


Evaluation of vitamin E potency

Dear Sir:

Vitamin E (α-tocopherol) is available as both RRR-α-tocopheryl acetate (RRR-α-TA) and all-rac-α-tocopheryl acetate (all-rac-α-TA). Differences in biological activity of these preparations are expressed by an officially accepted potency factor, according to which RRR-α-TA is 1.36 times as potent as all-rac-α-TA (1). Burton et al (2) suggested deriving the potency factor from the relative availability of RRR- and all-rac-α-tocopherol and concluded that the ratio should be closer to 2:1. In most of their studies, an equimolar mixture of d3-RRR-α-TA and d6-all-rac-α-TA was ingested before biokinetics in human plasma and tissue concentrations in patients were monitored. Single and multiple dosing studies with comparatively high loads of α-TA were carried out. Relative availability was expressed as the ratio of d3-α-tocopherol to d6-α-tocopherol. It seems premature to conclude that the ratio is 2:1, however, because of the following 2 unresolved issues.

1) Is relative bioavailability an indicator of biopotency? The weight potency factor of vitamin E is a relative measure of the functional activity of α-tocopherol and its homologues and stereoisomers. Potency reflects the functional response of a compound and usually is expressed in terms of the EC50 (concentration or dose of an effector that produces 50% of the maximal possible effect). The weight potency factor for RRR-α-TA is the ratio of the EC50 of RRR-α-TA to the EC50 of all-rac-α-TA and was assessed by the resorption-gestation assay in rats. Assessment of the weight-potency ratio included administering and testing either RRR-α-TA or all-rac-α-TA, a design denoted as the 2-independent-experiments approach. Additional assays based on the prevention or cure of muscular dystrophy in rats, chicks, and rabbits and on the prevention of encephalomalacia in chicks provided almost identical values for the factor. I appreciate the difficulties of conducting similar studies in humans.

Availability of the active compound at the target site is a prerequisite for its efficacy, and therefore potency is dependent on availability. Concentrations in tissues and plasma may be easily related to biopotency, provided the relation between concentration and effect can be described linearly. However, a sigmoidal concentration-effect response curve restricts proportionality to a range of intermediate concentrations only. At high doses, absorption or transport usually becomes a limiting factor and as a result availability does not parallel potency. Thus, changes in bioavailability can result in varying efficacy. Such data are not meaningful for the assessment of potency, which is defined as a property of the compound. Moreover, the functional dose response of the individual α-tocopherol stereoisomers was found to be synergistic and not additive (3). These synergistic effects are probably difficult to relate to availability. Obviously, several factors may complicate the evaluation of biopotency from data on relative bioavailability and appropriate validation of such an approach is mandatory.

2) Is the method valid for assessing relative availability? Burton et al (2) concluded that bioavailability of all-rac-α-tocopherol is roughly half that of RRR-α-tocopherol, at least in the long term. However, the new method provided a range of values for relative availability because the d3-d6 ratio changed with time from ~1.5 to 2.0.

The classical approach to assessing relative availability is based on 2 separate applications: 1 for the test and 1 for the reference compound (4). Therefore, classic relative availability has to coincide with availability as determined by the competitive dosing approach, thereby demonstrating the validity of the new approach. For RRR-α-TA and all-rac-α-TA, both approaches would be expected to provide identical relative availabilities under conditions of first-order (or dose linear) kinetics.

There are, however, several indications in the paper that the tocopherol plasma kinetics were not linear (eg, lack of dose proportionality in the multiple dosing experiments, washout kinetics of unlabeled tocopherol after administration of high doses of labeled tocopherol, and variation of the d3-d6 ratio with time after multiple dosing). These findings are not surprising because at high doses nonlinear kinetics would be expected for a system exerting biodiscrimination. Stereoselectivity is usually mediated by proteins displaying binding saturation. For the discrimination of the 8 stereoisomers of α-tocopherol, the hepatic tocopherol transfer protein (TTP) is assumed to play a major role in adjusting plasma and tissue tocopherol concentrations (5). I agree with Burton et al (2) that the configuration at the C-2 position of the molecule is the major determinant of biological differences between the α-tocopherol stereoisomers. Discrimination between 2S and 2R forms of α-tocopherol is assumed to take place because of lower binding affinity of the 2S forms to TTP (6). Thus, presentation of both RRR- and SRR-α-tocopherol to TTP will result in preferential binding of RRR-α-tocopherol. Increasing the relative abundance of RRR-α-tocopherol will lower the probability of SRR-α-tocopherol binding to TTP, thereby enhancing the probability for SRR-α-tocopherol discrimination.

The dose administered in the studies by Burton et al (2) would contain 50% d3-2R-α-TA, 25% d3-2R-α-TA, and 25% d6-2S-α-tocopherol, so that 75% of the 2R forms would compete with 25% of the 2S forms (ratio of 3:1) for TTP binding. By contrast, if the 2 compounds were given separately, administration of all-rac-α-TA would result in 50% of the 2R forms competing with 50% of the 2S forms (ratio of 1:1). Thus, because of the relatively high abundance of the 2R forms when the 2 forms are given simultaneously, the 2R forms would compete more effectively and bioavailability of the 2S forms would be underestimated compared with that in experiments in which the compounds are given independently.

Because binding to TTP is saturable and stereoselective, the competitive dosing approach is not applicable unless tracer doses are used. At high doses the competitive dosing approach will tend to overestimate relative availability. I agree, however, with the authors that new methods have to be developed to reassess the potency factor in humans, perhaps based on an enhanced understanding of vitamin E function. Nevertheless, revision of
the currently accepted potency factor has to rely on an appropriate and validated method.

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REFERENCES

Reply to W Cohn

Dear Sir:

In reference to our recent publication (1), Cohn questions the validity of our method of measuring relative bioavailability of natural (RRR) and synthetic (all-rac) \alpha\-tocopherols in human tissues and whether our results are predictive of relative biopotency. In particular, Cohn states that giving both forms of vitamin E together in equal amounts, providing 75% 2R and 25% 2S forms, could overestimate the relative bioavailability of the 2R forms.

Cohn suggests that interactions between all-rac-\alpha\-tocopherol stereoisomers (eg, synergism) could invalidate the competitive method. However, it must be recognized that for many people supplementation with all-rac-\alpha\-tocopheryl acetate will itself be an uncontrolled, competitive exercise, carried out under conditions that are quite different from those used to determine animal biopotency. That is, the all-rac-\alpha\-tocopherol stereoisomers must compete with an unknown and variable amount of circulating, endogenous RRR-\alpha\-tocopherol, making it difficult to see how there could be a useful bioavailability ratio if the interactions are significant. We agree that validation of our method is important and welcome this opportunity to provide additional supporting evidence.

The official biopotency ratio for the acetate esters of natural and synthetic \alpha\-tocopherol (RRR-\alpha\-tocopherol acetate:all-rac-\alpha\-tocopheryl acetate = 1.36) is based on a small number of biological responses (primarily fetal gestation-resorption, as well as muscular dystrophy and encephalomalacia) measured in assays in a few species of vitamin E-deficient animals (rats, chicks, and rabbits). The general similarity between the different assay results, coupled with support from some high-dose, short-term bioavailability studies in humans, has been taken as evidence that a single number can adequately represent the relative biopotency of natural and synthetic vitamin E for most, if not all, animal species, including humans. Our recent study (1) provides evidence that the currently accepted biopotency value may significantly underestimate tissue bioavailability in humans, at least over the long term (ie, 1–2 y and beyond).

We applied the competitive dosing technique in a companion study of relative bioavailability in male rats (N Hidiroglou, DO Foster, GW Burton, KU Ingold, unpublished observations, 1989). Concentrations of RRR-\alpha\-tocopherol and all-rac-\alpha\-tocopherol were measured at various times in plasma and tissues of rats fed exclusively a 1:1 mixture of the acetates (at a relatively high dose of 36 mg/kg diet) over a 5-mo period. The ratio of RRR-\alpha\-tocopherol to all-rac-\alpha\-tocopherol (RRR:rac; averaged over 10 animals) varied little between tissues (except liver and plasma) and over time: 1.40 ± 0.06 in brain, 1.46 ± 0.06 in heart, 1.36 ± 0.07 in kidney, 1.47 ± 0.06 in lung, 1.46 ± 0.07 in muscle, 1.41 ± 0.07 in testis, 1.16 ± 0.06 in liver, and 1.62 ± 0.08 in plasma.

Recently, the Hoffmann–La Roche group published the results of their application of chiral chromatographic methods to determine the relative concentrations of all 8 stereoisomers of all-rac-\alpha\-tocopherol in plasma and several tissues of vitamin E-deficient female rats dosed only with all-rac-\alpha\-tocopheryl acetate daily for ≤3 mo (2). Averaging their calculated RRR:rac over time gives 1.38 for brain, 1.41 for adipose tissue, 1.18 for liver, and 1.61 for plasma. Clearly, there is good agreement between the 2 methods when comparisons are possible, despite the fact that, as Cohn remarked (1), the 2R:2S dose ratio is 3:1 in our competitive method and 1:1 in the traditional method. Thus, the suggestion that the competitive method overestimates the relative RRR:rac bioavailability is not supported by these experimental findings.

Furthermore, it is apparent that the relative bioavailability values in nonhepatic rat tissues are all close to rat biopotency values [eg, 1.26–1.41 for fetal resorption and 1.43–1.52 for myopathy (3)]. The closeness of these tissue bioavailability ratios to the biopotency ratios is consistent with bioavailability being the dominant cause of the difference in biopotency in rats. This strengthens the observation of Weiser et al (2), with respect to their own findings, that “the results are in agreement with the hypothesis that for \alpha\-tocopherol stereoisomers, biopotency differences are related to corresponding differences of \alpha\-tocopherol concentrations.”

In rats the maximum RRR:rac is observed in plasma (1.5–1.7), in both continuous feeding and single-dose, competitive studies, and tissue values do not exceed ≈1.5 over a period of ≥5 mo of continuous dosing. In humans, the ratio in plasma starts at ≈1.5 during dosing and rises to a limiting value of ≈2 several days after dosing, regardless of the total dose (30, 150, or 300 mg/d) and dosing period, and is maintained over a wide range of plasma concentrations (up to the analytic sensitivity of the method, ≈3 mo after 8 continuous days of dosing at 300 mg/d) (1). Nonhepatic tissue RRR:rac values (≈1.4–1.6) after short-term dosing (≤5 wk) generally reflect the lower plasma values observed during dosing and the proportion of labeled \alpha\-tocopherol greatly lags behind the corresponding proportion in plasma. In contrast, the results obtained from 2 terminally ill patients indicate, especially for the patient receiving 300 mg/d for almost 2 y, a long-term trend toward equilibration of the labeled \alpha\-tocopherol between plasma and tissues, with regard both to the RRR:rac, which tends to a maximum value of ≈2, and to the fraction of labeled \alpha\-tocopherol. Also noteworthy is