

Antioxidant therapy for the prevention of cardiovascular disease

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Introduction

Coronary artery disease (CAD) is the major cause of death in developed countries.¹ It may present as a typical 'heart attack', as sudden death, or it may be detected at a later date and be described as a silent infarct. Good coronary care units, the use of thrombolytic and anti-arrhythmic drugs, and accurate methods for assessing cardiac function and coronary artery pathology have all successfully reduced in-hospital mortality. However, about 75% of those who have a myocardial infarction (MI) die outside hospital,² and sudden death is the first and only manifestation in about 20% of all those who present with CAD.^{3,4} Therefore, treatment is not available for most patients, and the aim must be to prevent the development and progression of CAD.

There are five conditions that predispose to premature death from CAD. These are: hypertension;^{5,6} hypercholesterolaemia;^{7,8} the post-menopausal state;^{9,10} a thrombotic tendency;^{11,12} and the risk of developing ventricular arrhythmias.¹³ Patients with any of these conditions may be offered appropriate pharmacological treatment. In addition, those known to have CAD because they have had a MI or suffer from angina or strongly suspected of having CAD, because they have cerebrovascular or peripheral vascular disease, should also be offered secondary preventive treatment with, for example, lipid-lowering therapy and aspirin.

All the above patient groups and the general population can be offered lifestyle advice, which may be considered under five headings: (i) smoking

cessation;^{14,15} (ii) weight control;^{16,17} (iii) taking reasonable exercise;^{18,19} (iv) eating a proper diet;^{20,21} and (v) taking antioxidant therapy.^{22–24}

The purpose of this review is to consider the role of antioxidants as a means of reducing the risk of premature death from CAD. Hitherto antioxidants have been considered as one of the explanations for the beneficial effects of the healthy, Mediterranean-type diet. That it is now possible to assess free-radical damage, measure antioxidant activity and prescribe a pharmaceutical preparation which will produce a measurable effect, means that the role of antioxidants can be assessed more scientifically.

Free radicals and antioxidants

Oxidative damage

Antioxidant therapy may inhibit atherosclerosis and thereby prevent the clinical complications of the disease such as CAD, and in particular, MI.²⁵ In healthy individuals, antioxidants protect components of the body against free-radical damage.²⁶ Free radicals are species containing one or more unpaired electrons in their outer atomic orbital. This electron imbalance renders them highly reactive and capable of widespread oxidation of lipids, proteins, DNA and carbohydrates. This eventually causes disruption of cell membranes, leading to release of cell contents and death.²⁷ Free radicals are formed by several exogenous processes such as radiation and tobacco

smoke, and are the endogenous natural by-products of cellular metabolism²⁸ (Figure 1). When oxygen is reduced in the electron transport chain, oxygen-derived free-radical intermediates are formed. The superoxide radical (O_2^-) and hydrogen peroxide (H_2O_2) intermediates can escape from the system, and in the presence of transition metal ions (e.g. Fe^{2+} , Cu^{2+}) form the far more damaging hydroxyl radical (OH^-).²⁷ One example of this oxidative damage is lipid peroxidation.²⁹ Free radicals may attack polyunsaturated fatty acids within membranes, forming peroxy radicals. These newly-formed free radicals can then attack adjacent fatty acids within membranes causing a chain reaction of lipid peroxidation. The lipid hydroperoxide end-products are also harmful, and may be responsible for some of the overall effect, which can lead to tissue and organ damage.

Oxidative damage in cardiovascular disease

The oxidative modification of circulating lipoproteins by free radicals, particularly low-density lipoproteins (LDL), is important for the development of atherosclerosis.³⁰ Smaller, denser LDL particles, which are known to be a risk factor for cardiovascular disease,³¹ may promote atherogenesis for several reasons.³² These modified LDL particles do not bind readily to the endogenous LDL receptor and are therefore not cleared from the circulation by this mechanism. They penetrate the arterial intima more easily, are more readily oxidized, possibly because they contain less antioxidant protection, and are taken up by the macrophage scavenger receptors,

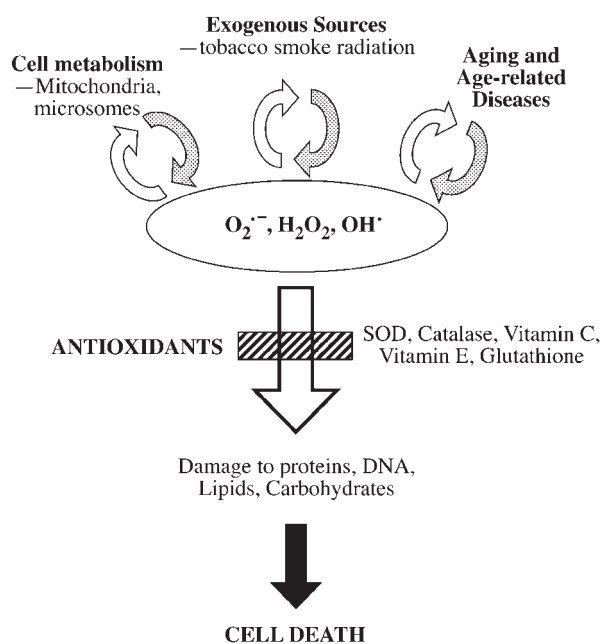


Figure 1. Mechanisms of oxidative stress.

accelerating foam-cell formation (Figure 2). This early histological feature leads to the development of atherosclerotic plaques.^{30,32}

Antioxidants

Under normal circumstances, there are large numbers of antioxidants, synthesized within the body or taken in the diet, that form a natural defence against free-radical induced damage.³³⁻³⁵ Antioxidants react with the free radicals to neutralize their effect by donating electrons, thereby forming much less reactive radicals. The imbalance between protective antioxidants and damaging free radicals is termed oxidative stress. Antioxidants are enzymes, such as superoxide dismutase, catalase and glutathione peroxidase, which mainly act within the intracellular compartments, and low-molecular-mass antioxidant scavengers in the circulation that protect lipoproteins from oxidative modification in the extracellular fluid.³⁶

Vitamin E is the major lipid-soluble antioxidant present in cell membranes and lipoproteins that protects against oxidative modification. *In vitro* studies have shown that LDL oxidation does not occur appreciably until the endogenous vitamin E has been oxidized.^{37,38} LDL supplemented with vitamin E both *in vitro*³⁹ and *in vivo*⁴⁰ shows increased resistance to oxidation. The major antioxidant of the aqueous phase is vitamin C, which acts as the first line of defence during oxidative stress. Its concentration *in vivo* is solely dependent on dietary intake, but once it is oxidized it can be reduced back to its antioxidant form intracellularly by the antioxidant glutathione.⁴¹ Vitamin C is also important for maintaining levels of antioxidant vitamin E by reducing the vitamin E radical (the oxidized form of vitamin E). Thus scavenger antioxidants are important not only because they react with free radicals directly but also because they act synergistically with one another.^{42,43} However, although the experimental evidence is encouraging, clinical trials are needed to demonstrate their efficacy and usefulness.

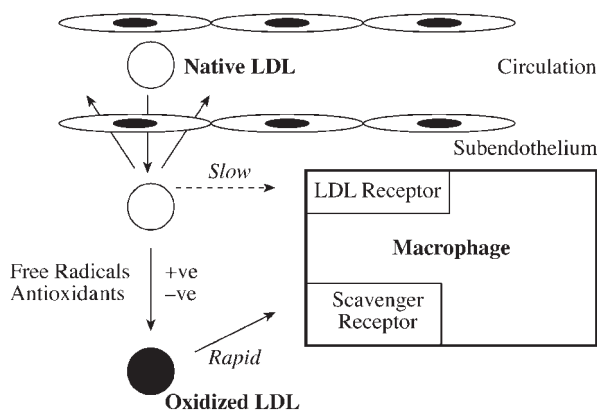


Figure 2. The oxidation of low-density lipoprotein (LDL).

A protective role for antioxidants in CAD

Several large-scale epidemiological studies have observed that an increase in antioxidant level limits the clinical expression of CAD. Individuals with high dietary intakes of vitamin C, vitamin E and β -carotene have a lower risk of CAD and may have a greater life expectancy.⁴⁴ There is also increasing evidence from trials using antioxidant supplements to support this theory. For example, in a recent randomized, controlled clinical trial performed on a total of 2002 patients with angiographically-proven CAD, vitamin E supplementation (400 or 800 IU) prevented the combined end-point of non-fatal myocardial infarction and cardiovascular death, with an overall risk reduction of 47%,⁴⁵ but did not reduce total mortality.

Several major, randomized trials of antioxidant supplementation for the primary and secondary prevention of cardiovascular disease are currently in progress. The Heart Outcomes Protection (HOPE) study is investigating vitamin E supplementation in men and women with known CAD, and the Women's Health Study (WHS) is a primary prevention trial of vitamin E and β -carotene. A combination of vitamin E, vitamin C and β -carotene is being used in the Supplementation Vitamins, Minerals, and Antioxidant (SU.VI.MAX) trial in France, the Women's Antioxidant Cardiovascular Disease Trial (WACDT) in the USA and in the Heart Protection Study Oxford, UK. These will provide more reliable data and help to define the role of antioxidants as primary and secondary preventive measures for cardiovascular disease.

Currently vitamin E seems to have the most cardioprotective potential of all the antioxidants. Although *in vitro* studies suggest that a combination of antioxidants will provide the most benefit, the evidence for other antioxidants such as vitamin C and β -carotene remains uncertain.⁴⁶⁻⁴⁹ Further intervention trials will be required before any firm conclusions can be made regarding antioxidants as a potential therapy for reducing mortality from CAD.

Antioxidant activity of red wine

The incidence of cardiovascular disease in Mediterranean countries is lower than expected, and it has been suggested that this may be partly due to greater consumption of red wine, an observation that has been termed the 'French paradox'.^{50,51} Phenolic compounds in red wine are known to protect LDL from oxidation,^{52,53} which may explain why regular consumption of red wine may reduce the development of cardiovascular disease.⁵⁴ Although there is

good evidence for the protective antioxidant effects of red wine *in vitro*,^{52,53,55} data to confirm its clinical relevance are limited.

Using an enhanced chemiluminescent assay for measurement of total antioxidant activity in serum,⁵⁶ Maxwell *et al.*⁵⁷ studied the effects of red wine *in vivo* to determine whether a physiologically relevant increase in antioxidant activity could be demonstrated. This assay indicates the total amount of antioxidant defence, including those known to have biological significance, such as vitamins C and E, as well as those whose antioxidant function *in vivo* is less certain, for example, urate and albumin. Healthy volunteers were given 5.7 ml Bordeaux per kg, and blood was sampled regularly over 4 h for antioxidant activity. The antioxidant capacity of serum rose dramatically, reaching a maximum at 90 min post-dose, with a gradual decline thereafter. Levels were still significantly raised at 4 h.

This study demonstrated that red wine has a measurable protective antioxidant effect *in vivo*. However, the variable antioxidant activity of different red wines means that the potential therapeutic doses are unpredictable.⁵⁸ In addition, whilst many would find red wine a very acceptable way of taking antioxidants to reduce their cardiovascular risk, it may not be acceptable or appropriate for all individuals. An attractive option would be to harness the protective components of red wine and administer them as a safe, therapeutic and non-alcoholic preparation in combination with those antioxidant vitamins thought to be protective against CAD.

Antioxidant activity of a pharmaceutical preparation: a preliminary study

One such commercially available pharmaceutical preparation (Seresis Pharmaton, 144.1 mg active treatment per capsule) contains a combination of natural water- and lipid-soluble antioxidant substances, including vitamins C and E (60 mg and 6.7 mg, respectively), β -carotene (2.4 mg) and selenium (25 mg). In addition, it contains the polyphenolic compounds extracted from grapeseed, which are used to produce red wine (50 mg).

In a preliminary placebo-controlled crossover study, the total antioxidant activity⁵⁶ of two capsules of this preparation was determined in ten healthy individuals (5 male and 5 female, mean \pm SE age 25.4 ± 1.0 years; mean height 175 ± 2.5 cm; mean weight 70.3 ± 3.5 kg). All were non-smokers with a moderate alcohol intake, and none was taking vitamin or herbal supplements. Volunteers each took placebo (day 1), a single dose

of preparation (day 2) and 5 days of supplementation (day 3) and blood was sampled over 24 h, for measurement of serum total antioxidant capacity.

A single dose of active treatment (day 2) demonstrated a clear and significant increase in serum antioxidant activity. This peaked at 2 h post-treatment (501.5 ± 50.1 vs. 420.4 ± 31.6 $\mu\text{mol/l}$ trolox eq., $p < 0.01$ compared with baseline) and remained elevated above baseline at 24 h (482.1 ± 24.4 $\mu\text{mol/l}$ trolox eq., $p < 0.01$) (Figure 3). There was a greater change in peak antioxidant activity after 5 days supplementation (542.2 ± 42.6 $\mu\text{mol/l}$ trolox eq.). Compared with day 2, there was also a significant increase in antioxidant activity at 24 h on day 3 (482.1 ± 24.4 vs. 543.5 ± 41.2 $\mu\text{mol/l}$ trolox eq., $p < 0.05$) suggesting a gradual build up of antioxidant capacity with regular supplementation (Figure 3).

Figure 4 shows a comparison of the change in total antioxidant capacity obtained after a single administration of red wine⁵⁷ and a single administration of the antioxidant capsules, compared with their respective baseline values. These results are presented to give some insight into the relative potencies of a pharmaceutical antioxidant preparation and red wine. The data must be interpreted with great caution

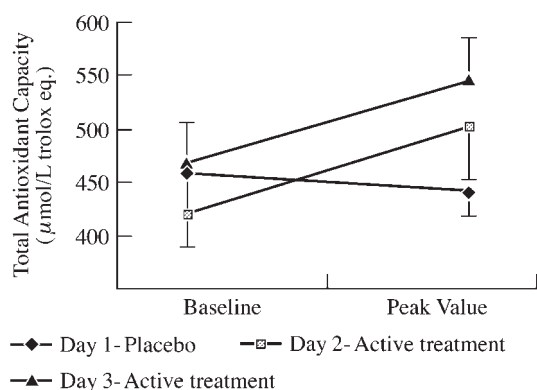


Figure 3. Change in total antioxidant capacity from baseline to peak value for placebo and days 2 and 3 of supplementation with active treatment. Values are means \pm SEM.

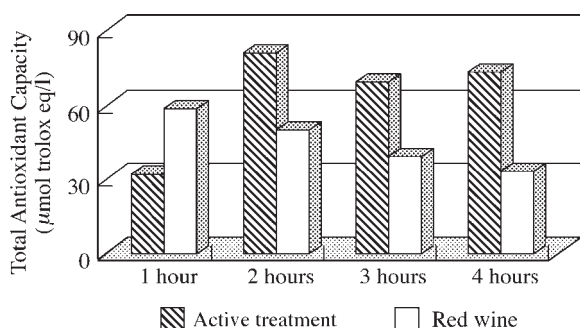


Figure 4. Change in total antioxidant capacity for a single dose of red wine vs. active treatment compared with baseline values. Values are means \pm SEM.

since they have been derived from two separate studies with different groups of volunteers. The change in total antioxidant capacity in serum increases significantly for both treatments compared with baseline values. Although red wine achieves an initial rapid rise in antioxidant defence, reaching a maximum at 1 h post-dose, there is a gradual decline thereafter. The antioxidant capsules produced a slower initial increase. However, the maximal increase (achieved at 2 h post-dose) is greater than with the red wine (81.2 vs. 59.0 $\mu\text{mol/l}$ trolox eq., compared with baseline) and is effective over a longer period of time.

This preliminary study has shown that the daily intake of a pharmaceutical preparation, containing the active components of red wine and antioxidant vitamins, can potentially increase an individual's defence against the oxidative stresses that occur naturally and as a result of disease. The comparison of results obtained from a previous study of red wine, using the same method for measurement of antioxidant activity,⁵⁶ demonstrates that this antioxidant combination has, at least, comparable effects in human serum.

Conclusions

The potential role of antioxidants in the prevention and treatment of disease, particularly coronary artery, cerebrovascular and peripheral vascular disease, needs to be determined. This review indicates how agents may achieve their beneficial effects and also presents some early evidence that combinations of antioxidants, available in the form of pharmaceutical preparations, may produce measurable changes in serum, and presumably tissue, antioxidant defences. The next stage will be to define a desirable level of antioxidant activity and demonstrate that achieving such a level can produce meaningful clinical effects.

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