The epidemiology of dietary antioxidants and atherosclerotic disease

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Recent evidence suggests that oxidative damage, particularly to low density lipoprotein, may be involved in the development and progression of atherosclerosis. Dietary antioxidants such as alpha tocopherol, acorbic acid, and carotenoids represent one possible defence against ocidative stress, raising the possibility that these agents may prevent or delay the development of atherosclerotic disease. A growing body of observational data suggests an inverse association between dietary intake or plasma levels of dietary antioxidants and cardiovascular disease. In addition, limited randomized trial data further suggest these agents may reduce the risk of subsequent cardiovascular events. While epidemiologic evidence supports the possibility that dietary antioxidants may play a role in the prevention of atherosclerosis, these agents represent a promising but unproven means of reducing the risk of cardiovascular disease.

Introduction

Basic science, clinical observation, and epidemiologic studies have all contributed to an emerging body of evidence on the role of antioxidants in prevention of coronary disease. Each of these disciplines have contributed to the cholesterol hypothesis of atherogenesis, first identifying the atherogenic potential of total cholesterol, then low density lipoprotein (LDL), and, recently, oxidatively modified low density lipoprotein (Ox-LDL). The study of antioxidants, which may inhibit the oxidation of LDL, may help further elucidate the role of LDL and oxidative damage in atherosclerotic disease.

Proposed mechanism of action of antioxidants

Elevated LDL is clearly associated with increased risk of cardiovascular disease but, until recently, the mechanism by which LDL acts was unclear. Data from in vitro and in vivo studies suggest that oxidative damage to LDL significantly increases LDL's atherogenicity[1]. Oxidized LDL (Ox-LDL) may have several different mechanisms of promoting atherogenicity. First, Ox-LDL may directly alter both the structure and function of endothelial cells[2,3]. Second, Ox-LDL may chemotactically attract monocyte/macrophages to the subendothelium[4]; and these monocyte/macrophages then develop into lipid-laden foam cells of an atheromatous plaque[5,6]. Third, Ox-LDL is taken up into foam cells via a scavenger receptor more rapidly than unoxidized LDL[7,8]. Fourth, Ox-LDL may stimulate the synthesis of auto-antibodies which may play a role in atherogenesis[9]. By several mechanisms, then, Ox-LDL may initiate and propogate a cascade of reactions which result in atherosclerosis.

In vitro studies have identified antioxidants, agents which may prevent the oxidation of LDL, thereby impeding the progression of atherosclerosis. Three important dietary antioxidants are vitamin C (ascorbic acid), vitamin E (tocopherol and tocotrienols), and β-carotene. These three are naturally occurring, dietary antioxidants; vitamin C is found in many fruits and vegetables; vitamin E is in liver, egg yolks, milk fat, cereal grains, nuts and several vegetable oils; and β-carotene (BC) is found in carrots, green leafy vegetables, squash, melons, and tomatoes. Vitamin C is water-soluble, whilst vitamin E and β-carotene are fat soluble, so can reside in circulating lipoproteins and lipid membranes.

When incubated, LDL will become oxidized[10,11,12], but this oxidative damage may be inhibited by dietary antioxidants. One human study indicated that LDL taken from patients treated with vitamin E, is resistant to in vitro oxidization compared to LDL from untreated patients[13].

Epidemiological studies

Several animal studies have shown that antioxidants impede the progression of atherosclerosis, but the usefulness of this data is limited, given the difficulties in applying the results to humans. Epidemiologic studies, on the other hand, are limited by the inability to observe humans in the same strictly controlled conditions possible in animal and laboratory research. Conclusions can only be drawn from a careful evaluation of all the available evidence. A number of researchers using different methodologies have provided evidence on the possible role of antioxidants in the prevention of cardiovascular disease.

Descriptive studies

Six descriptive, or ecological, studies have shown a correlation of per capita consumption of dietary anti-

oxidants or mean plasma levels of various antioxidants with cardiovascular disease rates within a given population. The consumption of fresh fruit and vegetables was inversely associated with the risk of heart disease in two British studies[14,15]. Verlangieri hypothesized that cardiovascular mortality is declining in the United States, in part, due to greater year-round availability of fruit and vegetables[16]. Ginter found vitamin C intake inversely associated with US mortality rates[17].

A study of 11 European countries by Gey found that vitamin E levels were inversely associated with cardiovascular mortality[18,19,20]. Riesmersma found an insignificant, but apparent, inverse association between vitamin E intake and cardiovascular mortality[21]. These descriptive studies are useful for formulating hypotheses, but analytic studies are necessary in order to rigorously test these hypotheses.

Cross sectional studies

Two case-control or cross sectional studies reported significant inverse associations between heart disease and antioxidant level. In one, leukocyte ascorbic acid levels were significantly lower among those with angiographically documented coronary disease compared to controls[22]. In the second, Riemersma compared plasma antioxidants levels in angina patients with those of healthy controls[23,24]. Vitamin E levels were significantly lower in angina cases than controls. There was a similar trend for β-carotene but no relationship between vitamins A and C or selenium and angina. A causal relationship between antioxidant intake and heart disease cannot be inferred from these studies, however, since the disease could be the result or the cause of the antioxidant levels.

Prospective studies

Prospective studies are less subject to the biases of case-control studies, since the exposure of interest, in this case antioxidant status, is measured prior to the development of cardiovascular disease. In three nested case-control studies, blood samples were collected and frozen at baseline. Subjects who later developed cardiovascular disease were matched with healthy controls, and their baseline blood samples were compared. Street et al. found a significant inverse association between baseline β-carotene levels and subsequent myocardial infarction[25]. Two other nested case-control studies found no association between antioxidant levels and vascular mortality[26,27]; however, the blood samples in these two studies were stored at -20° celsius, and the stability of antioxidants at this temperature is questionable.

Several observational, prospective cohort studies have examined the relationship between dietary intake of antioxidants and heart disease, and all have shown a risk reduction associated with antioxidant intake. Researchers with the Nurses' Health Study followed a cohort of 121 000 US female nurses aged 30–55[28,29,30]. A semiquantitative food frequency questionnaire was administered in 1980 to 87 245 subjects who were free of cancer, stroke, and heart disease. As of 1 June 1988, there were 552 cases of coronary disease, including 150 deaths and 436 non-fatal myocardial infarctions.

Women who consumed the most antioxidant vitamins were compared with women who consumed the least; women in the highest quintile of β-carotene consumption had a 22% risk reduction (RR=0.78 95%CI=0.59–1.03; p for trend across quintiles=0.02) when compared to women in the lowest quintile. Vitamin E had an even more significant risk reduction; the relative risk was 0.66 (95%CI=0.50–0.87) in the highest intake quintile (p,trend=0.001), and this effect can be attributed almost entirely to vitamin E supplementation, rather than diet. The relative risk for vitamin C is 0.80 (95%CI=0.58–1.10), but across quintiles there was no significnat trend across quintiles after controlling for vitamin E intake which was highly correlated with vitamin C consumption (p,trend=0.15). When the intake of β-carotene, vitamin E, and vitamin C are combined into a total antioxidant score, the relative risk for coronary disease is 0.54 (95%CI=0.40–0.73) among those in the highest quintile compared to the lowest (p,trend=0.001).

Another prospective cohort study, the Health Professionals Follow-up Study, examined dietary antioxidants based on four year follow-up data[31]. Of 39 000 men who had no history of vascular disease or other condition which would have necessitated dietary changes, there were 667 major coronary events (360 revascularizations, 209 non-fatal myocardial infarctions, and 106 fatal myocardial infarctions). When men in the highest quintile of intake of β-carotene were compared with men in the lowest quintile, the relative risk was 0.75 (95%CI=0.57–0.99;p,trend=0.04). Men in the highest quintile of vitamin E consumption had a relative risk of 0.68 (95%CI=0.51-0.90;p,trend=0.01) when compared with men in the lowest quintile. Vitamin C intake was not related to risk reduction in this study.

The Massachusetts Elderly Cohort Study also examined dietary information, obtained through in-person interviews[32]. The 1299 participants were followed for an average of 4.75 years through annual mailings, and were interviewed in 1976 and again in 1980. Of the participants, 151 died from cardiovascular deaths, and 47 of these were fatal myocardial infarctions. The relative risks of cardiovascular death from lowest to highest quartile of β-carotene are 1.00 (referent), 0.75, 0.65, and 0.57, respectively (p,trend=0.016), after controlling for confounders such as age, sex, smoking, alcohol consumption, cholesterol intake, and functional status. The corresponding relative risks for fatal MI are 1.00 (referent), 0.77, 0.59, 0.32 (p,trend=0.02).

An observational study of U.S. men and women, the NHANES-I study, examined vitamin C intake[33]. The 11 349 participants in the study, aged 25–74 were followed for a median of ten years, and the standardized cardiovascular mortality rate was 34% lower (RR=0.66, 95%CI=0.53–0.82) than expected among participants with the highest vitamin C intake. Vitamin C supplement use explained most of the association. This study did not examine the correlation of Vitamin C supplementation with other vitamin supplementation, as other studies which showed that controlling for supplement use reduced the significance of vitamin C to nothing. In a prospective cohort of Swedish women, estimates of vitamin C intake from a 24 hour recall dietary history were inversely correlated with CVD event rates; however, these findings did not persist after controlling for age[34].

The limits of observational data

While the data from both prospective blood-based and dietary intake studies are compatible with a possible benefit of antioxidants, the available observational data are sparse and not all consistent. Additional observational data would certainly be a valuable contribution to the totality of evidence concerning antioxidants and cardiovascular disease. However, regardless of the number or sample size of such studies, or the consistency of their findings, observational investigations are limited in their ability to provide reliable data on the most plausible small to moderate benefits of antioxidants. It may be, for example, that greater dietary intake of antioxidants, measured by blood levels or a diet assessment questionnaire, is only a marker for some other dietary practice or even non-dietary lifestyle variable that is truly protective. It is, in fact, plausible that intake of antioxidant-rich foods is indeed protective, but the benefit results not from their antioxidant properties, but some other component these foods have in common. In addition, the intake of individual dietary antioxidants is often highly correlated, making it difficult to determine the specific benefit of any one.

Observational studies can control for the effects of known potential confounding variables but they cannot take into account unknown or unmeasured confounding factors. In searching for small to moderate effects, the amount of uncontrolled confounding in observational studies may be as large as the likely risk reduction. For these reasons, reliable data can only emerge from large scale randomized trials, in which investigators allocate subjects at random to either active treatment or placebo.

Clinical randomized trials

Large clinical trials avoid the limits of observational studies by distributing the known and unknown confounding variables among treatment groups. However, no data from large trials is available on antioxidant use. In four small trials, three studies[35,36,37] of claudication reported a positive benefit of vitamin E supplementation while on trial[38] reported no benefit of vitamin E in angina pectoris after six months of treatment.

A subgroup analysis within the Physicians' Health Study enrolled 333 doctors who had a history of chronic stable angina or who had a prior coronary revascularization procedure[39]. The Physicians' Health Study is a randomized, double-blind, placebo-controlled two-by-two factorial trial of 22 071 US male physicians aged 40–84, testing aspirin in the primary prevention of cardiovascular disease and β-carotene in the primary prevention of cancer.

In this subgroup analysis, two endpoints were defined: major coronary events and major vascular events. Major coronary events included coronary revascularization, fatal coronary disease, and non-fatal MI. Major vascular events included non-fatal and fatal stroke. Among subjects who received β-carotene, there was a 51% reduction (RR=0.49, 95%CI=0.29–0.88) in risk of major coronary events, and a 54% reduction (RR=0.46, 95%CI=0.24–0.85) in rise of major vascular events. Furthermore, the effect of β-carotene was time-dependent, consistent with the theory that antioxidant intake slows the progression of atherosclerosis. Relative risk was analysed by year of follow-up, and no effect appeared during the first year, but did appear in the second year and persisted thereafter.

More randomized trials needed

The US National Heart, Lung, and Blood Institute recently issued a summary statement resulting from a conference on 'Antioxidants and the Prevention of Human Atherosclerosis'[40]. The summary statement supported further randomized trials examining the role of vitamins C and E and β-carotene in the primary and secondary prevention of cardiovascular disease.

There are currently several large scale randomized trials of antioxidants testing their role in the prevention of cardiovascular disease as well as cancer. The Physicians' Health Study is testing β-carotene in 22 000 healthy US male physicians. The Women's Health Study is testing β-carotene and vitamin E in the primary prevention of cardiovascular disease and cancer in 44 000 healthy, US female nurses. The interactions between vitamin E and β-carotene will be examined in this study. The CARET study is testing a combination of β-carotene and retinoic acid in 18 000 asbestos workers. The Finnish alpha tocopherol/β-carotene study and a Chinese antioxidant vitamin cocktail study results were recently presented revealing an apparent reduction in the risk of cancer, but no meanginful conclusions were drawn in relation to cardiovascular disease. Several secondary prevention trials among high risk individuals are currently in the planning stages.

Conclusion

In summary, available epidemiological evidence supports the possibility that antioxidants may have a protective effect in cardiovascular disease, and basic research supports a plausible mechanism for the involvement of oxidative stress in atherogenesis. More reliable data which should be forthcoming in the near future, will further elucidate the role of antioxidants in the primary and secondary prevention of heart disease.

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膳食的抗氧化劑與動脉粥樣硬化的流行病學

摘 要

最近證明，氧化損害，特別是低密度脂蛋白的氧化損害也許與動脉粥樣硬化的形成與發展有關。膳食的抗氧化劑如α-生育酚，抗壞血酸和類胡蘿卜素代表一種可能的防衛， 以對抗氧化損害，也許這些抗氧化劑可預防和延緩動脉粥樣硬化的形成。作者從生長機體的觀察數據指出， 膳食中或血漿中的抗氧化劑水平與心血管疾病成負相關。再者，隨機試驗數據進一步指出這些抗氧化劑也許可減少心血管疾病的危險，流行病學支持膳食的抗氧化劑有預防動脉粥樣硬化的可能性，但從減少心血管疾病來說，這只代表一種有希望的，而未被證明的事實。