol, which has been associated with a reduced risk of heart disease, had a much lower antioxidant potency than resveratrol, inhibiting LDL oxidation by only 40% and 19%. These studies suggest that the combination of phenolic compounds in wine may protect against atherogenesis by their antioxidant effects over a prolonged period of consumption.

Specific Gene Effects (Aging)

Longevity regulatory genes have been identified in many eukaryotes, including rodents, flies, nematode worms and in the single-celled organism yeast. These genes seem to be part of an evolutionarily conserved longevity pathway that evolved to promote survival in response to unfavorable conditions. Sir2-family proteins (Sirtuins) are Class III protein deacetylases conserved from prokaryotes to mammals. Resveratrol has been reported to act as a Sirtuin activator, and this property has been proposed to account for its anti-aging effects. In many organisms, calorie restriction slows the pace of aging and increases maximum lifespan. In the budding yeast, *Saccharomyces cerevisiae* calorie restriction extends lifespan by increasing the activity of Sir-2 (20-05-1), a member of the conserved Sirtuin family of NAD^+-dependent protein deacetylases. This family includes Sir-2.1, a Caenorhabditis elegans enzyme that regulates lifespan, and SIRT1, a human deacetylase that promotes cell survival by negatively regulating the p^{53} tumor suppressor. Howitz et al. (20-05M) demonstrated that resveratrol lowers the Michaelis constant of SIRT1 for both the acetylated substrate and NAD^+, and increases the survival of cells by stimulating SIRT1-dependent deacetylation of p^{53}. In yeast, resveratrol mimics calorie restriction by stimulating Sir2, increasing DNA stability and extending lifespan by 70%.

Synergy

Recent studies examined the possible synergistic interactions among different plant polyphenols. Mertens-Talcott et al. (22-05M) investigated the interactions of ellagic acid and quercetin with resveratrol in human leukemia cells. They showed that polyphenolic compounds interact synergistically in the induction of caspase-3 activity. Results of these studies indicate that the anticarcinogenic potential of foods containing polyphenols may not be based on the effects of individual compounds, but may involve synergistic enhancement of the anticancer effects.

Chan et al. (20-05M) studied the interaction of grape polyphenols on NO production by macrophages, mediators of vascular damage in atherosclerosis. They found that quercetin and

resveratrol at a micromolar range suppressed iNOS gene expression and NO production. Ethanol, at a moderate level, did not produce any appreciable level of reduction of INOS or NO activity. However, when alcohol is present at 0.1 to 0.75% level, it increased the effect of grape phenols in a concentration-dependent manner. Ethanol was also shown to act synergistically with quercetin and resveratrol. Thus, the synergistic interaction between ethanol and grape components quercetin and resveratrol may have potential clinical implications. Therefore, grape polyphenols containing complex mixture of compounds may have a great promise in combating cardiovascular diseases and cancer.

Summary and conclusions

Current evidence supports a central role for inflammation in all phases of atherosclerosis. Pathologic mechanisms operating in the atherosclerotic lesion are largely chronic inflammatory responses. Epidemiological studies indicate that the consumption of red wine reduces the mortality and morbidity from CAD. Polyphenols are important ingredients of diets consumed by humans and animals. Muscadine grapes contain high levels of antioxidants, including resveratrol, a polyphenol with multiple cardioprotective effects. These antioxidants are thought to be the effective agents behind the so-called 'French Paradox,' the low mortality rate from CAD amongst the French population despite their high-fat diet and smoking. Resveratrol has been shown to inhibit lipid peroxidation of LDL, prevent the cytotoxicity of oxidized LDL, protect cells against lipid peroxidation, reduce platelet aggregation, and inhibit vascular SMC proliferation. The cardioprotective effect of polyphenols has also been attributed to an increase in the plasma level of high density lipoprotein cholesterol and a decrease in the prostanoid synthesis from arachidonate. These polyphenolic compounds in muscadine grapes also reduce platelet adhesiveness and inhibit inflammatory mediators. Polyphenols provide cardioprotection by increasing the production of the vasodilating factor nitric oxide, endothelium-derived hyperpolarizing factor and prostacycline and inhibiting the synthesis of vasoconstrictor endothelin-1 in endothelial cells. Resveratrol is reported to slow aging and has been suggested as a potential calorie restriction mimetic. It has been shown to stimulate Sirtuins and this property has been suggested to account for its anti-aging effects. Different polyphenols present in grapes may interact with each other and this synergistic action may be responsible for the

enhanced health benefits. Muscadine grape powder containing the complete mixture of polyphenols may have great promise in combating heart disease and its complications.

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References