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A New Wrinkle on Topical Vitamin E and Photo-inflammation: Mechanistic Studies of a Hydrophilic γ -Tocopherol Derivative Compared with α -Tocopherol

Raymond L. Konger^{1,2}

The antioxidant function of vitamin E is thought to mediate its photo-protective effects. Cyclooxygenase-2 (COX-2) is an important mediator of early photo-inflammation. Thus, the ability of γ -tocopherol to inhibit COX-2 activity independently of its antioxidant function raises important questions regarding potential roles that this form of vitamin E plays in photo-protection and skin cancer chemoprevention.

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Since Linus Pauling published his widely read and publicly debated treatise *Vitamin C and the Common Cold* in 1970, there has been tremendous lay interest in the potential protective effects of antioxidants in a variety of ailments. This interest did not escape the cosmetic industry. Currently, pharmacy shelves are well stocked with skin-care products, sunblocks, and sun-tanning

lotions that tout the remarkable antioxidant properties of vitamin E. Given that there is good evidence that vitamin E may have protective functions within the epidermis, this widespread use cannot be simply trivialized as a long-term marketing ploy. However, there is no good consensus regarding the clinical utility of the various naturally occurring forms of vitamin E, nor

the widely used thermostabile esterified forms of vitamin E. This is partly because, beyond its antioxidant effect, very little is known regarding the cellular mechanisms through which the different biologically relevant forms of vitamin E work. Yoshida and colleagues (2006, this issue) demonstrate that a novel water-soluble vitamin E derivative, γ -tocopherol-*N,N*-dimethylglycinate hydrochloride (γ -TDMG), may be superior to the widely used α -tocopherol (α -Toc) form of vitamin E in suppressing UVB-induced photo-inflammation. Moreover, they demonstrate that γ -TDMG inhibits the production of two inflammatory mediators, prostaglandin E₂ (PGE₂) and nitric oxide. Finally, Yoshida *et al.* provide evidence that the ability of γ -TDMG to block PGE₂ production may be independent of its antioxidant properties. This has important implications not only for epidermal photo-protection and chemoprevention, but also for current studies examining the chemopreventive effects of vitamin E on other epithelial malignancies, particularly prostate, colon, and lung cancer.

Historical perspective

Vitamin E was first described in 1922 by Herbert M. Evans and Katharine Bishop. In their studies, an unknown factor that they called vitamin E was found to be necessary for fetal development in rats. Fetal development was found to be aborted in female rats fed a defined diet. However, fetal development was restored by the addition of plant material, the most effective being wheat germ oil. Vitamin E was subsequently isolated in 1936 and was given the name tocopherol, or “the childbirth-producing alcohol” (Greek *tokos*, childbirth; *pherein*, to bear; *ol*, an alcohol). In 1938, Paul Karrer synthesized vitamin E and demonstrated its function as a lipid-soluble antioxidant. By the 1940s and 1950s, the antioxidant activity of vitamin E was well established, and it was recognized as an essential nutrient in 1968. Natural plant-derived vitamin E is composed of eight different natural derivatives (α -, β -, γ -, and δ -tocopherols and the related α -, β -, γ -, and δ -tocotrienols). Whereas γ -tocopherol (γ -Toc) is the most abundant tocopherol found in the diet,

¹Department of Pathology and Laboratory Medicine, Indiana University School of Medicine, Indianapolis, Indiana, USA; and ²Department of Dermatology, Indiana University School of Medicine, Indianapolis, Indiana, USA

Correspondence: Dr. Raymond L. Konger, Department of Pathology and Laboratory Medicine, Indiana University School of Medicine, Fesler Hall, Room 403, 1120 South Drive, Indianapolis, Indiana 46202, USA. E-mail: rkonger@iupui.edu

α -Toc represents the most abundant vitamin E derivative found in most human tissues and sera, with γ -Toc being the only other tocopherol found in biologically relevant amounts. This discrepancy between dietary consumption of α - and γ -Toc and the levels of these tocopherols in tissue and blood is due to differences in metabolism, transport, and excretion that favor retention of α -Toc (Jiang *et al.*, 2001). Thus, given its higher tissue levels, α -Toc has been the primary tocopherol used in dietary supplements and in research studies over the past 30 to 40 years. Various studies during this period indicate that the incidence of a number of malignancies, including lung, colon, and prostate, exhibits an inverse correlation with blood levels of vitamin E. However, with the exception of prostate cancer, more recent randomized controlled studies have failed to show a correlation between dietary supplementation with α -Toc and reduced cancer risk (Ritenbaugh *et al.*, 2003). This past focus on α -Toc may have resulted in the failure to recognize the potential unique contributions of γ -Toc to human pathophysiology (reviewed by Jiang *et al.*, 2001). First, human and animal studies indicate that plasma levels of γ -Toc exhibit greater inverse correlation with the incidence of cardiovascular disease and prostate cancer. Second, whereas α -Toc exhibits a modest increase in the ability to suppress lipid peroxidation, γ -Toc is a more effective trap for lipophilic electrophiles, such as reactive nitrogen oxide species (for example, peroxyxynitrite and nitrogen dioxide). Third, oral supplementation of α -Toc has been shown to suppress tissue γ -Toc levels, whereas oral γ -Toc supplementation increases the tissue levels of both α -Toc and γ -Toc. Finally, γ -Toc and its water-soluble metabolite 2,7,8-trimethyl-2-(β -carboxyethyl)-6-hydroxychroman (γ -CEHC) have been shown to inhibit the enzymatic activity of the inducible form of cyclooxygenase (COX-2) in macrophages and epithelial cells and after carrageenan-induced inflammation in rats (Jiang *et al.*, 2001; Jiang and Ames, 2003). This suppression of COX-2 activity was not dependent on the antioxidant function of either γ -Toc or γ -CEHC, and this

effect was not observed when equivalent concentrations of α -Toc were used. Given the important role of COX-2 in inflammation and tumorigenesis, this suggests that γ -Toc may exhibit superior anti-inflammatory and chemopreventive properties as compared with α -Toc.

γ -TDMG ... directly inhibits COX-2 activity independently of its ability to scavenge reactive oxygen species.

Vitamin E as a photo-protectant

The epidermis, acting as the primary barrier to fluid loss and environmental insult, is constantly exposed to UV light and other oxidative stressors. This constant oxidative barrage leaves its mark on the epidermis, resulting in phototoxicity, immunosuppression, photoaging, and cutaneous neoplasia (Fuchs, 1998). Because of its potent antioxidant and lipophilic properties, vitamin E is thought to play a key role in protecting epidermal cell membranes and lipids from oxidative damage. Numerous studies have demonstrated a role for topical vitamin E in reducing both acute and chronic UV-mediated skin responses, such as erythema and edema, sunburn-cell formation, DNA photo-adduct formation, immunosuppression, and photo-carcinogenesis (Thiele *et al.*, 2005). However, because of the inherent instability of α -Toc, both topical preparations and oral supplements often use inactive α -Toc esters (for example, α -tocopherol acetate). This may serve to increase shelf-life, but there is considerable debate as to whether appreciable bioconversion of these inactive esters to the active nonesterified form occurs in the skin (Fuchs, 1998; Thiele *et al.*, 2005). Moreover, there is some evidence that the use of these esterified tocopherols may actually enhance photo-carcinogenesis (Gensler *et al.*, 1996).

The role of γ -tocopherol in epidermis

Whereas there is evidence that topical α -Toc is a potent cutaneous photo-

protectant, there is much less information concerning γ -Toc in epidermal biology. Thus, it is interesting that in human skin, γ -Toc levels exceed those of α -Toc (Jiang *et al.*, 2001). This contrasts with the higher level of α -Toc found in other tissues and in serum and suggests that γ -Toc plays an important role in epidermal protection from oxidative stress. In addition, studies using γ -Toc have been limited by its near-complete insolubility in water and the fact that it is readily oxidized by atmospheric oxygen. These physico-chemical restraints appear to have been remedied by the synthesis of the water-soluble γ -Toc derivative γ -TDMG (Takata *et al.*, 2002). By adding an *N,N*-dimethylglycine ester group to the phenolic functional group of γ -Toc, the investigators achieved a hydrophilic prodrug that is resistant to oxidation and is readily converted to the active γ -Toc after systemic administration. This group subsequently demonstrated that topically administered γ -TDMG was absorbed and efficiently bioconverted both into γ -Toc and, subsequently, into γ -CEHC (Yasuoka *et al.*, 2005). Notably, the bioconversion was much greater than that observed for two commonly used α -Toc esters (α -Toc acetate and α -TDMG). In this same report, γ -TDMG exhibited significantly greater ability to prevent sunburn-cell formation, lipid peroxidation, and edema and inflammation than the two α -Toc esters. However, this study did not clarify whether the improved biological outcome with the use of γ -TDMG was simply due to its higher rate of bioconversion.

As they report in this issue, this same group of investigators expanded on the earlier studies by applying doses of γ -TDMG and α -Toc acetate (α -TA) that result in equivalent levels of active γ -Toc and α -Toc within the epidermis (Yoshida *et al.*, 2006). They then examined the effect of both agents on UVB-induced photo-inflammation. When applied 1 hour before UVB irradiation, both α -TA and γ -TDMG showed similar abilities to inhibit UVB-induced edema and PGE₂ production. Notably, when applied 1 hour after UVB irradiation, γ -TDMG was still highly effective, whereas the α -TA response was considerably suppressed. This is an important distinction, as the authors also confirm

earlier reports that α -Toc pretreatment exhibits a greater capacity to block UVB-induced lipid peroxidation. Thus, the ability of γ -TDMG, but not α -TA, to maintain anti-inflammatory activity when applied after UVB irradiation suggests that this ability is not mediated solely by antioxidant activity.

This idea is further supported by several additional studies. First, the authors show that pretreatment with α -TA was more effective in suppressing UVB-induced COX-2 mRNA and protein expression as compared with γ -TDMG (Yoshida *et al.*, 2006). This suggests that α -TA, via its greater antioxidant properties, has a greater capacity to reduce UVB-mediated COX-2 induction. This is not surprising, given that oxidative stress has previously been shown to trigger COX-2 expression (Zhang *et al.*, 2005). In contrast, γ -TDMG pretreatment was more effective than α -TA in inhibiting UVB-induced COX-2 enzymatic activity. This indicates that γ -TDMG, presumably acting via γ -Toc and γ -CEHC, directly inhibits COX-2 activity independently of its ability to scavenge reactive oxygen species. These results support the earlier findings of Jiang and colleagues that γ -Toc, but not α -Toc, directly inhibits COX-2 activity (Jiang *et al.*, 2001; Jiang and Ames, 2003). It should be noted that these additional studies examining COX-2 expression and activity only addressed γ -TDMG and α -TA pretreatment. Yet γ -TDMG markedly suppressed UVB-induced PGE₂ production even when γ -TDMG was applied 1 hour after UVB irradiation. As UVB-induced reactive oxygen species are significantly elevated up to 90 minutes after an acute exposure (Wang and Kochevar, 2005), it would be interesting to determine the degree to which this post-treatment response is dependent on manipulation of COX-2 expression or inhibition of COX-2 enzymatic activity.

In addition to COX-2, nitric oxide and inducible nitric oxide synthase contribute to UVB-induced photo-

inflammation. Moreover, α -TA has been previously shown to suppress COX-2 activity via its ability to block peroxynitrite production formed from the reaction of nitric oxide with O₂⁻ radicals (Beharka *et al.*, 2002). As γ -Toc has been reported to have greater capacity to block reactive nitrogen oxide species formation, this may help to explain the increased capacity of γ -Toc to inhibit COX-2 activity. However, this idea is not supported by the next experiment of Yoshida *et al.* (2006). In this experiment, they demonstrated that γ -TDMG and α -TA were equally effective in blocking nitrite production and that γ -TDMG was only slightly more effective in inhibiting inducible nitric oxide synthase expression.

In conclusion, the study by Yoshida *et al.* (2006) indicates that γ -TDMG is superior to α -TA in blocking UVB-induced photo-inflammation. In particular, the effectiveness of γ -TDMG in blocking UVB-induced photo-inflammation was maintained when it was applied after the UVB dose. This is important given the typical patterns of sunscreen use, in which sunscreen is applied just before or at some point after initial sun exposure. These data also raise several important questions. First, how long after UVB irradiation can γ -TDMG provide photo-protection? Given the ability of γ -TDMG to suppress PGE₂ levels 1 hour after UVB irradiation, additional studies are needed to verify whether post-treatment with γ -TDMG also inhibits UVB-induced COX-2 activity, lipid peroxidation, reactive nitrogen oxide species formation, and inducible nitric oxide synthase suppression. In addition, the mechanism through which γ -TDMG suppresses COX-2 expression and activity is unclear. Most importantly, these findings suggest that γ -TDMG may be a superior investigational tool for UVB photo-carcinogenesis studies.

CONFLICT OF INTEREST

The author states no conflict of interest.

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