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Analysis of the variable effect of dietary vitamin E supplements on experimental atherosclerosis

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Summary

Vitamin E inhibits processes thought to be important in the development of atherosclerosis but clinical trials to determine its effect on cardiovascular disease have given variable results, the majority being negative. The reasons for this are unclear. Animal trials can be better controlled and use more rigorous measures of lesion progression than human trials. The present study reviewed trials using rabbits and mice to determine whether they also are variable and, if so, to uncover methodological differences that may account for the different outcomes. A large number of trials examining the effect of vitamin E supplements on experimental atherosclerosis were identified. Using rigorous selection criteria, a well-defined group was selected for further investigation. Almost all the mice trials showed a significant effect of vitamin E, but only around one-third of the rabbit trials did so. When the rabbit trials were divided into those that did and those that did not observe significant effects, no single factor was found that could account for the dichotomy. However, when the percentage reduction in disease was considered, rather than the within-trial significance level, there were clear dose-dependent effects of vitamin E on disease severity in heritable hyperlipidaemic rabbits, and in genetically normal rabbits made hyperlipidaemic with cholesterol alone; the dose dependence was different in the two groups, the heritable hyperlipidaemic rabbits showing a near ten-fold lower sensitivity. The high doses required to affect experimental atherosclerosis may, if applicable to other species, help explain the absence of effects in many human trials.

Abbreviations: IU, international units; LDL, low-density lipoprotein; SD, standard deviation

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Introduction

Atherosclerosis, the disease underlying most heart attacks and strokes, is characterised by the focal accumulation of lipids, cells and fibrous proteins within the intima and inner media of large and medium-sized systemic arteries. The mechanisms by which it develops have not been established, but key steps appear to include entry of circulating lipoproteins into the wall, their oxidation within the wall, dysfunction of the endothelial cells lining the inner surface of the artery, endothelial expression of adhesion molecules leading to the accumulation of monocyte/macrophages in the wall, proliferation of smooth muscle cells that have migrated into the intima, and formation of blood clots following plaque rupture.

In vitro or in vivo studies have demonstrated that all of these processes are inhibited by vitamin E (e.g. Devaraj and Jialal, 1998). Consequently, there is an expectation that increased levels of dietary vitamin E should protect against atherosclerosis. Although several large epidemiological studies found that high-level vitamin E intake or supplementation for at least 2 years is associated with a reduction in cardiovascular disease (Jha et al., 1995), the results of prospective clinical trials have been mixed. Recent reviews (Steinberg and Witztum, 2002; Jialal and Devaraj, 2003) conclude that while some double-blind, placebo-controlled trials have had positive outcomes, others – particularly the larger ones – have not. The reasons for this variability are unclear.

Investigations of the effects of vitamin E on experimental atherosclerosis in animals can be better controlled and can use more rigorous measures of lesion progression than investigations in people. The present study reviews trials in animals with the aims of determining whether these trials are similarly variable and, if so, of uncovering systematic methodological differences between those showing and those failing to show beneficial effects. Such differences might assist understanding of the variation between human trials.

Methods

Studies of the effects of vitamin E on experimental atherosclerosis in rabbits and mice were selected from a larger group identified using the National Centre for Biotechnology Information's "PubMed" database (http://www.ncbi.nlm.nih.gov/entrez/query.fcgi). Each group of search terms included an animal, an intervention and an outcome. All combinations of the following terms were used: mouse, mice, rabbit or rabbits for the animal; vitamin E, tocopherol, tocopherols, tocotrienol or tocotrienols for the intervention; and atherosclerosis or lesions for the outcome. The papers identified by these searches were considered for further investigation if the PubMed abstract reported that the extent of lesions had been assessed. Reviews were excluded, as were papers in languages other than English. The cut-off date for publication was 31.12.2004.

Studies involving species other than rabbits or mice were not examined because there have been too few of them to permit a rigorous analysis. This paper concentrates on the rabbit studies since there was considerable disagreement between them and hence more scope for analysis. The mouse literature was more consistent and is only briefly discussed.

For trials that used various antioxidants but had an experimental group given vitamin E alone, that group and its control were included. Trials using vitamin E solely as part of an antioxidant mixture were excluded. Papers that compared a normal diet with a vitamin E deficient diet, or used animals in which the α-tocopherol transfer protein gene was disrupted, were excluded since they are only peripherally related to the issue of whether supplementing a normal diet with additional vitamin E might reduce atherosclerosis.

Limitations were placed on study outcomes. Inclusion was restricted to commonly accepted forms of experimental atherosclerosis, arising solely from spontaneous or engineered genetic hyperlipidaemias, or from hyperlipidaemia induced by supplementing diets with atherogenic lipids or proteins. Studies of lesions triggered by mechanical or electrical injury, with or without hyperlipidaemia, were excluded (since they are more relevant to restenosis following angioplasty than to classical atherosclerosis), as were two studies where vitamin E was given to pregnant animals and spontaneous lesions assessed in their offspring. Studies using rabbits were included only if the severity of experimental atherosclerosis was measured in a way that allowed ratios of disease severity with and without vitamin E to be calculated, since this was an essential part of the analysis. Quantification based on arbitrary scales (e.g. 0 for no lesions, 1 for mild lesions, etc.) was not adequate for this purpose. Accepted outcomes were (i) lesion area as a fraction of arterial surface area, (ii) cholesterol concentration in arterial tissue, and (iii) quantitative morphometry of the area, length or thickness of lesions in histological sections. The