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Regulation of vascular endothelial function by red wine procyanidins: implications for cardiovascular health

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ABSTRACT

The effects of oligomeric procyanidins (OPC) on vascular endothelial function provide a plausible explanation for the reduced incidence of coronary heart disease in red wine drinkers. Grape variety is an important influence on the amounts of OPC in wines. Red wines made from Aglianico, Sagrantino or Tannat grapes typically have higher OPC levels. The endothelial response to OPC induces atheroprotective changes in vascular function, which are likely mediated via oxidant signalling mechanisms originating from the mitochondrial electron transport chain.

1. Introduction

Moderate daily consumption of wine is associated with a lower risk of coronary heart disease than drinking beer or other alcoholic drinks.[1] In part this may be due to the healthier lifestyles of wine drinkers. Despite this, an explanation for the lower heart disease mortality of wine drinkers has been sought for many years. While some consider all alcoholic drinks confer the same benefit and attribute these effects mainly to the metabolic actions of alcohol,[2] research on the health benefits of red wine has focused increasingly on the actions of the polyphenol constituents.[3]

Although the effects of red wine polyphenols on vascular function have been investigated for more than half a century,[4] the absence of a plausible mechanism for why red wine causes a greater reduction in cardiovascular disease slowed progress in resolving the question whether polyphenol constituents are more important than alcohol. Epidemiological studies often report no differences in the relative risk of cardiac events in drinkers of red wine compared to white wine.[5] But the polyphenol composition of red wines is complex and variable, such that many red wines may have insufficient amounts of key polyphenols, and therefore confer no greater improvements in cardiovascular health than white wine. An important confounder is that white wine drinkers may also consume key polyphenols from other dietary sources.[6]

In order to establish a cause and effect relationship between specific red wine polyphenols and a reduction in cardiovascular disease the following criteria should be met: (1) a plausible mechanism of action; (2) adequate amounts in wine to confer the proposed effect; (3) bioavailability of the active compounds or active metabolites; (4) evidence of a dose–response relationship in experimental models and human studies comparing red wines with high amounts of the active polyphenol compared to red wines with low amounts; and (5) placebo-controlled clinical trials of red wine containing the active polyphenols, or analogous products containing these molecules, to show a long-term reduction in cardiovascular disease.

Progress in understanding how red wine consumption reduced coronary heart disease made an important step forward in 1993 when Fitzpatrick et al. showed red wine and grape extracts caused endothelium-dependent vasodilatation of isolated blood vessels through nitric oxide (NO) release.[7] This vasodilator response was confirmed by several research groups, and linked to the polyphenolic components of grapes.[8–11] Consistent with these observations red wine and de-alcoholized red wine increased flow-mediated dilatation of the forearm in human volunteers.[12] This is an endothelium-dependent response that could not be reproduced with alcohol alone.[12] Based on these findings sufficient red wine polyphenols are absorbed from the gastrointestinal tract to cause

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a functionally relevant change in endothelial function.[12] But the polyphenol composition of the red wine used in this study was not described.

Atherosclerotic disease is an insidious process starting early in life, that is, preceded by endothelial dysfunction.[13] The concept that red wine polyphenols improve endothelial function was a particularly significant insight because a full understanding of the dietary factors inhibiting atheroma formation could improve evidence-based disease prevention. The effect of red wine on atherosclerotic lesion development has been evaluated in animal models. Some studies reported a reduction in lesions,[14,15] but others failed to confirm such findings.[16] One explanation for these conflicting observations was that red wines contain a specific active constituent responsible for the anti-atherosclerotic actions, which was not present in sufficient amounts in red wines that lacked protective activity.

To explore this question we investigated the effects of red wine extracts on endothelin-1 (ET-1) synthesis by endothelial cells, as this 21 amino acid vasoconstrictor peptide had been linked to the mechanisms underlying atherosclerotic lesion formation.[17] Treatment of cultured endothelial cells with red wine extracts resulted in a marked inhibition of ET-1 synthesis due to reduced gene expression.[17] The magnitude of this effect correlated with the polyphenol content of the wine samples.[17] Red wine is a particularly complex mixture of polyphenols, but the sensitivity of this experimental approach made it a useful bioassay for screening polyphenols purified from red wine extracts in order to isolate biologically active molecules inhibiting ET-1 synthesis. This identified oligomeric procyanidins (OPC) as the active components in a red wine polyphenol extract.[18] For a range of red wine samples suppression of ET-1 synthesis closely correlated with OPC levels.[18] In agreement with these studies, OPC purified from a grape seed extract had previously been shown to induce endothelium-dependent vasodilatation of isolated blood vessels.[19] The structure–activity profile of grape seed OPC producing endothelium-dependent vasodilatation was very similar to that of OPC purified from red wine extracts.[18,19]

In addition, our studies of other red wine polyphenols, including (−)-epicatechin, resveratrol and anthocyanins, showed little or no effect on ET-1 synthesis in amounts present in red wine,[20] which made it unlikely that these molecules accounted for the cardioprotective effects of red wine. Others also reported that (−)-epicatechin does not produce endothelium-dependent vasodilatation of isolated vessels,[19,21] or prevent atheromatous lesion formation in apolipoprotein E deficient mice.[22] Nevertheless, under some experimental conditions (−)-epicatechin may contribute to the vascular effects of polyphenol-rich products such as red wine and cocoa as supplementation can improve endothelial function and lower blood pressure.[23]

Many reports have suggested the actions of resveratrol underlie the cardioprotective effect of red wine. However, resveratrol is only a minor polyphenol in red wines with amounts typically <5 mg/L.[24,25] Indeed, a recent report found no evidence of a link between resveratrol consumption from wine and a reduced risk of cardiovascular disease.[26]

In contrast to resveratrol, OPC levels in some wines exceed 1000 mg/L.[18] OPC are part of the wider group of oligomeric flavan-3-ol oligomers called proanthocyanidins.[27] Proanthocyanidins are present in grape seeds and skins, with seeds being the main source of OPC in red wines. Grape seed derived OPC consist mainly of dimers to pentamers composed of (−)-epicatechin subunits, which are frequently galloylated.[19,27] Other dietary sources of OPC include cocoa products and apple. High dietary consumption of proanthocyanidins is associated with lower cardiovascular mortality[6] and better renal function in elderly women.[28] Moreover, the importance of OPC in mediating the health benefits of red wine is supported by the blood pressure lowering effects observed in clinical trials of grape seed extract,[29,30] as these extracts consist mainly of OPC.

To investigate whether there was an association between OPC levels in red wines and reduced coronary heart disease mortality we investigated the OPC concentrations of red wines from areas of France and Sardinia with low levels of heart disease and greater longevity.[18] In both cases OPC levels were higher in local wines than those of neighbouring areas.[18] The difference was most striking in southwest France, and was linked to the prevalence of wines made from Tannat grapes.[18] Tannat is a traditional grape variety grown in southwest France, which is rarely grown in other regions of France. Compared to other grape varieties it has one of the highest levels of polyphenol synthesis, particularly flavan-3-ols including OPC.[31]

An association between reduced heart disease and higher dietary intake of OPC due to consumption of Tannat wines is consistent with the experimental evidence that OPC are the components in red wine conferring the greatest atheroprotective effect through their actions on vascular endothelial function. To further develop insights into the link between red wine consumption and vascular health we describe in this communication results from OPC measurements of a wide selection of red wines made from different grape varieties. We also consider some of the factors affecting the amounts of OPC present in red wine such as winemaking practices and environmental influences in vineyards.

A further area described in this communication is investigations into how OPC modify endothelial function, as this may reveal further insight into the role of dietary polyphenols in vascular health, or identify important therapeutic targets for drug development. A number of mechanisms have been put forward to explain the actions of red wine polyphenols on endothelial function. These include evidence of activation of oestrogen receptors leading to stimulation of NO production,[32] and induction of intracellular signalling mechanisms via superoxide anions or hydrogen peroxide.[33] Here we have compared actions of OPC with those of resveratrol, as its effects have also been linked to oestrogen receptor activation,[34] as well as to intracellular signalling mechanisms via SIRT1.[35] This comparison has enabled us to show that the actions of OPC and resveratrol on the endothelium are mediated via distinct signalling pathways.

2. Results and discussion

2.1. Comparison of grape varieties

Total polyphenols (TPP) and OPC were analysed for 818 red wines. Wines were classified by grape variety if the predominant variety was ≥70% of the blend. The reference group (blends/miscellaneous) consisted of wines from around the world made from blends of common varieties, and grape varieties where <5 samples of that grape variety had been analysed. Bordeaux wines were grouped separately because of their origin and common use of Cabernet Sauvignon, Cabernet Franc and Merlot grape varieties. Results for wine samples are shown as box (median with 25th and 75th percentile) and whisker (10th and 90th percentile) plots.

For each grape variety there was considerable variation in the observed TPP and OPC concentrations (Fig. 1). While grape variety can have an important influence on these levels, it is likely that winemaking practices can have an even greater impact. More subtle influences such as vine management, vineyard environmental factors (terroir), and grape ripeness may also be important.

Wines made from the Sagrantino, Aglianico and Tannat grape varieties had higher concentrations of TPP ($P=<0.001$) and OPC ($P=<0.001$) than the reference group (Fig. 1). The values for all other grape varieties were not significantly different from the reference group. TPP and OPC levels in Sagrantino wines were greater

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