

Bioavailability of a Novel, Water-Soluble Vitamin E Formulation in Malabsorbing Patients

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Abstract In cystic fibrosis (CF), pancreatic insufficiency and a diminished bile acid pool cause malabsorption of important nutrients and dietary components leading to deficiency, poor nutritional status, and oxidative stress. Of particular significance is the malabsorption of fat-soluble nutrients and antioxidants, which are important for normal immune and neurologic function. Patients with CF often are deficient in these compounds despite supplementation with the current standard of care therapy. The objective was to compare the pharmacokinetic profile of this water-soluble vitamin E formulation (Aqua-E) with an oil-based softgel formulation in a malabsorbing patient population. Patients with CF who had documented malabsorption were recruited for participation in this pharmacokinetic study. Patients who met inclusion and exclusion criteria discontinued vitamin E supplementation, except for that in a multivitamin, for 7 to 21 days before the day of dosing. Patients were randomized to

a single dose of 20 ml of Aqua-E or three oil-based softgels, which contained equivalent amounts of tocopherols. Blood was drawn from patients at time 0, 2, 4, 8, 24, 48, and 168 hr and analyzed for tocopherols. Eight patients were enrolled in the study and randomized to Aqua-E or softgels. The primary outcome, the absorption of γ -tocopherol in Aqua-E ($AUC = 115 \mu\text{g/ml}^*\text{hr}$), was significantly greater than that of oil-based softgels ($AUC = 25.3 \mu\text{g/ml}^*\text{hr}$; $P = 0.013$). Total-tocopherols ($\alpha + \gamma + \delta$) in Aqua-E ($AUC = 294 \mu\text{g/ml}^*\text{hr}$) showed a strong trend toward increased absorption compared with that of oil-based softgels ($AUC = 117 \mu\text{g/ml}^*\text{hr}$; $P = 0.09$). In conclusion, this novel, water-soluble formulation showed a marked and statistically significant increase in absorption of γ -tocopherol in malabsorbing patients with CF compared with an oil-based formulation.

Keywords Vitamin E · Tocopherol · Malabsorption · Bioavailability · Water-soluble · Cystic fibrosis · Nutrition · Cholestasis · Vitamin E deficiency · TPGS

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Introduction

Several disease states, especially those that impair biliary and pancreatic function, are associated with malabsorption of fat-soluble macro- and micronutrients. In cystic fibrosis (CF), increased viscosity of pancreatic secretions causes obstruction of pancreatic ducts that ultimately leads to the destruction and fibrosis of the exocrine pancreas in 85% of the patients with CF. The resulting failure of secretion of pancreatic digestive enzymes causes steatorrhea and malabsorption. Improvements in life expectancy have led to an increasing recognition that CF often is associated with a number of hepatic and/or biliary abnormalities, such as chronic cholestatic liver disease [1–3].

While in the absence of biliary abnormalities the bile acid pool of patients with CF may appear normal, differences in bile acid composition, absorption, and fecal excretion may affect the digestion and absorption of lipids [4–6]. Insufficient pancreatic secretions and biliary abnormalities impair micellar solubilization, which is essential for absorption of lipid soluble compounds.

This impairment in the absorption of nutrients, specifically fat-soluble vitamins and antioxidants, leads to deficiencies in these compounds as well as a poor nutritional and antioxidant status. Pancreatic enzyme replacement therapy frequently fails to completely correct malabsorption of lipids because of incomplete intraluminal solubilization and/or reduced mucosal uptake of lipids [7]. Malabsorption contributes to significantly lower blood levels of lipophilic nutrients in patients with CF than in normal controls even with the current standard supplementation [8–11].

Malabsorption of vitamin E is particularly important because of its role as an essential nutrient and as a major component of the antioxidant system. Severe deficiency as a result of malabsorption can cause varying degrees of neurologic damage [12, 13]. Even metabolic and subclinical deficiency leads to increased oxidative stress, which weakens the immune system and increases the risk of complications associated with CF [8, 10, 14, 15]. Supplementation with high doses of vitamin E (as α -tocopherol or its acetate ester) has been shown to increase blood levels and reduce oxidative stress in patients with CF [16–18].

Because malabsorption, especially when associated with hepatobiliary complications, impairs normalization of vitamin E status in some patients with CF despite standard supplementation, there is a need for specially formulated products that overcome this nutrient malabsorption. It is preferable to formulate vitamin E supplements supplying all eight tocols (4 tocopherols and 4 tocotrienols) comprising vitamin E in foods because emerging research indicates that these tocol have unique antioxidant and other biologic effects. For example, γ -tocopherol, a dominant form in the diet, seems to neutralize nitrogen radicals effectively [19] and may be important for patients with CF because infections and inflammation increase substantially the formation of nitrogen radicals. Reactive nitrogen oxides species have been suggested as contributing factors to neurodegenerative diseases [20].

A novel, water-soluble vitamin E formulation, which uses a vehicle that can form micelle like particles and contains the entire vitamin E complex, has recently been made commercially available. We undertook a study to determine the pharmacokinetic profile of this water-soluble vitamin E formulation compared with the typical oil-based softgel formulation in a malabsorbing CF patient population.

Materials and methods

Study patients and protocol

Patients with cystic fibrosis who had documented malabsorption were recruited for participation in this pharmacokinetic study. Eligible patients included patients 7 to 35 years of age with an FEV₁ >35% and no recent hospitalizations (within 3 weeks). Patients were excluded if they had significant liver disease (AST, ALT, or GGT >2 × upper limit of normal), diabetes mellitus requiring insulin therapy, significant anemia (hemoglobin <9 gm/dl), were pregnant or lactating or had poor compliance with medical regimens as assessed by CF clinic care providers. Written, informed consent was obtained from the parent/guardian of minors and written assent was obtained from minors. The East Tennessee State University Institutional Review Board approved the study protocol and consent/assent forms.

Patients who met inclusion and exclusion criteria discontinued vitamin E supplementation, except for that in a multivitamin, for 7 to 21 days before the day of dosing. Patients were randomized to a single dose of 20 ml of “Aqua-E®” or three oil-based “softgels.” Twenty milliliters of Aqua-E and three softgels contained approximately equivalent amounts of γ -tocopherol (235 mg ± 5%) as well as total tocopherols as determined by two independent analyses. On Day 0, the physician performed a history and abbreviated physical exam, placed an IV, and drew a baseline blood sample. Patients were given a standardized breakfast as well as their pancreatic enzymes and had blood samples drawn at 2, 4, and 8 hr on Day 0. Patients returned on the morning of Days 1, 2, and 7 for fasting blood draws. At each subsequent visit an interval history, abbreviated physical exam, and monitoring for adverse events was conducted. After the Day 7 blood draw, patients were instructed to restart their previous vitamin regimen.

Vitamin E formulations

The Aqua-E manufactured for the clinical study was formulated to contain equivalent ratios of the tocopherols as present in the Vitamin E Factor® 100/100 (Yasoo Health, Johnson City, TN) softgel preparation. Both products contain natural tocopherol extracts, which are inherently variable in their composition from batch to batch. In addition, Aqua-E contains TPGS, a derivative of α -tocopherol. The analysis for α -tocopherol in Aqua-E requires a separate analysis for TPGS, which contributes a significant amount of α -tocopherol to the preparation. The analysis of TPGS introduces an additional source of variation in balancing the α -tocopherol content of the two forms. For these reasons, the target was to achieve dosing within a 10% difference based on duplicate analyses of the tocopherols in the preparations. The α -tocopherol

was the sum of the free α -tocopherol from these analyses plus the amount contributed by TPGS, which was analyzed by Eastman Chemical Company. The analytical results indicated that there was a <5% difference in the dosing of γ -tocopherol, the primary outcome (231 mg in 20 ml of Aqua-E and 228 mg in the 3 oil-based softgels). The analytical results showed that 20 ml of Aqua-E or three oil-based softgels contained: 281 mg and 245 mg of α -tocopherol, 68 mg and 69 mg of δ -tocopherol, and 586 mg and 559 mg of total tocopherols, respectively.

Tocopherol analysis

Aqua-E and softgel

Aqua-E and softgels were analyzed for tocopherol content by high-pressure liquid chromatography (HPLC). Samples were dissolved in DMSO and methanol and chromatographed in the reverse phase mode using a Aquasil C18 bonded phase column with a gradient consisting of acetonitrile and phosphoric acid. Detection is achieved photometrically at 290 nm. Concentrations are expressed as weight percentages against standards whose purity is verified by NMR. TPGS was analyzed by HPLC. Briefly, TPGS was extracted in doubly denatured ethanol. Samples were chromatographed on an Inertsil C8 HPLC column (Keystone Scientific, Inc.) with a gradient consisting of acetonitrile followed by isopropyl alcohol and water. Detection is achieved photometrically at 280 nm. Standards of the tocols were obtained from Merck KGaA (Darmstadt, Germany).

Blood analysis

Blood was obtained in pediatric tubes with EDTA. Samples were centrifuged and the plasma was removed and stored at -80°C . Plasma samples were shipped on dry ice to a third party contractor (Craft Technologies, Wilson, NC) for analysis of tocopherols.

Tocopherols were measured by HPLC. After thawing, 150- μl aliquots of plasma were diluted with 150 μl of water and deproteinated by vortexing with 300 μl of ethanol containing tocol as an internal standard and butylated hydroxytoluene (BHT) as an antioxidant. The samples were extracted twice with 1 ml of hexane and the combined supernatant was evaporated under nitrogen. The residue was dissolved with vortexing in 30 μl of ethyl acetate, was diluted with 100 μl of mobile phase, and ultrasonically agitated for 15 sec before placement in the autosampler. A 20- μl volume was injected.

The HPLC system consisted of a computer data system, an autosampler maintaining samples at 20°C , a column heater at 31°C , a programmable ultraviolet visible detector, and a fluorescence detector (Thermo Separation

Products, Fremont, CA). The separation was performed isocratically on a Spherisorb ODS2 column (3 μm , 4.6×150 mm). The mobile phase consisted of acetonitrile/dioxane/isopropanol/triethylamine (79.1/14.8/6/0.1) at a flow rate of 1.5 ml/min. The alcohol component contained 100 mM of ammonium acetate.

Linear calibration curves were prepared consisting of three concentrations of analytes, which spanned the physiologic levels of micronutrients in serum. The calibrants included α -, γ -, and δ -tocopherols. Quantitation was performed by internal standard calibration using peak area ratios.

Data analysis

Patient tocopherol measurements from times 0, 2, 4, 8, 24, 48, and 168 hr were used to determine the AUC (area under the curve over 168 hr). For each of α -, γ -, δ - and total tocopherols the Aqua-E and softgel group means were compared with the *t*-test (heterogeneous variance). The group means also were compared with analysis of covariance using subject weight as a covariate (Minitab Statistical Software, Release 13.31). The noncompartmental pharmacokinetic elimination rates were derived using a SAS software program described in the article "Noncompartmental Pharmacokinetics and Bioequivalence Analysis" by Arturo Soto Matos-Pita and Bernardo de Miguel Lillo. Group mean elimination rates were compared with the *t*-test. Probability levels ≤ 0.05 were used to indicate statistical significance.

Results

Eight patients were enrolled in the study and randomized to "Aqua-E" or "softgels." There were no withdrawals and no severe adverse events noted. Patient characteristics are summarized in Table 1. All patients were taking ADEK supplementation and pancreatic enzymes. The most common concomitant medications included albuterol, dornase alfa, and cromolyn sodium.

The bioavailability of the tocopherols, measured as AUC, are summarized in Table 2. The primary outcome, the bioavailability of γ -tocopherol in Aqua-E, was significantly greater than that of oil-based softgels (115 $\mu\text{g/ml*hr}$ vs. 25 $\mu\text{g/ml*hr}$; $P=0.013$) as shown in Figure 1. Total-tocopherols ($\alpha + \gamma + \delta$) in Aqua-E (AUC = 294 $\mu\text{g/ml*hr}$) showed a strong trend toward increased absorption compared to that of oil-based softgels (AUC = 294 $\mu\text{g/ml*hr}$ vs. 117 $\mu\text{g/ml*hr}$, $P=0.09$). α -Tocopherol (AUC = 179 $\mu\text{g/ml*hr}$) and δ -tocopherol (AUC = 10 $\mu\text{g/ml*hr}$) in Aqua-E also showed a trend toward greater absorption (AUC = 96 $\mu\text{g/ml*hr}$; $P=0.18$ and AUC = 4.5 $\mu\text{g/ml*hr}$, $P=0.24$ respectively). Adjustment for

Table 1 Patient characteristics

	Softgel (<i>n</i> = 4)	Aqua-E (<i>n</i> = 4)
Age (yr)	12.3 ± 4	10.5 ± 3
Weight (kg)	35.2 ± 10.3	29.7 ± 7.4
BMI (kg/m ²)	16.1 ± 0.6	16.7 ± 2.2
FEV ₁ (% predicted)	73% ± 8%	87% ± 10%
Creatinine (mg/dl)	0.5 ± 0.1	0.5 ± 0.1
Total bilirubin (mg/dl)	0.6 ± 0.4	0.5 ± 0.1
AST (units/l)	29 ± 11	28 ± 2
ALT (units/l)	25 ± 11	26 ± 5
Supplement use containing vitamin E	100%	100%
Pancreatic enzyme use	100%	100%

Note. BMI body mass index; FEV₁ forced expiratory capacity in 1 second; AST aspartate amino transferase; ALT amino alanine transferase.

Values are mean ± standard deviation.

weight of the patients did not affect the results for α -, γ - and total tocopherols. The weight adjusted covariance showed a borderline significance ($P = 0.053$) for δ -tocopherol.

Differences in the elimination rate constant of δ -tocopherol between Aqua-E ($K_{el} = 0.0198$) and the oil-based softgels ($K_{el} = 0.0165$) was not significant. Full pharmacokinetic modeling of both the absorption and elimination phases was not possible with the number of data points available.

Discussion

The results indicate that this water-soluble preparation of all eight tocopherols comprising vitamin E is more bioavailable than a lipid-soluble supplement in this malabsorbing population. Despite the small sample size of this pilot study, the difference in the absorption (AUC) of γ -tocopherol was statistically significant and approached significance for total tocopherols. This water-soluble preparation normalized both α -tocopherol and γ -tocopherol in patients who were deficient. This study is significant because studies have demonstrated repeated deficiencies despite aggressive supplementation. In addition, other water-dispersible formulations have not demonstrated and improved bioavailability of fat-soluble vitamins. The key difference of this preparation is the use of TPGS to achieve micelle-like particles.

The prevalence of multiple vitamin deficiencies has been documented in a large number of infants (45.8%) diagnosed with CF between ages 4 to 8 weeks [21]. Despite oral supple-

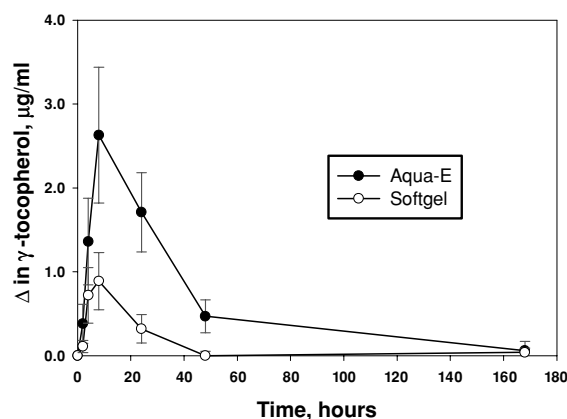


Fig. 1 Changes in γ -tocopherol plasma levels above baseline over time in patients receiving a single dose of Aqua-E or oil-based softgel (mean ± standard error of the mean).

mentation with vitamins A, D, E, and K or other multivitamins, recurrence of deficiencies was rather common (vitamin A, 11.1%; vitamin D, 12.5%; and vitamin E, 57.1%). Furthermore, those having normal vitamin status initially, developed deficiency states during the course of the study (vitamin A, 4.5%; vitamin D, 14.4%; and vitamin E, 11.8%). In a recent study, serum 25-hydroxyvitamin D levels in 109 of 134 patients were below the Cystic Fibrosis Foundation consensus treatment guidelines goal of 30 ng/ml and only 8 percent responded to the recommended repletion therapy course of 400,000 IU oral ergocalciferol. Furthermore, none of the 33 who completed a second course of 800,000 IU reached the serum vitamin D goals [22]. These studies underscore the need for a technology that improves the levels of fat-soluble micronutrients in cystic fibrosis.

Whereas water-soluble or water miscible forms can be formulated using different technologies, absorption may not be enhanced. Soltani-Frisk et al. [23] showed a nonsignificant decrease in the AUC of α -tocopherol with a water-miscible form of α -tocopheryl acetate compared with the fat-soluble form. Water-miscible preparations that use polysorbate 80 or other surfactants do not necessarily form micelles that are critical in enhancing the absorption of fat-soluble vitamins in those with some degree of biliary insufficiency.

TPGS, an amphiphilic compound that forms micelle-like particles, has been used to enhance intraluminal absorption of lipophilic compounds in conditions associated with malabsorption caused by incomplete micellar solubilization. In addition, TPGS may enhance bioavailability of lipophilic

Table 2 Area under the curve of tocopherol plasma levels

	Area under the curve ($\mu\text{g/ml}\cdot\text{hr}$)			
	γ -tocopherol	α -tocopherol	δ -tocopherol	Total tocopherols
Aqua-E (<i>n</i> = 4)	115 ± 19	179 ± 45	10.6 ± 4.1	294 ± 70
Softgels (<i>n</i> = 4)	25.3 ± 10	96 ± 29	4.5 ± 1	117 ± 39
	$P = 0.013$	$P = 0.18$	$P = 0.24$	$P = 0.09$

Note. Values are mean ± standard error of the mean.

compounds by inhibiting P-glycoprotein [24]. TPGS has been used to enhance absorption of poorly soluble drugs, such as cyclosporine or amprenavir (Agenerase® capsules). Argao et al. [25] showed that co-administration of TPGS and vitamin D₃ increased serum 25(OH)D in cholestatic patients.

The role of TPGS, however, in normalizing the vitamin E status in malabsorption may be greatly enhanced by utilizing its ability to achieve micellar solubilization and increase absorption of the natural tocopherols. In normal subjects, α -tocopherol may be more bioavailable than TPGS [26]. This suggests that it is preferable to enhance the absorption of the naturally occurring, unesterified tocopherols, especially for cystic fibrosis patients in whom the function of digestive enzymes, such as esterases may be suboptimal. Furthermore, TPGS supplies only α -tocopherol, one of the eight tocopherols comprising vitamin E in foods. Emerging research suggests important biologic functions of the other tocopherols and tocotrienols.

γ -Tocopherol, the major form of vitamin E in the diet in the United States, has been shown to have potent and beneficial antioxidant and nonantioxidant properties. It was found to be more effective than α -tocopherol in protecting lipids against peroxynitrite, a free radical formed from the reaction between nitric oxide released by macrophages and oxygen [19]. γ -Tocopherol and its physiologic metabolite 2, 7, 8-trimethyl-2-(beta-carboxyethyl)-6-hydroxychroman (γ -CEHC) inhibits cyclooxygenase-catalyzed synthesis of prostaglandin E₂ (PGE₂) in activated macrophages and epithelial cells [27]. Furthermore, γ -tocopherol, but not α -tocopherol, decreases proinflammatory eicosanoids and inflammation damage in rats [28]. In hemodialysis patients, administration of γ -enriched but not α -enriched mixed tocopherols lowered median C-reactive protein [29]. Other studies suggest a role of γ -tocopherol in reducing the risk of prostate cancer and neurodegenerative diseases [30, 31]. These properties of γ -tocopherol, especially as they relate to inflammation, merit further evaluation in cystic fibrosis, which is associated with oxidative and inflammatory stress. Tocotrienols have been receiving attention for various biologic effects, including atherosclerosis [32], suppression of some tumors [33], and protection of neuronal cells from glutamate induced death [34].

Large doses of α -tocopherol have been shown to deplete other tocopherols and tocotrienols and specifically γ -tocopherol [35, 36]. This imbalance, which occurs in normal subjects, may be further exacerbated in patients with cystic fibrosis by supplying large amounts of absorbable α -tocopherol only. Although the effects of such imbalance are not understood, the increasing evidence on the biological function of the other tocopherols and tocotrienols suggests that it is preferable to provide supplements which contain a tocopherol profile similar to foods. In this study, plasma levels of γ -tocopherol were significantly lower than values found

in normal subjects in seven of eight patients, whereas α -tocopherol levels were borderline or in the low normal range in all patients. The lower relative levels of γ -tocopherol may be partly the result of receiving supplements of α -tocopherol, although differences in diet in this population or other factors cannot be excluded.

In conclusion, this water-soluble formulation is significantly more effective than fat-soluble forms in normalizing vitamin E status in malabsorbing cystic fibrosis and especially in providing the complete vitamin E complex of natural tocopherols plus tocotrienols as found in foods.

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