FINAL REPORT

UWA 97/2

GRAPE ANTIOXIDANT PHENOLICS: ABSORPTION AND AND INHIBITION OF LIPID PEROXIDATION IN HUMANS

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Summary

The oxidative damage of lipoproteins in the body is thought to play a critical role in cardiovascular disease. There has been evidence from test tube studies that phenolic compounds in red wine can act as antioxidants to protect lipoproteins from damage. The objects of this project was to examine the absorption and antioxidant action of red wine phenolic compounds using controlled human intervention trials.

In acute studies, it was shown that specific phenolic acids such as caffeic acid and gallic acid (as the 4-O-methyl derivative) were absorbed and concentrations increased in blood within 2-4 hours following consumption of red wine. Despite the incorporation of these compounds into the blood stream there was no effect of red wine consumption on the ex vivo oxidation of low density lipoprotein. In longer term studies conducted with red wine, dealcoholised red wine and white wine in smoking subjects, protection against lipid peroxidation was noted only during the consumption of dealcoholised red wine. These studies suggest that any cardioprotective effects of red wine are unlikely to be due to the antioxidant activity of the wine polyphenols.

Background

In the early 1990's experiments were carried out which showed that red wine added to low density lipoprotein (LDL) in the test tube prevented it from undergoing oxidation [1]. This was a potentially important observation because oxidative damage to LDL, the so-called 'bad cholesterol', is thought to be an important process in the development of heart disease [2]. These early observations received widespread publicity and together with evidence that a Mediterranean diet, which included red wine, was associated with a lower risk of heart disease, led to the popular view that red wine was good for heart disease. It has been well established that moderate alcohol intake may reduce the risk of heart disease [3], possibly by raising the concentration of the protective high density lipoprotein (HDL) in the blood. Whether red wine offers any additional benefit to that of any other alcohol beverage with regard to the risk of coronary heart disease still remains to be determined. This has become an area of keen interest with the American Heart Association recently presenting a position statement on 'Wine and Your Heart' [4].

Our research programme in this area began in the mid 1990's with a research grant from the National Heart Foundation with initial studies designed to identify some of the main antioxidant components in red wine and if they are absorbed into the bloodstream after consumption. This was followed by a three year grant from the Grape and Wine Research and Development Corporation to extend these studies to conduct controlled intervention studies in human volunteers.

Objectives

1 To determine if specific phenolic antioxidant compounds can be absorbed and detected in plasma following ingestion of wine.

2 To utilize a highly sensitive marker for in vivo lipid peroxidation (isoprostanes) to determine if ingestion of wine influences oxidative stress.

Introductory Technical Information

It became clear from test tube studies that the antioxidant activity of beverages such as red wine or grape juice depended directly upon the polyphenol content of the beverage [5]. This was confirmed when the polyphenols were removed from the beverage by passing it through a polyvinylpyrolidone column and the resulting 'phenol stripped' beverage no longer retained any antioxidant activity. In an attempt to discover some of the specific antioxidant compounds in grape juice and red wine we separated extracts on thin later chromatography plates and determined the antioxidant activity of individual fractions. Mass spectrometeric analysis of the most active fractions resulted in identification of several relatively simple phenolic acids such as caffeic acid and protocatechuic acid. From studies using the pure compounds we were able to determine that caffeic acid for example could significantly inhibit LDL oxidation at concentrations as low as 1 micromole per litre.

Research Methodology

Our next major task was to determine if these compounds could be absorbed into the blood stream after drinking red wine. To test this we recruited 12 volunteers to consume either red wine, phenolic-stripped red wine, dealcoholized red wine or water [6]. Volunteers visited out laboratory once each week to consume the beverages in random order. Blood samples were taken before each drink and at 1, 2 and 4 hours following the drink. Analysis of blood by using highly sensitive gas chromatography-mass spectrometry equipment revealed that caffeic acid is present in plasma within 2 hours of drinking either red wine or dealcoholized red wine, but was not present after consumption of 'phenol-stripped' red wine. Another metabolite that showed a marked increase in plasma following red wine was 4-0-methylgallic acid. This metabolite is probably formed from gallic acid, an important component of tannins. The level of these components in plasma was the same for either red wine or dealcoholized red wine, indicating that alcohol probably has little effect on the absorption of the polyphenols.

To test the effects of red wine polyphenol consumption over a longer time period we designed a longer term intervention study in which volunteers drank approximately 2 glasses of red wine per day for 2 weeks [7]. For comparison, volunteers also drank white wine with the same alcohol content as the red wine, but with lower concentration of polyphenols, and in another two week period they drank a dealcoholized version of the red wine. For this cross over design study we chose volunteers who were smokers, since smoking is known to increase

oxidative stress and smokers have lower levels of blood antioxidant vitamins. In this case we felt that any antioxidant effects of red wine phenolics would be maximized in such subjects. Another major obstacle to overcome was to find a method that could determine oxidant damage in the body rather than rely on measures of isolated blood samples carried out in the test tube. The method we have developed in our laboratory is to measure a group of compounds called isoprostanes. These are formed by the free radical oxidation of fatty acids in the body, which can be measured by a very sensitive and specific gas chromatography mass spectrometry method, either in the plasma or excreted in the urine.

Results

Despite the clear evidence for the absorption of polyphenols there was no effect on oxidation of lipoproteins isolated from volunteers after consumption of red wine. This may indicate that the level of compounds absorbed into the blood is too low to have antioxidant effects or that the metabolism of the compounds effects their antioxidant activity. This is supported by the fact that all gallic acid detected in blood was in the methylated form not the free form, and that the antioxidant activity of the methylated form is significantly reduced [6]. Another possible explanation is that these compounds may only have antioxidant activity after a longer period of consumption.

Results from the two week intervention study showed that Isoprostane concentrations in plasma and urine decreased following consumption of

dealcoholized red wine but did not change after consumption of either the red or white wine [7]. This effect was seen without any significant alteration to plasma antioxidant vitamins such as vitamin E or C. This result indicates that only the polyphenols in dealcoholized wine had an antioxidant effect in smoking subjects. The reason for this somewhat surprising result is not clear, but may be related to the known prooxidant effect of alcohol metabolism [8], which in this study may have counteracted any antioxidant action of the wine polyphenols.

Discussion and Research Outcomes

If there are any special protective effects of red wine drinking on cardiovascular disease these may not be due to antioxidant effects of the polyphenols. There may be alternative actions of grape derived polyphenols, which could influence heart disease. These could include effects on blood platelets to help prevent clotting or to improve the functioning of blood vessels [9,10]. It remains a key area of current research to determine the possible health effects of polyphenolic compounds derived either from grapes or other dietary sources such as fresh fruit and vegetables [11].

Analysis of research outcomes compared to objectives:

1 Both major objectives of the project were addressed. Results indicate that specific phenolic compounds in red wine can be absorbed into the blood stream following consumption of red wine. However, the antioxidant effects of these compounds are only observed in the absence of alcohol, at least in smoking subjects. Red wine phenolic compounds may have

bioactivity other than antioxidant effects and therefore it cannot be ruled out that these compounds will not affect other import components of the cardiovascular system such as platelet activation or vascular function.

2 The major findings of this project have been published in peer-reviewed international journals (see appendix), as well as in wine industry magazines.

3 The findings of this project have been shared with the medical research community at National Scientific meetings such as the Australian Atherosclerosis Society and the Society for Free radical Research.

Implications and Recommendations

This research has helped to define the potential impact of wine consumption on human health. We conclude that any cardiovascular benefit of wine is unlikely to be due to the antioxidant activity of the polyphenol constituents. It is recommended that further research is necessary to define other potential biological effects of these wine constituents particularly on blood platelets and vascular function. According to a recent position paper by the American Heart Association called Wine and Your Heart [4], “there is little current justification to recommend alcohol (or wine specifically) as a cardioprotective strategy.” Clearly then further work is required if moderate consumption of wine is to be considered as a healthy supplement to the normal diet.

Intellectual Property

There are no issues of intellectual property arising from this work.

Summary of Information Developed

This research has led to the discovery of new knowledge and method development relating to the absorption and metabolism in man of polyphenolic compounds from red wine. The technical details of these developments are summarized in the attached publications arising from this work (see Appendix).

Financial Statement

The following financial statement has been provided by the Research Grants Office at the University of Western Australia.

GRAPE AND WINE RESEARCH AND DEVELOPMENT CORPORATION

STATEMENT OF INCOME & EXPENDITURE

PROJECT: UWA 97/2

	Income	Expenditure	Balance
Year Ending 30th June 1998	20,108.00	3,597.00	16,511.00
Year Ending 30th June 1999	10,556.00	38,476.00	-27,920.00
Year Ending 30th June 2000	10,556.00	22,123.00	-11,567.00
Year Ending 30th June 2001	48,122.00	781.00	47,341.00
Year Ending 31 December 2001	0.00	24,365.00	(24,365.00)

Totals	**89,342.00**	**89,342.00**	**0.00**
			0.00

BREAKDOWN OF EXPENDITURE

	Salary	Operating	Total
Year Ending 30th June 1998	3,597.00	0.00	**3,597.00**
Year Ending 30th June 1999	34,478.00	3,998.00	**38,476.00**
Year Ending 30th June 2000	5,127.00	16,996.00	**22,123.00**
Year Ending 30th June 2001	0.00	781.00	**781.00**
Year Ending 31 December 2001	24,365.00	0.00	**24,365.00**
Totals	**67,567.00**	**21,775.00**	**89,342.00**

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4. Goldberg IJ, Mosca L, Piano MR, Fisher EA. Wine and your heart: A science advisory for healthcare professionals from the nutrition committee, council on epidemiology and prevention, and council on cardiovascular nursing of the American Heart Assoc. *Circulation*. 2001; 103:472-475.
5. Abu-Amsha R, KD Croft, IB Puddey, JM Proudfoot, LJ Beilin. Phenolic content of various beverages determines the content of inhibition of human serum and low-density lipoprotein oxidation in vitro: identification and mechanism of action of some cinnamic acid derivatives from red wine. *Clinical Science* 1996; 91: 449-458.
6. R Abu-Amsha, KD Croft, LJ Beilin, IB Puddey. Ingestion of red wine significantly increases plasma phenolic acid concentrations but does not acutely effect ex vivo lipoprotein oxidizability. *American Journal of Clinical Nutrition* 2000; 71: 67-74.

7. R Abu-Amsha Caccetta, V Burke, TA Mori, LJ Beilin, IB Puddey, KD Croft. Red wine polyphenols, in the absence of alcohol, reduce lipid prooxidative stress in smoking subjects *Free Radical Biology and Medicine* 2001; 30: 636-642.
8. Meagher EA, Barry OP, Burke A, Lucey MR, Lawson JA, Rokach J, et al. Alcohol-induced generation of lipid peroxidation products in humans. *J Clin Invest* **104:** 805-13; 1999.
9. Paceasciak CR, Rounova O, Hahn SE, Diamandis EP, Goldberg DM. Wines and grape juices as modulators of platelet aggregation in healthy human subjects. *Clin Chim Acta* **246**: 163-182; 1996.
10. Stein JH, Keevil JG, Wiebe DA, Aeschlimann S, Folts JD. Purple grape juice improves endothelial function and reduces the susceptibility of LDL cholesterol to oxidation in patients with coronary artery disease. *Circulation* **100**: 1050-1055; 1999.
11. LW Morton, R Abu-Amsha Caccetta, IB Puddey, KD Croft.Chemistry and biological activity of dietary phenolic compounds: Relevance to cardiovascular disease *Clinical and Experimental Pharmacology and Physiology* 2000; 27: 152-159.

Appendix – Publications Resulting from this project (reprints attached)

R Abu-Amsha, KD Croft, IB Puddey, JM Proudfoot, LJ Beilin. Phenolic content of various beverages determines the content of inhibition of human serum and low-density lipoprotein oxidation in vitro: identification and mechanism of action of some cinnamic acid derivatives from red wine. *Clinical Science* 1996; 91: 449-458. (Part Funded by the National Heart Foundation)

R Abu-Amsha, KD Croft, LJ Beilin, IB Puddey. Ingestion of red wine significantly increases plasma phenolic acid concentrations but does not acutely effect ex vivo lipoprotein oxidizability. *American Journal of Clinical Nutrition* 2000; 71: 67-74.

R Abu-Amsha Caccetta, V Burke, TA Mori, LJ Beilin, IB Puddey, KD Croft. Red wine polyphenols, in the absence of alcohol, reduce lipid prooxidative stress in smoking subjects *Free Radical Biology and Medicine* 2001; 30: 636-642.