Cardioprotective actions of grape polyphenols
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Abstract

The aim of this review is to discuss the accumulating evidence that suggests that grape extracts and purified grape polyphenols possess a diverse array of biological actions and may be beneficial in the prevention of some inflammatory-mediated diseases including cardiovascular disease. The active components from grape extracts, which include the grape seed, grape skin, and grape juice, that have been identified thus far include polyphenols such as resveratrol, phenolic acids, anthocyanins, and flavonoids. All possess potent antioxidant properties and have been shown to decrease low-density lipoprotein–cholesterol oxidation and platelet aggregation. These compounds also possess a range of additional cardioprotective and vasoprotective properties including antiatherosclerotic, antiarrhythmic, and vasorelaxation actions. Although not exclusive, antioxidant properties of grape polyphenols are likely to be central to their mechanism(s) of action, which also include cellular signaling mechanisms and interactions at the genomic level. This review discusses some of the evidence favoring the consumption of grape extracts rich in polyphenols in the prevention of cardiovascular disease. Consumption of grape and grape extracts and/or grape products such as red wine may be beneficial in preventing the development of chronic degenerative diseases such as cardiovascular disease.

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Abbreviations: ApoE, apolipoprotein E; BP, blood pressure; COX, cyclooxygenase; CVD, cardiovascular disease; FMD, flow-mediated dilatation; LDL, low-density lipoprotein; NO, nitric oxide; p70S6K, p70 ribosomal protein S6 kinase; PECAM-1, platelet endothelial cell adhesion molecule-1; PI3K, phosphatidylinositol 3-kinase; ROS, reactive oxygen species.

1. Introduction

Cardiovascular disease (CVD) is one of the leading causes of death in many economically developed nations as well as in emerging economies. Although some of the major risk factors for CVD are not “modifiable”—age, sex, genetic predisposition—diet and lifestyle issues are recognized as the major modifiable risk factors. For instance, the negative effects of excessive dietary intake of saturated fats and cholesterol in the development of CVD via changes in plasma low-density lipoprotein–cholesterol (LDL-c) are well established [1]. It is noteworthy however that certain nonlipid risk factors too can influence the development of CVD including coronary heart disease because about one half of all coronary heart disease deaths occur in individuals with normal cholesterol levels [2]. Accordingly, current knowledge favors the notion that risk factors other than raised plasma cholesterol play an important role in the development of CVD [3].

Indeed, recent findings have highlighted the importance of oxidative stress, vascular inflammation, and endothelial dysfunction (as central) to the development of CVD (Fig. 1) [4]. Such advancements in the knowledge of the disease process have also provided new avenues to develop novel pharmaceutical and/or dietary strategies to curb the development of vascular diseases. On this point, the recent...
expansion and the growing popularity of functional foods and nutraceuticals aimed at promoting heart health can be viewed as specific examples. It is of interest to note that epidemiological observations have played a significant part in the development of such products, with the most notable examples being the findings in relation to the lower ischemic heart disease incidences in the Eskimos and the better vascular health and lower cardiac mortality rates in the French [5]. In both instances, research based on the original epidemiological observations have progressed rapidly and resulted in the identification and confirmation of respective dietary protective agents, namely, the long-chain n-3 polyunsaturated fatty acids (Eskimos) and the polyphenols of *Vitis vinifera* in the case of the French. For the latter, Renaud and de Lorgeril [5] suggested wine intake as one possible explanation for the lower than expected coronary heart disease mortality rates in France (the “French Paradox”). Many studies to date have investigated the French paradox, and recent evidence demonstrates that consuming alcohol in the form of (red) wine might confer a protection against coronary heart disease above that expected from its alcohol content alone, with the benefit being attributed to the presence of polyphenols. Similarly, grape seed and red wine extracts are also known to contain high levels of polyphenols, and the beneficial health effects have been attributed to consumption of these compounds [6-13].

Some of the potential mechanisms of preventing CVD after consumption of grape polyphenols could be related to their antioxidant activity [14]. The protective effect of polyphenols is in part due to their ability to retard the development and progression of early atherosclerotic lesions to advanced atherosclerotic plaques. Antioxidant flavonoids found in red wine for example can reduce the oxidation of LDL-c, which is a key and early event of the atherogenic process [15]. Other potential mechanisms by which grape polyphenols may exert cardioprotective effects include a reduction in oxidative stress, modulation of the inflammatory cascade, improvement in vascular endothelial function (eg, flow-mediated dilatation [FMD]), and protection against atherothrombotic episodes including myocardial ischemia and inhibition of platelet aggregation [14,16-22]. In this review, we discuss the beneficial cardiovascular effects of grape polyphenols.

### 2. Grape composition

Products such as red wine extract, grape seed, and grape skin extracts as well as grape juice are all known to contain a diverse array of potent antioxidants in the form of polyphenols, which include phenolic acids (eg, gallic acid), anthocyanins, and simple and complex flavonoids (eg, proanthocyanidins). The quantity, structure, and degree of polymerization of grape proanthocyanidins differ, depending on their localization in the grape tissues [23]. For example, grape seeds contain higher concentrations of monomeric, oligomeric, and polymeric flavan-3-ols compared with grape skins [23]. In a study by Monagas et al [23], grape seeds were reported to contain approximately 2.3 to 8.2 mg/g of monomeric, oligomeric, and polymeric flavan-3-ols such as (+)-catechin, (−)-epicatechin and their gallates. However, grape skin proanthocyanidins have a higher degree of polymerization than those from the seeds and as a result are more easily transferred to wine [23,24]. Grape skins contain approximately 20-fold less (on a milligram per gram basis) monomeric, oligomeric, and polymeric flavan-3-ols compared with grape seeds [23]. It is also well known that grape polyphenol composition and content varies between different cultivars and is influenced by geographic location and the climatic conditions [25].

Although the skin and seeds of grapes have been reported to contain “cardioprotective” polyphenolic antioxidants, a recent animal study demonstrated that extracts from the flesh of grapes possessed cardioprotective actions [21]. The total polyphenolic index was lower in the grape flesh compared with the grape skin; however, the anthocyanins were exclusively in the grape skin, whereas the reactive oxygen scavenging activities were similar in the 2 groups. The results suggested that the flesh of grapes may be equally cardioprotective despite the fact that the grape flesh does not contain anthocyanin activity.

As mentioned above, there is mounting evidence to indicate the potential cardioprotective effects of red wine and red grape juice consumption, and this has been attributed to specific polyphenolic constituents of the grapes (see other sections below and Table 1) [25]. One of the active components of red wine is resveratrol (trans-3,5,4′-trihydroxystilbene), a naturally occurring phytoalexin originating mainly from the skin of grapes. Phytoalexins including...
resveratrol are antibacterial and antifungal chemicals produced by plants as a defense against infection by pathogens [26]. The amount of resveratrol found in grape skins also varies with exposure to fungal infection and its geographic origin. The resveratrol levels in the final “product,” for example, for wine, can also be affected by the vinification process (e.g., the amount of fermentation time a wine spends in contact with grape skins) and the grape cultivar and can be as high as 5.87 mg/L in merlot wines [27].

### 3. Oxidative stress/LDL oxidation

Oxidation of lipoproteins such as LDL-c is an important step in the development of atherosclerosis, and therefore, a
sufficiently high plasma antioxidant status may be protective. Oxidized LDLS stimulate endothelial cells to produce chemokines and other factors that have direct chemotactic activity for monocytes to adhere to the endothelium. Oxidized LDL is preferentially taken up by macrophage cells via scavenger receptors, and they consequently become loaded with lipids and convert into “foam cells” [28]. These foam cells that tend to accumulate in fatty streaks are early indicators of atherosclerosis. Therefore, LDL oxidation has been identified as a key event in atherosclerosis, thus suggesting that it may be possible to reduce the risk of atherosclerosis by dietary supplementation with antioxidant preparations enriched in polyphenols such as red wine, grape seed, and grape skin extracts. Indeed, short-term ingestion of purple grape juice decreased LDL susceptibility to oxidation in coronary artery disease patients [29] and in hypercholesterolemic human subjects supplemented with grape seed proanthocyanidin extract [9,30]. In addition, it was shown that the skin and flesh of grapes contain similar and significant reactive oxygen species (ROS) scavenging activity, suggesting that grape flesh may be equally cardioprotective as grape skin [21].

4. Atherosclerosis

High blood levels of cholesterol, particularly LDL-c, contribute to the development of atherosclerosis [15]. Apolipoprotein E<sup>−</sup> (ApoE<sup>−</sup>)-deficient mice are characterized by accelerated development of atherosclerosis and an increase in “oxidative state” (ie, more susceptible to oxidative stress) and represent a good model for atherosclerosis. Fuhrman et al [31] investigated atherosclerotic lesions using ApoE<sup>−</sup>-deficient mice following the dietary supplementation of freeze-dried extracts of fresh grapes. For ApoE<sup>−</sup>-deficient mice that consumed 150 μg total polyphenolics per day for 10 weeks, a 41% reduction in the atherosclerotic lesion area was observed compared with control (no supplements) or placebo (glucose and fructose supplementation) groups. This effect was associated with a significant reduction in serum oxidative stress as indicated by an 8% reduction in plasma lipid peroxide concentration and an increase in antioxidant capacity (16%-20%) as well as a reduction (33%) in macrophage uptake of oxidized LDL [31]. In addition, a study by Frederiksen et al [32] using Watanabe heritable hyperlipidemic rabbits demonstrated that the beneficial effects of grape seed extract were independent of the antioxidant effects (the plasma antioxidant capacity was measured by the Randox method), that is, a 11% decrease in plasma cholesterol and 77% increase in endothelium-dependent relaxation of aortic rings and 68% reduction of atherosclerosis.

A study by Stein et al [29] involved administering purple grape juice (Welch’s 100% Concord Grape) to 15 adults with documented coronary artery disease, for 14 days. Flow-mediated vasodilation was calculated from various parameters after ultrasound of the brachial artery and was found to be significantly increased above baseline values. Although the effects of the flavonoids from the grape juice products including red wine have been known to exert anti-inflammatory, antioxidant, platelet inhibitory, and arterial

5. Vascular relaxation/flow

A wealth of data in the literature show that grape and wine polyphenols possess strong vasorelaxant actions both in the large conductance (aorta) and smaller resistance (mesenteric) vessels ex vivo [13,29,34]. The primary mode of action has been reported to be inhibition of the release of nitric oxide (NO), and supporting data are also available to suggest induction of endothelial NO synthase by polyphenols is reduced [35,36].

Soares De Moura et al [20] demonstrated that oral administration of grape skin extract significantly reduced systolic, mean, and diastolic arterial pressure in a hypertensive rat model. In addition, red wine polyphenols administered in drinking water (150 mg kg<sup>−1</sup> day<sup>−1</sup>) prevented increases in systolic blood pressure (BP) but not heart rate in male Wistar rats after infusion with the potent vasoconstrictor, angiotensin II [37].

Grape skin extract was shown to concentration-dependently inhibit lipid peroxidation and induce endothelium-dependent vasodilation in norepinephrine-induced contracted mesenteric vessels ex vivo [20]. The results demonstrated that the beneficial effect of moderate red wine consumption could be partly due to antioxidant actions. However, a recent study [38] using cholesterol-fed hamsters demonstrated that the beneficial effects of grape seed extract were independent of the antioxidant effects (the plasma antioxidant capacity was measured by the Randox method), that is, a 11% decrease in plasma cholesterol and 77% increase in endothelium-dependent relaxation of aortic rings and 68% reduction of atherosclerosis.
relaxing effects, Concord grape juice was also shown to reduce systolic BP by 7.2 mm Hg and diastolic BP by 6.2 mm Hg in a study involving 40 hypertensive patients [39]. Other cardiovascular parameters after consumption of grape seed proanthocyanidins or grape extract have been shown to be altered, including improvement in coronary flow, aortic flow, and developed pressure [8,14]. Furthermore, these results were associated with a significant reduction of the generation of oxygen free radicals.

Interestingly, Clifton [19] reported no differences in systolic and diastolic BP, serum lipids, oxidized LDL, and other cardiovascular measures after daily consumption of grape seed extract by 36 adults for a 4-week treatment period. However, there was a significant difference in FMD as measured by ultrasound in subjects who had above average cardiovascular risk factors, which included high cholesterol, smoking, or high BP. Therefore, sufficient grape seed antioxidant polyphenols from grape seed extract were absorbed to influence FMD [19]. It is conceivable that “bioavailability” of the polyphenols may have been a determinant in the failure of grape seed extract to reduce BP despite improvement in FMD in this study [19]. Reported bioavailabilities of the flavonoids range from about 10% to 50% [40]. A recent in vitro study confirmed the limited bioavailability of dietary polyphenols when the polyphenols were in a complex with carbohydrate polymers and lipids [41]. In this regard, a recent pharmacokinetic study with healthy volunteers demonstrated that only high doses of oral resveratrol, tha is, 5 g, led to peak plasma concentrations of resveratrol of 2.4 μmol/L after 1.5 hour postdose [42]. In addition, urinary excretion was rapid, with approximately 77% of all resveratrol species excreted within 4 hours (of the lowest dose tested). Therefore, consumption of very high doses of resveratrol may be required to elicit changes in physiological parameters such as BP. Finally, the type of polyphenol as well as the nature and extent of its glycosidic conjugation may also influence its bioavailability [40,43,44].

6. Platelet aggregation

It has been previously suggested that the inhibition of platelet reactivity by wine may partly explain some of its cardioprotective effects, for example, the French paradox as mentioned earlier [5]. Red wine and grape juice, but not white wine, when infused intravenously or intragastrically into anesthetized dogs improved coronary artery blood flow parameters and inhibited platelet aggregation [45]. This early study suggested that the protective cardiovascular effects observed could be related to the phenolic flavonoids in the red wine and grape juice. In a study involving only 10 subjects, the effects of drinking purple grape juice, grapefruit juice, or orange juice for 1 week were examined [7]. Platelet aggregation responses to collagen were significantly reduced in the grape juice group only and appear to reflect the total phenolic contents of the test components. For instance, the total phenolics (gallic acid equivalents) were 2.26, 0.75, and 0.86 g/L for purple grape juice, orange juice, and grapefruit juice, respectively. In addition, purple grape juice contained flavonols, anthocyanidins, and proanthocyanidins, but there was no detectable levels of these components in the orange or grapefruit juices. Another (human) study demonstrated a decrease in platelet aggregation, increase in platelet-derived NO release, and a decrease in superoxide formation, both in vitro and after oral supplementation with purple grape juice, confirming the absorption and bioavailability of the bioactives from purple grape juice [6].

Platelet endothelial cell adhesion molecule-1 (PECAM-1) is a tyrosine phosphoprotein highly expressed in endothelial cells. It is an important component in the regulation of neutrophil transendothelial migration [46]. A recent study showed that a possible mechanism involved in the beneficial effects of grape extracts containing polyphenols on platelet activation involves the activation of PECAM-1 [47]. Therefore, activation of PECAM-1 by grape polyphenols may partly explain the positive effects of red wine consumption, and this has been proposed to further explain the French paradox [47]. Shanmuganayagam et al [22] compared the effects of combining grape seed and grape skin extracts on platelet activity in dogs in an ex vivo feeding study or using an in vitro human platelet incubation study protocol. The major finding was that the concentrations of grape seed extract (5 mg/kg body weight) and grape skin extract (20 mg/kg body weight) had little effect on ex vivo platelet activity in dogs. In humans, only the highest test dose of grape seed extract (100 mg/L blood) inhibited platelet aggregation. However in both models, a greater antiplatelet effect was observed when a combination of grape seed and grape skin extract was used [22].

The antiaggregating effects of resveratrol have been observed at concentrations as low as 1.2 μg/L (of red wine diluted 1000-fold), which inhibited platelet aggregation by 41.9% [48]. Therefore, reducing the level of platelet aggregation may be a contributing factor associated with the cardioprotective effects of polyphenols.

7. Myocardial ischemia/reperfusion

Ventricular arrhythmias remain an important cause of death in ischemic heart disease, and ROS have been implicated in the pathogenesis of ischemic heart disease [49]. Agents known to scavenge free radicals could prevent the deleterious effects of reperfusion-induced arrhythmias and/or improve the recovery of the myocardium after ischemia. Recent studies in experimental animal models have demonstrated protective actions of grape and wine polyphenols against experimentally induced myocardial ischemia and reperfusion [8,21,35,50-54]. For instance, both the grape and wine polyphenols have been reported to reduce the mortality and the severity of cardiac arrhythmia after myocardial ischemia/reperfusion, both after acute infusion and after oral administration [53,54]. Using the Langendorff isolated rat heart preparation, Sato et al [50] identified grape seed proanthocyanidins (in a 3-week
prefeeding dietary intervention trial) to be cardioprotective against myocardial ischemia and reperfusion as evidenced by reduced infarct size and improved recovery of posts ischemic contractile functions [50]. The grape seed procyanidin supplemented group had a significant reduction in the myocardial infarct size as well as a relatively higher aortic flow compared with controls, and this was associated with a significant increase in the hydroxyl radical scavenging activity. A more recent study reported both grape flesh and grape skin extracts (oral administration) improved aortic flow and protected hearts from ischemic reperfusion, and both dietary interventions reduced myocardial infarction size suggesting both grape flesh and grape skin are equally cardioprotective [21]. In another study investigating the potential cardioprotective effects of grape seed procyanidins, the extent of ischemia/reperfusion-induced cardiac arrhythmias were measured [8]. After 3 weeks on the grape seed procyanidin-supplemented diets, the incidence of reperfusion-induced arrhythmias was 25% in the supplemented group compared with 92% in the control group. Furthermore, resveratrol has also been shown to pharmacologically precondition the heart. For example, Hattori et al [35] demonstrated preconditioning of isolated, perfused working rat hearts with 10 μmol/L resveratrol, and this provided cardioprotection against posts ischemic ventricular recovery, reduced myocardial infarct size, and apoptosis. The results also showed that the action of resveratrol involved a cardioprotective mechanism that was NO dependent, which was supported by an increase in inducible NO synthase mRNA expression in the heart at only 30 minutes after reperfusion [35].

In more recent studies, resveratrol decreased experimentally induced arrhythmia duration, incidence of ventricular tachycardia, and animal mortality [51,52]. Results of electrophysiological studies revealed that resveratrol dose dependently (up to 100 μmol/L) decreased the action potential duration and inhibited the L-type calcium currents in isolated ventricular cardiomyocytes, thus partly explaining the mechanisms(s) of action [51]. In rat heart cardiomyocytes isolated from the right ventricle, resveratrol was shown to block the peak voltage gated sodium currents with an IC\textsubscript{50} of 77 μmol/L [55]. Resveratrol was also shown to reduce toxin-induced increases in diastolic calcium concentrations leading to a reversal of contractile dysfunction. Therefore, apart from resveratrol being well known for its antioxidant activity in vivo, it appears that resveratrol plays a more direct role on ion channels, controlling the excitability of the myocardium, which have been shown for other bioactives [56,57] and may partly explain the cardioprotective activity of resveratrol.

8. Cellular signaling

Sato et al [58] demonstrated that grape seed procyanidins may offer cardioprotection by inhibiting the regulatory genes cJUN and JNK1 [58]. In addition, grape seed extracts at low concentrations could inhibit agonist-induced vascular cell adhesion molecule-1 expression, a marker for cell adhesion that has been reported to be involved in chronic inflammatory conditions [59]. Resveratrol was shown to inhibit the release of chemokines such as interleukin-6 and other biochemical parameters involved in inflammatory processes, which have been reviewed elsewhere [16].

Because angiotensin II–induced hypertrophy of vascular smooth muscle cells is a key step in the development of CVD, Haider et al [60] investigated the potential beneficial effects of resveratrol on this process in vitro. Resveratrol at 50 μmol/L decreased phosphorylation of phosphatidylinositol 3-kinase (PI\textsubscript{3}K) and p70 ribosomal protein S6 kinase (p70\textsuperscript{S6K}) proteins, both of which are implicated in angiotensin II–induced protein synthesis (and vascular smooth muscle cell hypertrophy). The 2 proteins PI\textsubscript{3}K and
p70SGK are involved in the ERK 1/2 signaling pathway, downstream from ligand-activated G-protein–coupled receptors. Therefore, it is possible that resveratrol in addition to its effect on angiotensin II–induced hypertrophy could play an important role in other G-protein–coupled receptor signaling pathways that are involved in CVD.

Because different isoforms of cyclooxygenase (COX) enzymes have been strongly implicated in inflammatory processes (such as atherosclerosis [61]), this class of enzymes may also provide useful targets for resveratrol. Indeed, resveratrol isolated from grape skin was shown to inhibit the COX-1 enzyme by 98% at 100 μg/mL, but resveratrol was without effect against the closely related COX-2 enzyme [62]. In addition, viniferin and catechin (also from grape skin) inhibited COX-1 and COX-2 [62] and are therefore somewhat comparable to the pharmacological agents aspirin, naproxen, and ibuprofen in terms of their selectivity for inhibition of different isoforms of COX.

Peroxisome proliferator-activated receptors regulate transcription of various genes involved in cholesterol metabolism in the liver and other organs [63,64]. In a study by Ma et al [65], the grape seed proanthocyanidins were anti-inflammatory in human umbilical vein endothelial cells by a mechanism involving activation of PPARγ expression [65]. Thus, in addition to their cardioprotective effects, grape seed proanthocyanidins appear to reduce the inflammatory processes (see Fig. 2), which might partly explain the mechanism(s) for the amelioration of other chronic inflammatory conditions such as inflammatory bowel disease, cancer, and diabetes.

9. Summary

There is mounting evidence that crude grape extracts and red wine products contain bioactive ingredients that afford some protection against CVD. This is supported by data from in vitro, ex vivo, and in vivo animal studies and, albeit limited, human trials. The beneficial effects of these bioactive products appear to be mediated via a plethora of biochemical pathways and signaling mechanisms acting either independently or synergistically. The pleiotropic nature of the reported benefits of these polyphenols tends to suggest the modulation of multiple mechanisms and may explain their physiological efficacy. These properties render the polyphenols attractive candidates for nutraceutical and functional foods. Therefore, supplementation with grape seed, grape skin, or red wine products may be a useful adjunct to consider for a dietary approach in the prevention of CVDs, although additional research is required to support such a strategy.

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