Internat. J. Vit. Nutr. Res., 69 (2), 1999, 92–95

Relative Bioavailabilities of Natural and Synthetic Vitamin E Formulations Containing Mixed Tocopherols in Human Subjects

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Received for publication: July 16, 1998

Abstract: There are several reports in the literature on the relative bioavailabilities of *RRR* (natural) vs. *all-rac* (synthetic) forms of vitamin E in humans and animal models but none on the bioavailability of α-tocopherol in mixed vitamin E formulations. In the present study we examined the bioavailability of α-tocopherol in a typical commercially available product containing mixed tocopherols. We also tested a formulation containing all-rac-α-tocopherol with mixed tocopherols for purposes of comparison along with straight *RRR*-and *all-rac*-α-tocopheryl acetate as reference products. Normal male subjects were given one of the four formulations of vitamin E (800 IU per day in softgel capsule form for 10 days): 1. All-rac-α-tocopheryl acetate, 2. *RRR*-α-tocopheryl acetate, 3. *RRR*-α-tocopherol with mixed tocopherols, and 4. all-rac-α-tocopherol with mixed tocopherols. Both serum α- and γ-tocopherols were determined by HPLC at baseline, and at days 2, 4, 7 and 10. The values for α- at baseline and 10 days were 0.80, 0.80, 0.80 & 0.79 mg/dl and 1.67, 1.72, 1.76 & 1.62 mg/dl. The values for γ- were 0.28, 0.29, 0.30 & 0.29 mg/dl and 0.11, 0.08, 0.10 & 0.10 mg/dl. Thus the data show that a) the bioavailability of *RRR*-and *all-rac*-α-tocopherols is not affected by other tocopherols, and b) both *RRR*-and all-rac-α-tocopherol (free or esterified) significantly suppress the serum gamma tocopherol to the same extent. Furthermore, since there was no difference in the serum values of α-tocopherol between *RRR*-and all-rac-vitamin E given the same dose as IUs, the data also support the currently accepted ratio of 1.36 for the biopotency of *RRR*- vs. *all-rac*-α-tocopheryl acetate.

Key words: Natural vitamin E, synthetic vitamin E, formulations, mixed tocopherols, bioavailability

Introduction

Vitamin E as α-tocopherol functions as a major lipid-soluble antioxidant in mammalian systems. Its importance in human health has been well recognized in recent years, especially with respect to heart disease [1–3]. Vitamin E supplements have therefore become increasingly popular. Commercially available preparations of vitamin E are based upon either the synthetic (called *all-rac-* for all racemic, formerly *dl-*) or the natural (*RRR-*, formerly d-) forms. In addition, there are products containing *RRR*-α-tocopherol with mixed tocopherols. The synthetic contains an equimolar mixture of eight stereoisomers of α-tocopherol or α-tocopheryl acetate. While there are several reports in the literature comparing the bioavailabilities of natural and synthetic forms of vitamin E in human subjects, none have examined the bioavailability of vitamin E in products which also contain mixed tocopherols.

We therefore carried out a study to examine the relative bioavailability of α-tocopherol in a typical mixed vitamin E formulation based on *RRR*-α-tocopherol in human subjects. We also tested a mixed vitamin E formulation based on *all-rac*-α-tocopherol for purposes of comparison. Preliminary results were presented at the recent Experimental Biology meeting [4].

Materials and Methods

Subjects: Healthy male subjects, 24–45 years of age, were recruited for the study. The entry criteria included no smoking, no alcohol, no medication of any type and no vitamin or nutritional supplements. Furthermore, only those with a fasting plasma α-tocopherol value of 1.0 mg/dl or lower were entered. This was a randomized double-blind study.

Products tested: The subjects were divided into four groups of six subjects each as follows:

Group 1: Vitamin E as *all-rac*-α-tocopherol acetate
Group 2: Vitamin E as *RRR*-α-tocopheryl acetate
Group 3: Vitamin E as *RRR*-α-tocopherol with mixed tocopherols
Group 4: Vitamin E as *all-rac*-α-tocopherol with mixed tocopherols

The first three were commercially available products in the form of softgel capsules containing 400 IU of vitamin E per capsule. The fourth product was custom-made for this study to match Group 3 and contained, in addition to *all-rac*-α-tocopherol, *RRR*-α-tocopherol (8 mg), γ-tocopherol (26.7 mg) and δ-tocopherol (12.3 mg) for a total of 400 IU of vitamin E per capsule.

Dosage: The dosage was 800 IU a day (as α-tocopherol, 2 capsules of 400 IU each taken as a single dose) for 10 days.

Bioavailability study: The study was carried out at the New York Medical College OLM Medical Center in Bronx, NY. A fasting blood sample was drawn during the initial screening of the subjects. Following selection, a second sample was obtained for baseline plasma vitamin E analysis. The subjects were then given the dose of vitamin E to ingest in the presence of the study coordinator along with a standard breakfast. This process was repeated daily for 10 days. Additional blood samples were drawn on days 2, 4, 7 and 10. Serum was separated and frozen immediately at –85C. Both serum α- and γ-tocopherols were analyzed by reversed-phase HPLC within two weeks after sample collection [5]. Serum cholesterol was also determined using the Sigma kit [6].

Data analysis: Statistical analysis of the data was carried out using ANOVA (analysis of variance) and t-tests [7]. The area under the curve (AUC) for serum α-tocopherol, calculated by the trapezoidal rule, was used as a measure of relative bioavailability. The mean AUC following vitamin E supplementation was derived from the following equation

$$\sum_{i=1}^{n} (t_i - t_{i-1}) ([v_i + \sqrt{}_{i-1}] / 2 - v_0)$$

where t refers to the day following start of supplementation, i refers to the specific sampling period, v equals serum α-tocopherol value at t, and n refers to the number of data points during supplementation. The variable v at period one is the arithmetic mean of two baseline measurements.

Results

Serum vitamin E: The mean serum α-tocopherol values for the four groups at baseline and after 10 days of supplementation with vitamin E are shown in Table I. The baseline values were almost identical at 0.79–0.80 mg/dl. At the end of 10 days, the values had risen to more than double the baseline and ranged from 1.62–1.76 mg/dl with no significant difference between the groups.

Table II shows the serum γ-tocopherol data. The baseline values ranged from 0.28–0.30 mg/dl and after 10 days of supplementation there was a marked decrease ranging

Table I: Mean serum α-tocopherol values at baseline and after 10 days

	Serum α-tocopherol (mg/dl)*				
Group	Day 0	Day 2	Day 4	Day 7	Day 10
1.	0.80 ± 0.12	0.92 ± 0.13	1.22 ± 0.22	1.46 ± 0.29	1.67 ± 0.36
2.	0.80 ± 0.12	0.94 ± 0.12	1.31 ± 0.14	1.52 ± 0.24	1.72 ± 0.29
3.	0.80 ± 0.14	0.96 ± 0.20	1.28 ± 0.34	1.54 ± 0.52	1.76 ± 0.73
4.	0.79 ± 0.14	0.96 ± 0.21	1.21 ± 0.32	1.44 ± 0.43	1.62 ± 0.50

* Mean ± standard deviation

Table II: Mean serum γ-tocopherol values at baseline and after 10 days

Group	Serum γ-tocopherol (mg/dl)* Day 0	Day 2	Day 4	Day 7	Day 10
1.	0.28 ± 0.09	0.25 ± 0.08	0.23 ± 0.09	0.14 ± 0.07	0.11 ± 0.09
2.	0.29 ± 0.09	0.27 ± 0.08	0.21 ± 0.07	0.15 ± 0.06	0.08 ± 0.04
3.	0.30 ± 0.10	0.27 ± 0.08	0.22 ± 0.07	0.14 ± 0.07	0.10 ± 0.05
4.	0.29 ± 0.11	0.26 ± 0.10	0.18 ± 0.08	0.12 ± 0.06	0.10 ± 0.05

* Mean ± standard deviation

from 0.08–0.11 mg/dl, with no significant differences between the four groups.

Area under the serum vitamin E curve (AUC): The AUC data for both α- and γ-tocopherols following vitamin E supplementation (corrected for the baseline values) are shown in Table III. There was no significant difference between the groups for either α- or γ-tocopherol. The negative values for γ are due to a decline from baseline values during the supplementation period.

Serum cholesterol: The serum cholesterol values remained essentially constant (and in the normal range) during the 10-day vitamin E supplementation period in all the four groups (Table IV). Since there was practically no change in the serum cholesterol values as well as in the tocopherol values, the vitamin E/cholesterol ratios are not shown.

Table III: Mean area under the curve (AUC) for serum α- and γ-tocopherols

Group	α-Tocopherol AUC (mg/dl)	×	γ-Tocopherol (time in days)*
1.	4.556 ± 1.312		–0.856 ± 0.404
2.	5.048 ± 1.430		–1.025 ± 0.383
3.	5.143 ± 3.048		–1.006 ± 0.392
4.	5.528 ± 2.313		–0.987 ± 0.446

Mean ± standard deviation

Table IV: Mean serum cholesterol at baseline and after 10 days

Group	Serum cholesterol (mg/ml)* Baseline	10 days
1.	188 ± 26	188 ± 25
2.	184 ± 34	181 ± 30
3.	183 ± 23	182 ± 22
4.	186 ± 25	188 ± 24

Mean ± standard deviation

Discussion

Since there was no difference in the serum α- tocopherol values following 10 days of supplementation or in the serum α- tocopherol AUC values between the four treatment groups, this demonstrates that the four vitamin E products have comparable or equal relative bioavailability. These data also show that the bioavailability of α-tocopherol from the vitamin E products is not influenced by the presence of other tocopherols (primarily γ) at the dosages used in this study.

Both α-tocopherol or α-tocopheryl acetate in high doses were found to suppress serum γ-tocopherol, and this is consistent with previous observations [8–11]. There was a reciprocal relationship between serum α- and γ-tocopherols. As serum α-tocopherol values increased, γ-tocopherol values showed a corresponding decrease. One explanation for this effect is the preferential secretion of α- by the liver into plasma [12] although competition for uptake at the intestinal mucosal level cannot be excluded. The presence of mixed tocopherols with a substantial amount of γ-tocopherol (53.4 mg) had no effect on this phenomenon. It is possible that much higher amounts of γ, perhaps in the equimolar range, might be necessary to counteract the effect of α-tocopherol. Also, it would be interesting to examine whether high doses of γ-tocopherol would have any effect on the absorption of α-tocopherol.

The terms bioavailability, biological activity and biopotency are often used interchangeably in the literature dealing with the absorption of vitamin E. Bioavailability is the rate and extent of a substance from a dosage form to reach circulation whereas biological activity or biopotency refers to a quantifiable biological effect such as fetal resorption and serum pyruvate kinase activity used in the functional bioassay for vitamin E using rats.

Earlier studies based primarily upon rat bioassays had clearly established the biopotency ratio of *RRR*-tocopheryl acetate to *all-rac*-tocopheryl acetate as 1.36 [13, 14]. However, in a few recent studies a pharmacokinetic approach was employed using deuterated tocopherol compounds with a very limited number of subjects, and the data indicated a bioavailability ratio of *RRR*- to *all-rac*- greater than

1.36 [12, 15–17]. This same ratio was presumed to reflect their differences in biopotency as well.

In the present study, the dosage of vitamin E was based on biopotencies in terms of IUs rather than their actual weights (in mg) and was exactly the same in all the four groups, and therefore the lack of difference in the relative bioavailabilities also confirms the currently accepted biopotencies for *RRR*- and *all-rac* vitamin E (1.36 IU/mg and 1.00 IU/mg for α-tocopheryl acetate and 1.49 and 1.10 IU/mg for α-tocopherol, respectively) under the conditions of the study. This is consistent with a similar observation made previously comparing *RRR*- with *all-rac*-α-tocopheryl acetate in human subjects [11].

Acknowledgement

The authors express their appreciation to Dr. Edward P. Norkus, New York Medical College, Our Lady of Mercy Medical Center, Bronx, NY for his help with the human study and to Ms. Nancy Staron for her help in the preparation of the manuscript.

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