Failure of Vitamin E in Clinical Trials: Is Gamma-Tocopherol the Answer?
Sridevi Devaraj, PhD, and Ishwarlal Jialal, MD, PhD

Oxidative stress and inflammation play a crucial role in atherosclerosis. However, prospective clinical trials of dietary antioxidants with anti-inflammatory properties, such as α-tocopherol (AT), have not yielded positive results. AT supplementation decreases γ-tocopherol (GT) levels. GT is an antioxidant with potent anti-inflammatory activity, and plasma GT levels are inversely associated with cardiovascular diseases. Thus, studies using pure GT, alone or in conjunction with AT, will elucidate its utility in cardiovascular disease prevention.

Key words: antioxidant, tocopherol, inflammation, oxidative stress, nitrative stress

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INTRODUCTION

Several lines of evidence support a role for oxidative stress and inflammation in atherogenesis. Epidemiologic studies suggest that low levels of antioxidants are associated with an increased risk of cardiovascular disease and that increased intakes appear to be protective. Vitamin E occurs in nature in eight structurally related forms, of which four are tocopherols and four are tocotrienols. Tocopherols have a saturated phytol side chain with three chiral centers that are in an “R” configuration at positions 2’, 4’, and 8’ in the naturally occurring forms. Tocopherols differ in the number of methyl groups they have at the 5’- and 7’-positions of the chromanol ring. For instance, γ-tocopherol (GT) is unsubstituted at the C-5 position, whereas α-tocopherol (AT) is fully substituted in the chromanol ring (Figure 1).

In supplementation studies in humans, AT, the major form of vitamin E, has been shown to significantly decrease biomarkers of oxidative stress and inflammation. The results of prospective vitamin E clinical trials, however, have been disappointing, as discussed previously.1 All of the studies discussed were carried out with supplements containing AT. It is well known that AT supplements decrease plasma GT concentrations2 as a result of the function of the hepatic tocopherol transfer protein, which preferentially incorporates AT into the plasma.3 This may explain the null results observed with AT supplementation in the majority of prospective clinical trials. In this brief review, we will discuss the potential beneficial effects of GT.

GT

GT is the most prevalent form of vitamin E in plant seeds and products derived from them.4 Vegetable oils such as corn, soybean, and sesame and nuts such as walnuts, pecans, and peanuts are rich sources of GT. Because of the widespread use of these plant products, GT represents 70% of the vitamin E consumed in the typical US diet.5 Furthermore, plasma and tissue GT are decreased by AT supplementation.6 However, GT has received little attention since the discovery of vitamin E in 1922 and is not included in the current dietary intake recommendations. This is mainly due to the lower bioavailability and bioactivity of GT compared with AT.7 However, recent evidence suggests that GT has certain biological properties that are not shared by AT and may be important to human health.

ABSORPTION AND AVAILABILITY

Studies using deuterium-labeled tocopherols have led to an understanding of the absorption and metabolism of GT. AT and GT dietary fat are taken up without preference by the intestine and secreted in chylomicron particles together with triacylglycerol and cholesterol. During the subsequent lipoprotein lipase-mediated catabolism of chylomicron particles, some of the chylomicron-bound vitamin E appears to be transported and transferred to peripheral tissues such as muscle, adipose tissue, and brain.8 The resulting chylomicron remnants are subsequently taken up by the liver. GT appears to be degraded largely to the hydrosoluble γ-carboxyethyl-hydroxychroman (γ-CEHC)9 by a cytochrome P450-dependent process,10 and is then primarily excreted in urine.11 Plasma γ-CEHC concentrations are reported to be 50 to 100 nmol/L in humans.7 In human urine, γ-CEHC exists predominantly as a glucuronide conju-
gate with concentrations ranging from 4 to 33 \( \mu \text{mol/L} \), which have been shown to increase to over 100 \( \mu \text{mol/L} \) after supplementation with GT.\(^8\) Finally, biliary excretion may be an alternative route for eliminating excess GT.\(^13\) Excess GT secreted into feces during supplementation may play a role in eliminating fecal mutagens and thus reduce colon cancer. Thus, the biological disposition and retention of GT appear to be regulated by a metabolism that is quite different from that of AT.

**GT AS AN ANTIOXIDANT**

The antioxidant activity of tocopherols is mainly due to their ability to donate phenolic hydrogens (electrons) to lipid radicals. Because it lacks one of the electron-donating methyl groups on the chromanol ring, GT is somewhat less potent in donating electrons than is AT and is therefore a slightly less powerful antioxidant.\(^13\) However, the unsubstituted C-5 position of GT appears to make it better able to trap lipophilic electrophiles such as reactive nitrogen species. In pioneering studies, Cooney et al.\(^14\) found that GT is superior to AT in detoxifying nitrogen dioxide. They showed that GT reduces nitrogen dioxide to the less harmful nitric oxide or traps nitrogen dioxide to form 5-nitro-\( \gamma \)-tocopherol.\(^15\) Jiang et al.\(^16\) showed that supplementation of rats with 90 mg GT/kg diet significantly inhibited protein nitration, as evidenced by decreased levels of 3-nitro-tyrosine in plasma, liver, and kidney.

**OTHER BENEFICIAL CELLULAR EFFECTS OF GT**

GT has been shown to inhibit smooth muscle cell proliferation by inhibiting protein kinase C activity, while \( \beta \)-tocopherol had no effect, indicating that this effect is independent of antioxidant activity. Recently, Jiang et al.\(^10\) found that both GT and \( \gamma \)-CEHC possess anti-inflammatory activity. Both inhibited prostaglandin E2 synthesis via inhibition of cyclooxygenase-2 activity in lipopolysaccharide-stimulated macrophages, while AT slightly decreased prostaglandin E2 (PGE2) and, in this study, had no significant effect on cyclooxygenase activity. In a subsequent study of carrageenan-induced inflammation in rats, GT supplementation (33 or 100 mg/kg) but not AT supplementation (33 mg/kg) decreased PGE2 synthesis at the site of inflammation in addition to decreasing 8-isoprostanes, tumor necrosis factor, and total nitrates/nitrites.\(^16\)

**GT AND CARDIOVASCULAR DISEASE**

Several animal studies provide some evidence that GT might be beneficial against cardiovascular disease. Saldeen et al.\(^17\) investigated the effects of AT and GT supplementation on platelet aggregation and thrombosis in Sprague-Dawley rats, and found that GT supplementation (100 mg/kg/d) led to a greater decrease in platelet aggregation and delay of arterial thrombogenesis than did AT supplementation. GT supplementation also resulted in stronger inhibition of superoxide generation and lipid peroxidation. Subsequently, they reported that GT was significantly more potent than was AT in enhancing superoxide dismutase activity in plasma and arterial tissue and in increasing the arterial protein expression of both manganese superoxide dismutase and copper/zinc superoxide dismutase.\(^18\) Also, GT supplementation was associated with an increase in endothelial cell nitric oxide synthase (eNOS). However, the relevance of these studies is unclear, since most of them used either mixed tocopherol preparations or GT-enriched supplements rather than purified GT alone.

Although much less is known about GT than about AT, much evidence suggests that GT may be important in the defense against cardiovascular disease. Plasma GT concentrations are inversely associated with increased morbidity and mortality due to cardiovascular disease.\(^19\) Ohrvall et al.\(^20\) and Kontush et al.\(^21\) reported that serum concentrations of GT, but not of AT, were lower in cardiovascular disease patients than in healthy control subjects. In a concomitant cross-sectional study of Swedish and Lithuanian middle-aged men, Kristenson et al.\(^22\) found that plasma GT concentrations were twice as high in the Swedish men, who had a 25% lower incidence of cardiovascular disease-related mortality. In a 7-year follow-up study of 34,486 postmenopausal women, Kushi et al.\(^23\) concluded that the intake of dietary vitamin E (mainly GT), but not of supplemental vitamin E (mainly AT), was significantly inversely associated with in-

**Figure 1.** Chemical structure of \( \alpha \)-tocopherol and \( \gamma \)-tocopherol.
creased risk of death from cardiovascular disease. Recently, these investigators further showed that dietary vitamin E was associated with a reduced incidence of death from stroke in postmenopausal women. Regular consumption of nuts, which are an excellent source of GT, lowers the risk of myocardial infarction and death from ischemic heart disease.

GT SUPPLEMENTATION IN HUMANS

Despite the promises of GT as an effective antioxidant and anti-inflammatory agent in vitro, there is not much evidence on GT supplementation in humans. In a clinical trial, Himmelfarb et al. enrolled 15 uremic patients undergoing dialysis. Five patients were supplemented with RRR-AT (300 mg/d) and 10 received mixture of tocopherols (60% RRR-GT, 28% RRR-δ-tocopherol [DT], and 18% RRR-AT) for a duration of 14 days. Tocopherol administration increased serum CEHC concentrations in both healthy subjects and hemodialysis patients. Hemodialysis resulted in no change in the serum AT or GT concentrations, while decreasing serum α-CEHC and γ-CEHC levels by 63% and 53%, respectively (P = 0.001 compared with pre-dialysis).

A potentially important observation in this study is that the administration of the GT-enriched preparation, but not the AT preparation, significantly reduced C-reactive protein concentrations in hemodialysis patients. However, owing to the limitations of this study (its small sample size with respect to inflammatory biomarkers), further studies with larger sample sizes will be required to more definitively address these important end points. Furthermore, Liu et al. supplemented healthy subjects with placebo, all-racemic (all-rac) AT (100 mg/d), or mixed tocopherols (comprising 100 mg GT, 20 mg DT, and 20 mg AT) for 8 weeks. Mixed tocopherols but not AT supplementation decreased ADP-induced platelet aggregation. Both AT and mixed tocopherols supplementation resulted in reduced protein kinase C and increased superoxide dismutase and nitric oxide release. These two studies point to an important role for either GT or AT supplementation on biomarkers of oxidative stress and inflammation and cardiovascular disease; however, this needs to be carefully studied.

CONCLUSIONS

It is clear that while GT shows great promise as an antioxidant and anti-inflammatory agent, controlled intervention studies in humans are required to clearly establish the benefits of GT supplementation. Furthermore, potential synergistic effects between GT and AT and other antioxidants should also be explored. These efforts should help to clarify the role of GT in cardiovascular disease prevention and human health.

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