Effects of Elevated d-Alpha(RRR)-Tocopherol Dosage in Man

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Summary  A study was conducted to investigate the effects of a megadosage of free RRR-alpha-tocopherol in healthy college student volunteers. Of 19 volunteers, 14 were given daily doses of 600 mg (900 IU) of RRR-alpha-tocopherol for 12 weeks, and the remaining 5 were given identical placebo capsules. The investigation was performed by the single-blind method. Alpha-tocopherol levels were measured in plasma, red blood cells (RBCs), platelets, leucocytes (WBCs), and buccal mucosal cells. Alpha-tocopherol in plasma, RBCs, and WBCs rose, reached a maximum level 4 weeks after commencement of administration, and then remained at a plateau, while platelet and buccal cell levels reached a maximum level after 12 weeks of administration. The maximum levels in all the subjects were 2.5 to 3 times the baseline values. During the study, there were no changes in laboratory values for thyroid, liver, or kidney functions, and coagulation activity (including the vitamin K-dependent Hepaplastin test and PIVKA-II) or immunoglobulin levels. Healthy status continued without any abnormal symptoms, and without any subjective complaints on the questionnaire. In the control group also, no changes occurred during the investigation. Gamma-tocopherol changes were measured in plasma and RBCs. As plasma and RBC tocopherol levels rose after administration, the isomer levels were suppressed in both plasma and RBCs.

Key Words  d-alpha-tocopherol, side effects of vitamin E, plasma, erythrocytes, platelets, buccal mucosal cells

Magadose administration of vitamin E has been advocated to promote general well-being and prevent ischemic diseases of the heart and brain, and further to prevent aging. Large dosages of vitamin E preparations have thus been prescribed for treatment and prevention of recurrent aging diseases in medical clinics. However, vitamin E preparations generally used in Japanese clinics are all-rac(formerly dl)-alpha-tocopheryl esters such as the acetate, nicotinate, or succinate. Free RRR(formerly d)-alpha-tocopherol, which naturally occurs, is the most active

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among any tocopherol derivatives (1, 2) and there have been several reports in which the RRR form of tocopherol is more effective than the all-rac forms, especially than their esters, on the basis of the findings of elevated levels in plasma (3) and RBCs (4) after the administrations. However, there are no preparations of the free form of RRR-alpha-tocopherol for clinical use in Japan. Recently, there has been much interest in the clinical use of the elevated dosages (5) for the therapy of vitamin E deficiency resulting from chronic cholestatic liver diseases, abetalipoproteinemia, and hereditary anemia with hemoglobinopathies or enzymopathies, and for prevention of aging diseases. If RRR-alpha-tocopherol is biologically more active than the derivatives, it should be determined whether or not there are any hazard in its customary use at elevated dosages and for a long term. There have been several reports of undesirable effects of long-term extremely high dosages (6–10); they included development of general muscular weakness, lengthened prothrombin time, and a bleeding problem in vitamin K-deficient individuals, possible elevation of serum lipids, and reduced thyroid functions. But the bulk of evidence of date indicates that a daily intake in the range of 200 to 600 mg, which were all-rac-alpha-tocopheryl acetate, is innocuous in most people (11, 12). The present study examined bioavailability and potential hazard of an elevated dosage of a preparation of free form of RRR-alpha-tocopherol in healthy young adults.

MATERIALS AND METHODS

Preparation of RRR-alpha-tocopherol. Soft capsules, each of which was exactly filled with 100 mg of RRR-alpha-tocopherol and cotton seed oil, were provided by Eisai Co., Ltd. Identical placebo capsules were also provided. All capsules were coated with clear soft gelatin and kept in shading envelopes until use.

Subjects. Nineteen healthy male college student volunteers, aged 24 to 25 years old, were enrolled in this study after a healthy check. They were fully informed of the objectives and the procedure of the study at the beginning of the trials. The ethics committee at the hospital approved the study protocol. Fourteen of them were randomly assigned to the vitamin treatment group, while the five others served as the placebo group.

Experiment. Each subject was asked to take a total of 600 mg (900 IU) of RRR-alpha-tocopherol daily, in three equal doses (i.e. each dose = two 100 mg capsules) with meals, for 3 months. The control subjects received identical placebo capsules. The study was performed with a single-blind method. No subjects had any access to information of treatment assignment. The subjects had no limitation on living and food habits during the experiment. No effort was made to control or to measure nutrient intakes of subjects during the experiment expect for the avoidance of other supplementary or medicinal sources of vitamin E. The experiment for intake took place over a period of 3 months from April 15 to July 14, 1987. A fasting blood samples was taken by venipuncture at 8:00 a.m. at the beginning (day 0) and at one month, 2 months, and the end of intake (3 months). Additional blood
samples were taken at 3, 7, and 28 days after the termination of supplementation. Each subject was also asked to complete a simple questionnaire for subjective evaluation of treatment effects and other information. The questionnaire included fatigue, muscle weakness, gastrointestinal discomfort (including appetite loss, nausea, diarrhoea, and abdominal distress), headache, and abnormal vision.

**Laboratory analysis.** After an overnight fasting, 15 ml of heparinized blood was drawn, of which 3 ml was used for the analysis of tocopherol and lipid. The samples were centrifuged at 190 \( \times g \) for 10 min and then the platelet-rich plasma was obtained from the upper layer. The platelet-rich plasma was centrifuged at 800 \( \times g \) for 10 min to obtain the platelet pellet, which was washed twice with 10 ml of physiological saline. The sediment was then resuspended with 0.7 ml of 0.15M phosphate-buffered saline (pH 7.4), and sonicated at 20 Kc for 2 min. For the analysis of tocopherol, 0.5 ml of the suspension was used, and 0.1 ml of the remainder was used for protein assay. From the bottom layer, plasma and RBCs were separated by centrifugation at 1,600 \( \times g \) for 10 min. The RBC layer was taken out, from which RBCs were washed with three 10 ml portion of physiological saline. After the last washing, packed RBCs were resuspended by the same volume of phosphate-buffered saline (pH 7.4, 0.15M) and used for the analysis of tocopherol and hematocrit determination, as described in a previous paper (13).

From the 5 ml of heparinized blood, white blood cells were isolated by dextran sedimentation and hypotonic lysis of erythrocytes, by the modification of Henson’s method (14). The cells which contained 65–95% polymorphonuclear cells were suspended in Hank’s balanced salt solution.

Using the remaining blood, the cell counting was performed by an auto cell counter, and determination of cholesterol, triglycerides, phospholipids, aspartate ketoglutarate transaminase (GOT), and pyruvate ketoglutarate transaminase (GPT), creatinine phosphokinase (CPK), and blood urea nitrogen (BUN) was undertaken using an autoanalyzer. Plasma total lipids were estimated by addition of the three major lipids—total cholesterol, phospholipids, and triglycerides. In addition, thyroid hormones (T3, T4, and TSH), and prothrombin time, partial thrombin time, and “Hepaplastin test” as coagulation parameters, all of which were routinely determined in the laboratory center in our university hospital, were assayed. PIVKA (protein induced by vitamin K absence or antagonist)-II was determined with a commercial kit (Eisai Co., Ltd., Tokyo).

Besides analysis of blood, tocopherol contents in buccal mucosal cells were determined as described in a previous paper (15).

**RESULTS**

1. **Vitamin E status in parameters of plasma-, RBC-, platelet-, WBC-, and buccal cell-alpha-tocopherol levels**

Plasma and RBC alpha-tocopherol levels before supplementation were about 800 \( \mu g/100 \text{ ml} \) and 200 \( \mu g/100 \text{ ml} \) packed cells, respectively, which both were a
normal standard level in healthy adults as previously reported (16). In the experimental group, alpha-tocopherol levels in plasma and RBCs reached maximum levels after 4 weeks of supplementation, and continued at plateau levels thereafter, even in additional supplementation (Fig. 1). During the elevation of plasma and RBC tocopherol levels, gamma-tocopherol levels were drastically suppressed in both plasma and RBCs (Fig. 2). Particularly, gamma-tocopherol in RBCs decreased to an undetectable level during the elevation of their alpha-tocopherol levels. The elevated levels of plasma and RBC alpha-tocopherol rapidly decreased and returned to the baseline level at 7 days after the termination of treatment. The suppressed level of this isomer in plasma returned almost to the baseline levels at 28 days after the termination, while the RBC gamma-tocopherol level was still suppressed at 28 days after the termination. In the control group, there were no changes in alpha- and gamma-tocopherol levels in plasma RBCs throughout the experimental period.

Alpha-tocopherol levels in WBCs were also almost in parallel with the changes in those of plasma and RBCs. The tocopherol levels in plasma, RBCs, and WBCs
Fig. 2. Changes in gamma-tocopherol concentrations in plasma and RBCs after daily administration of 600 mg (900 IU) of RRR-tocopherol for 12 weeks in healthy male adults. Symbols are the same as in Fig. 1 legend.

increased to 2.5 to 3 times that of the baseline after the supplementation, without any further increase above these limited levels (Fig. 1). Determination of WBC tocopherol after the cessation of the administration was not done.

In platelets and buccal mucosal cells, the alpha-tocopherol levels elevated with duration of the supplementation, and after 8 and 12 weeks of the supplementation they reached a maximum level, respectively, which was also 2.5 or 3 times greater than the baseline levels (Fig. 1). Platelet alpha-tocopherol levels returned to the baseline levels 7 days later after cessation of supplementation, while alpha-tocopherol concentrations in buccal mucosal cells remained at elevated levels even one month later.

The plasma tocopherol level based on plasma total lipids (tocopherol/lipid ratio), which has been proposed by Horwitt et al. (17) as an index of nutritional status of vitamin E routinely available for clinical use, is also shown in Fig. 1. The changes in this ratio were in parallel with plasma tocopherol levels because of no changes occurring in plasma lipids with tocopherol supplementation.

2. Effect on laboratory parameters

The laboratory parameters before and after the supplementation are listed in Table 1. There were no significant changes in blood cell counts, and the values of GOT, GPT, CPK, and BUN. Immune globulins also did not alter after the supplementation, and the thyroid functions were neither suppressed nor enhanced, on the basis of the finding of T3, T4, and TSH. Blood clotting functions as shown in the values of prothrombin time, partial thrombin time, Hepaplastin test, and
Table 1. Results of selected clinical screening tests in subjects receiving daily doses of
600 mg (900 IU) of RRR-tocopherol for 12 weeks.

|                     | Control                  | Vitamin E                  |
|---------------------|--------------------------|----------------------------|
|                     | Before (0 day)           | After (12 weeks)           | Before (0 day)           | After (12 weeks)           |
| RBC count ($\times 10^4$) | 517±33                   | 519±38                     | 509±19                   | 511±19                     |
| Hemoglobin (g/dl)    | 15.9±1.1                 | 16.2±1.4                   | 15.4±0.6                 | 15.7±0.6                   |
| WBC count ($\times 10^3$) | 4.07±0.67               | 4.87±0.43                  | 5.17±1.81                | 5.89±1.57                  |
| Platelet count ($\times 10^4$) | 26.3±1.9             | 25.5±2.9                   | 26.7±5.7                 | 25.9±5.5                   |
| GOT (IU/ml)          | 18±5                     | 16±3                       | 15±3                     | 13±2                       |
| GPT (IU/ml)          | 25±15                    | 17±5                       | 13±9                     | 11±5                       |
| CPK (IU/ml)          | 159±106                  | 131±57                     | 129±96                   | 135±76                     |
| BUN (mg/dl)          | 14±3.0                   | 14±1.0                     | 14±3.0                   | 14±4.0                     |
| Cholesterol (mg/dl)  | 180±10                   | 168±24                     | 166±26                   | 175±19                     |
| Phospholipids (mg/dl) | 191±9                   | 191±17                     | 189±27                   | 195±20                     |
| Triglycerides (mg/dl) | 101±25                  | 117±41                     | 83±40                    | 86±37                      |
| Total lipids (mg/dl) | 473±35                   | 476±63                     | 438±73                   | 455±45                     |
| T₃ (ng/dl)           | 1.2±0.3                  | 1.2±0.2                    | 1.3±0.3                  | 1.2±0.1                    |
| T₄ (µg/dl)           | 9.0±1.2                  | 8.9±1.4                    | 8.9±1.4                  | 8.9±1.0                    |
| TSH (µU/ml)          | 3.1±1.8                  | 3.2±1.6                    | 2.6±1.4                  | 2.4±1.1                    |
| Prothrombin time (%) | 98±4                     | 98±2                       | 99±4                     | 97±4                       |
| Partial thrombin time (sec) | 34.9±2.6            | 35.1±2.7                   | 35.2±2.1                 | 35.0±2.4                   |
| Hepaplastin test (%) | 100±10                   | 106±11                     | 105±20                   | 109±15                     |
| PIVKA-II (µg/ml)     | <0.5                     | <0.5                       | <0.5                     | <0.5                       |

Mean±SD.

PIVKA (protein induced by vitamin K absence or antagonist)-II, did not change by
the tocopherol supplementation.

3. Effects on health status
Based on the questionnaire, in which muscle weakness, status of work
performance, gastrointestinal discomfort, headache, hypertension, visual changes,
and general well-being were examined, no changes in all the items were documented.

DISCUSSION
Free RRR-alpha-tocopherol(formerly $d$-alpha-tocopherol) is generally accept-
ed to be the most biologically active form among the eight kinds of isomers on the
basis of gestation and fetal reabsorption bioassay in rats ($1,2$) and to have a
biopotency of 1.49 U/mg. This superiority of biopotency of RRR-alpha-tocopherol
was also confirmed by the finding of changes in plasma and/or red blood cell
tocopherol levels after administration of the vitamins in humans ($3$) and rats ($4$).

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However, there are no preparations of vitamin E as a free RRR-alpha form (\(d\)-alpha-tocopherol), although the majority of the medical preparations of vitamin E in Japan are composed of all-rac-alpha-tocopheryl esters, such as acetate, nicotinate, and succinate. In this study, a soft gelatin capsule preparation of a free form of RRR-alpha-tocopherol was provided from the Eisai Co., Ltd., and the bioavailability was examined in human male young adults. There have been several reports that plasma vitamin E levels elevated by a factor of more than 1.5 to 2.0 times the normal baseline concentrations. Especially, Baker et al. (18) recently reported an increase in plasma tocopherol levels of 2,000 to 2,600 \(\mu g/100\text{ml}\) following supplementation with 800 IU daily of either all-rac- or RRR-alpha-tocopheryl acetate for 28 days in human male adults. Our result generally agreed with Baker's, and the maximum plasma level did not exceed 3 times the basal value. RBC and WBC tocopherol levels showed an increased level in parallel with the plasma levels, and the maximum levels in RBC and WBC reached 2.5 and 3 times the basal values, respectively. The elevation of tocopherol levels in platelets and buccal mucosal cells was relatively slower than in the above items, but the maximum levels in these cells ultimately reached 3 times the basal ones. This indicates that RRR-alpha-tocopherol administered could be well distributed in tissue cells, and the elevation was in an effective and limited concentration in all distributions even by the continuous oral administration. This may additionally explain the reason why oral megavitamin E supplementation is non-toxic. The elevated tocopherol concentrations in the cells as blood components, decreased to the baseline level within 7 days after cessation of supplementation (but there is no data for WBC tocopherol), while those in buccal mucosal cells remained at relatively higher levels one month later after the cessation of supplementation. This may indicate a slower turnover rate of tocopherol in tissue cells, as compared with that in blood cells. The precise mechanism in tocopherol turnover and transportation remains to be solved.

Although vitamin E is generally considered to be a relatively non-toxic nutrient in adults and a megadosage of vitamin E has been used for therapeutic and prophylactic purposes for a wide variety of disorders, there have been warnings about the toxic effects of megadosages of vitamin E, because this vitamin is fat soluble and may be difficult to be easily eliminated from the body after the absorption. Thus, reports of adverse symptoms from large doses of the vitamin abound in the literature (6–9), but they are largely subjective and based on limited observations. Farrel and Bieri (11) examined an average of three years administration in healthy adults with 100 to 800 IU/day, and reported that at the plasma tocopherol levels elevating to two times that of the basal values, no gross evidence of toxicity was apparent; in addition, the findings failed to reveal any disturbances in liver, kidney, muscle, thyroid gland, RBCs, WBCs, coagulation parameters, or blood glucose by performance of the 20 standard clinical blood tests. Tsai et al. (12) have studied the effect of megavitamin E supplementation (600 IU of all-rac-alpha-tocopheryl acetate daily for 4 weeks) in healthy adult volunteers, with a double-blind examination, and reported that the megavitamin E supplementation does not
have a significant effect, either beneficial or undesirable, on general health conditions, but it can cause a significant reduction of serum thyroid hormone levels and also an elevation of serum triglycerides levels. There have been several reports in experimental animals that thyroid function was depressed by consumption of large amount of vitamin E, being assumed from a reduction in the function of the hypothalamus-pituitary-thyroid system (12, 19–21). However, in the human experiment by Tsai et al. (12), the values of T3 and T4 after the supplementation were within normal range. In our study, there was no influence on thyroid function even with more-long-term administration of larger amount of vitamin E; this finding conflicted with that of Tsai et al. (12). Thus, no physiologically significant effect on thyroid function was confirmed in the subjects with long-term megavitamin E administration as a natural form RRR. One of the most toxic effects of vitamin E at elevated dosages is known to be the antagonism to vitamin K action and the enhancement of the effect of oral coumarin anticoagulant drugs, with overt hemorrhage (22, 23). Although Farrel and Bieri (11) and Tsai et al. (12) have reported no prolongation of prothrombin time, recent investigations have centered on the role of vitamin E in the vitamin K-dependent carboxylation reaction to produce gamma-carboxyglutamyl residues of thrombin protein, since vitamin E could interfere with the oxidation of vitamin K, in addition with competitive inhibition of the vitamin K-dependent carboxylation reaction by tocopheryl quinone of a metabolic by-product of vitamin E (24). Thus, we measured Hepaplastin test which examines the combined activities of coagulation factors of II, VII, and X, and PIVKA (proteins induced by vitamin K absence or antagonist)-II as well as prothrombin time and partial prothrombin time. All indices regarding vitamin K functions showed no reduction by the administration of 900IU of RRR-alpha-tocopherol daily for 3 months. In addition, among undesirable effects reported in a few cases of clinical observation with megavitamin E supplementations, general muscle weakness accompanied by increased plasma creatinine phosphokinase (CPK) activity has been reported (25, 26). No subjects claimed muscle weakness on their questionnaire nor did they have an elevated CPK activity. Other standard clinical tests including complete blood cell counting, liver function, and kidney function showed no changes after the supplementation. All subjects did not claim any symptoms and showed no abnormalities including fatigue, headache, gastrointestinal disturbances, or visual complaints on the questionnaire.

All the above findings show that no adverse phenomenon developed with administration of megadosages (daily 900 IU) of RRR-alpha-tocopherol for a long period (3 months), even though there was an elevated vitamin E level in effective sites of tissue cells.

In this study, plasma and RBC gamma-tocopherol levels were simultaneously determined. The findings of suppressed gamma-tocopherol levels resulting form the elevation of alpha-form in plasma after megavitamin E supplementation confirm the previous report by Baker et al. (18). These changes in plasma reflect the RBC levels in both tocopherol forms. Baker et al. (18) suggested that this finding shows a
competition of gamma-tocopherol with alpha-tocopherol binding sites in intestine in the absorption mechanism and the metabolic rate of gamma-tocopherol is very dependent on nutritional status of alpha-tocopherol, since previous work (27, 28) showed the existence of specific intestinal receptors for tocopherol uptake, which have higher affinity for alpha-tocopherol than for gamma-tocopherol. The recovery to the baseline levels was more delayed in RBCs than in plasma after the termination of the supplementation. This may be also due to a membrane affinity for tocopherols, because Kitabuchi and Wimalasena (29) have also reported a receptor of tocopherol in RBC membranes and the receptor had higher affinity for alpha-form than for gamma-form. However, existence of specific receptor for tocopherol has not yet been established, while it is generally considered that tocopherol in vivo moves among lipids in a passive manner with partitioning being determined by the total lipid level in the body components. To clarify the finding of competition between alpha- and gamma-tocopherols, further investigations are necessary.

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