*Free Radical Research*, August 2006; 40(8): 893–899

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# Changes in plasma alpha and gamma tocopherol levels before and after long-term local hyperthermia in cancer patients

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Accepted by Professor A. Azzi

*(Received 29 November 2005; in revised form 10 April 2006)*



**Abstract**
Local hyperthermia is one of the heat therapies for cancer patients. The effect of this therapy is recognized to affect the immune function. On the other hand, researchers have recently suggested that vitamin E has not only antioxidant but also other functions including the immune function. However, the association between local hyperthermia therapy and vitamin E level is not yet well understood. Comparing plasma alpha and gamma tocopherol levels before and after the therapy, the basal levels of both tocopherols in the cancer patients did not significantly differ from those in healthy subjects. However, the interindividual difference in the basal levels was very wide in the cancer patients. After long-term local hyperthermia (more than 70 days), the levels of both tocopherols were significantly higher than the basal levels. This result suggests that long-term local hyperthermia therapy influences plasma tocopherol level in cancer patients; thus, an increase in vitamin E level may play an important role in the therapy of cancer patients.

**Keywords:** *Local hyperthermia, vitamin E, cancer*

## Introduction

Local hyperthermia has been used in the heat therapy of cancer patients. The system of this therapy entails a direct heating of the tumor area and keeping it at 40–42.5°C for 30 min once or twice a week. The purpose of this therapy is to induce cell death and an immunostimulatory effect in the carcinoma region. Generally, this treatment has been used in combination with radiation therapy and chemotherapy. Recent investigations have demonstrated that this therapy greatly contributes to immune responses, by affecting the percentage of cluster of differentiation (CD) 4 and CD8 subsets, the CD4/CD8 ratio [1], as one of the index of cell-mediated immunity, thereby enhancing the activities of natural killer (NK) cells [2] and other cells [3–6]. However, the detailed mechanisms of these effects have not yet been reported.

Vitamin E (tocopherol) is a natural lipophilic vitamin. Its most important function is as an antioxidant, that is, a free-radical scavenger [7–9]. Recently, other possible beneficial functions of vitamin E have been considered, a preventive effect of neurodegenerative disorders, an anti-aging effect and others [10]. In previous studies, we found that alpha tocopherol administration in rodent models significantly protects the cognitive function, such as learning and memory [11–13]. Urano and coworkers reported about the function of the membrane permeability and fluidity in synaptic plasma membranes [14,15]. Sano et al. demonstrated that treatment with alpha

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ISSN 1071-5762 print/ISSN 1029-2470 online © 2006 Informa UK Ltd
DOI: 10.1080/10715760600750396

tocopherol and selegiline significantly suppresses the progression of Alzheimer's disease [16]. These reports indicate that vitamin E has not only an antioxidant function but also other biological functions, such as the so-called "beyond-antioxidant function". In addition, other researchers suggested that vitamin E affects the immune function; for instance, it enhances interleukin-2 (IL-2) production in mouse models [17] and inhibits IL-4 gene expression in peripheral blood T cells [18] among others [19–21].

The most interesting point to be noted is that both effects mentioned above affect the immune function. It is presumed that change in plasma vitamin E level is relevant to the effect of hyperthermia therapy including an immunostimulatory effect. However, little is known about the association between local hyperthermia therapy and vitamin E level in cancer patients. The purpose of this study is to investigate the change in plasma vitamin E level during chronic local hyperthermia therapy in cancer patients. To this purpose, we measured plasma alpha and gamma tocopherol levels using high-performance liquid chromatography (HPLC) in cancer patients before and after short- and long-term local hyperthermia therapies. Short-term local hyperthermia therapy, less than 70 days, did not affect plasma vitamin E level in cancer patients. However, long-term therapy, more than 70 days, significantly increased both plasma alpha and gamma tocopherol levels. These results indicate that this therapy may be effective over a period of more than 2 months, and that one beneficial effect of long-term local hyperthermia therapy, namely, the improvement of the immune system, may be attributable to increases in plasma alpha and gamma tocopherol levels.



## Subjects and methods

### *Subjects*

Fifty-six cancer patients and nine healthy individuals took part in this study. Table I shows the breakdown of the subjects. Before the start of this study, written informed consent was obtained from all the subjects.

### *Hyperthermia therapy*

Local hyperthermia therapy was performed using Thermotron RF-8 (Yamamoto Vinita Co., Ltd, Osaka, Japan) as described previously by Ostapenko et al. [1]. Electric power ranging from 100 to 1500 W was applied to the patient's carcinoma region. Blood pressure and pulse were monitored during the therapy. One session was about 30–60 min and was performed once or twice a week. In the case of long-term sessions, more than eight sessions were performed.

### *Plasma isolation*

To compare tocopherol levels before and after local hyperthermia therapy, we collected and analyzed 5–10 ml of peripheral blood from the subjects. The plasma fraction was isolated from these blood samples using a standard procedure, and centrifuged at 3500 rpm for 20 min. Finally, the upper layer was rapidly separated and stored in a deep freezer before use.

Table I. Breakdown of subjects. Fifty-six cancer patients and nine normal healthy subjects took part in this study. Each group were divided by carcinoma regions. Others denote the following carcinoma regions: rectum ($n = 3$), bladder ($n = 2$), ovary ($n = 2$), tongue ($n = 1$), skin ($n = 1$), gallbladder ($n = 1$), esophagus ($n = 1$) and cervix ($n = 1$). Before the start of this study written informed consent was obtained from all the subjects.

| Breakdown of subjects | |
|---|---|
| Cancer patients | 56 |
| Lung | 13 |
| Stomach | 6 |
| Breast | 7 |
| Liver | 6 |
| Pancreas | 6 |
| Colon | 6 |
| Others | 12 |
| Normal healthy subjects | 9 |

### *Measurement of alpha and gamma tocopherol levels*

Plasma alpha and gamma tocopherol levels were assessed as described previously [22,23], with some modifications. An aliquot of plasma (0.1 ml) was dissolved in a mixture of 6% pyrogallol solution (1 ml), 35% ethanol solution (0.1 ml), deionized water (0.1 ml) and tocol (20 μl) used as internal standard. After vortexing, 1.5 ml of hexane-ethyl acetate (9:1 v/v) was added to the mixture, which was then centrifuged at 3000 rpm for 15 min at 4°C. The extract was evaporated under nitrogen gas, and hexane (200 μl) was added to the residue to dissolve it. The resulting solution was analyzed by HPLC using an ultraviolet (UV) detector (SHIMADZU 2010-LC, SHIMADZU Corp., Kyoto, Japan). All the materials used were purchased from Wako Pure Chemical Co., Ltd, (Osaka, Japan). The tocol and alpha and gamma tocopherols used for making the standard curve were provided by Eisai Co., Ltd, (Tokyo, Japan).

### *HPLC conditions*

For the determination of plasma alpha and gamma tocopherol levels, the prepared samples were injected into a reverse-phase column (Wakopac Navi C30-5, $\varphi$4.6 × 250 mm, Wako) under the following conditions: 1.5 ml/min flow rate, 295 nm wave length and 100% acetonitrile eluent. The retentions times of the peaks of tocol and gamma and alpha tocopherols were 9.08, 13.98 and 17.40 min.

### *Statistical analysis*

The results are presented as mean ± SE. All data were assessed by the Student's *t*-tests, and a $p < 0.05$ was considered significant.

## Results

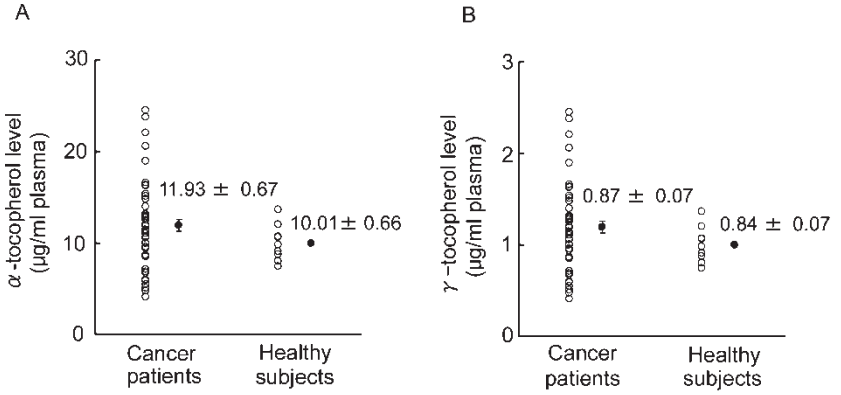
*Measurement of plasma alpha and gamma tocopherol levels before local hyperthermia therapy*

To determine the effect of local hyperthermia therapy, we measured basal plasma alpha and gamma tocopherol levels using the HPLC-UV detection method (Subjects and Methods). Both basal plasma tocopherol levels were not significantly different between the cancer and normal healthy subjects (Figure 1(A),(B)). The cancer subjects (11.93 ± 0.67 μg/ml) showed a slightly higher plasma alpha tocopherol level than the normal healthy subjects (10.01 ± 0.66 μg/ml). However, in the cancer subjects, the interindividual difference in plasma alpha tocopherol level was apparently wide (from 4.07 to 27.77 μg/ml). On the other hand, in the normal healthy subjects, no such wide interindividual difference in plasma alpha tocopherol level was observed (from 7.46 to 13.69 μg/ml). The same tendency was observed for plasma gamma tocopherol level. Next, we determined whether plasma vitamin E level is associated with tumor sites in cancer subjects. However, we could not find a significant difference in both plasma alpha and gamma tocopherol levels (Figure 1(C)). These results suggest that basal plasma vitamin E level does not significantly differ between cancer patients and normal healthy subjects, and that the interindividual differences in the levels of plasma alpha and gamma tocopherols in cancer patients are wide.

*Changes in plasma alpha and gamma tocopherol levels before and after local hyperthermia therapy*

To determine the effects of continuous local hyperthermia therapy and plasma alpha and gamma tocopherol levels on cancer, we assessed 29 cancer patients during such therapy. We collected blood samples from the subjects at various times after the start of the therapy (from 24 to 133 days). As shown in Figure 2, both plasma tocopherol levels were slightly increased in the cancer subjects after the first therapy. It was interesting that up to 70 days after the first therapy, both plasma tocopherol levels were slightly increased (alpha tocopherol, 105.4 ± 7.10%; gamma tocopherol, 111.0 ± 12.9%), but beyond 70 days, the levels were significantly increased compared with the basal levels (alpha tocopherol, 118.7 ± 11.5% $p < 0.01$; gamma tocopherol, 144.0 ± 20.0% $p < 0.0001$) (Figure 2(C)). These intriguing results indicate that long-term local hyperthermia therapy of at least 2 months may be more effective than short-term therapy, increasing the levels of plasma alpha and gamma tocopherols in cancer patients.

Furthermore, we chose five patients for the collection of peripheral blood at three different times

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| | α-tocopherol level | γ-tocopherol level |
|---|---|---|
| Cancer patients | 11.930 ± 0.672 | 0.870 ± 0.069 |
| Lung | 12.698 ± 1.393 | 0.854 ± 0.140 |
| Stomach | 13.624 ± 2.392 | 0.948 ± 0.302 |
| Breast | 10.723 ± 0.343 | 0.484 ± 0.073 |
| Pancreas | 9.858 ± 2.489 | 0.916 ± 0.212 |
| Liver | 15.753 ± 2.757 | 0.992 ± 0.164 |
| Colon | 10.381 ± 1.414 | 0.618 ± 0.182 |
| Others | 11.766 ± 1.650 | 0.765 ± 0.117 |
| Normal Healthy subjects | 10.013 ± 0.660 | 0.840 ± 0.070 |

(μg/ml plasma)

Figure 1. Measurement of basal plasma levels of alpha and gamma tocopherols in cancer patients and normal healthy subjects. Basal levels of (A) alpha and (B) gamma tocopherols (cancer; $n = 56$, healthy; $n = 9$). Black circles show the average of each parameter (clear circles). (C) Individual tocopherol levels of carcinoma regions. All data are given as mean ± SE.

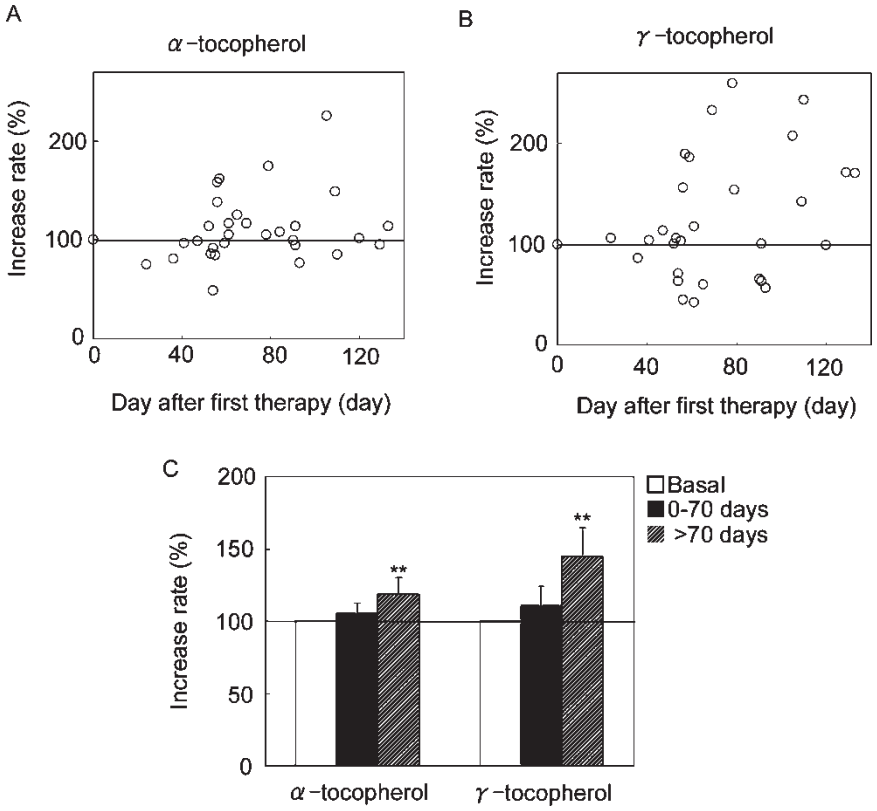


Figure 2. Changes in rates of increases in plasma alpha and gamma tocopherol levels less than and more than 70 days after local hyperthermia Comparison between basal and nonbasal levels of (A) alpha and (B) gamma tocopherol in cancer subjects ($n = 29$). The score of each subject was calculated from the rates of increases in the levels (against basal level = 100%). (C) Comparison between rates of increases in alpha and gamma tocopherol levels before and after 70 days after the start of the therapy. White, black and shadow bars show the basal level (100%), less than 70 days level (alpha tocopherol, 105.4 ± 7.10%; gamma tocopherol, 111.0 ± 12.9%), more than 70 day level (alpha tocopherol, 118.7 ± 11.5%; gamma tocopherol, 144.8 ± 20.0%). All data are given as mean ± SE. $^{**}p < 0.01$, significantly different from the basal level.

during the therapy (for the other 24 subjects, blood collection was performed only two times including that before the first therapy) (Table II). Comparing the basal plasma tocopherol levels with those of the blood samples from the third collection, we observed that almost all the subjects clearly showed increases in plasma alpha and gamma tocopherol levels (alpha tocopherol, 4/5 subjects; gamma tocopherol, 4/5 subjects). We could not find any correlations between these results and other parameters, such as age, sex, tumor area, and tumor stage (data not shown). These results suggest that the effectiveness of long-term local

Table II. Difference between rates of increases in plasma alpha and gamma tocopherol levels before and two blood collection days after the start of the therapy. For five subjects, peripheral blood was collected three times. Table II shows the rates of increases in alpha and gamma tocopherols two blood collection days after the start of the therapy.

| Case | Age | Sex | Tumor area | Day | α-Tocopheorl level (%) | γ-Tocopherol level (%) |
|---|---|---|---|---|---|---|
| 1 | 76 | Male | Lung | 0 | 100.0 | 100.0 |
| | | | | 24 | 75.0 | 106.6 |
| | | | | 55 | 84.4 | 103.6 |
| 2 | 62 | Female | Colon | 0 | 100.0 | 100.0 |
| | | | | 69 | 116.4 | 233.3 |
| | | | | 133 | 113.9 | 170.8 |
| 3 | 40 | Female | Cervix, uteri | 0 | 100.0 | 100.0 |
| | | | | 61 | 116.8 | 42.3 |
| | | | | 120 | 101.8 | 99.3 |
| 4 | 62 | Male | Lung | 0 | 100.0 | 100.0 |
| | | | | 65 | 125.0 | 61.0 |
| | | | | 109 | 148.0 | 142.8 |
| 5 | 64 | Male | Lung | 0 | 100.0 | 100.0 |
| | | | | 41 | 96.4 | 104.1 |
| | | | | 61 | 104.9 | 118.1 |

hyperthermia therapy is associated with increases in plasma alpha and gamma tocopherol levels.

## Discussion

In this study, we found that plasma alpha and gamma tocopherol levels gradually increase in cancer patients during long-term local hyperthermia therapy. In particular, after such therapy for more than 70 days, both plasma tocopherol levels were significantly higher than their basal levels.

Thus, we determined the function of increased plasma alpha and gamma tocopherol levels after the above-mentioned therapy. We could not determine the mechanism of this increase in this study. However, it may be presumed that increases in both plasma tocopherol levels have positive effects including their being immunostimulatory in the therapy. These effects might be one of the non-antioxidant functions of vitamin E. In previous study, we have proved that a deficiency in alpha tocopherol significantly correlates with cognitive dysfunction in rat models, using several maze tasks [11,12]. Recently, Yeh et al. have reported that alpha tocopherol has a protective effect against prostate cancer cells, via the down-regulation of phosphoinositide 3-kinase (PI3K)/Akt signaling [24]. Additionally, Maydani et al. reported that treatment with vitamin E leads to the enhancement of T cell production, and through the reduced production of prostaglandin E-2, to the suppression of peroxynitrite formation [25]. On the other hand, it is well known that many types of cancer cells are overproduction of reactive oxygen species (ROS) and nitric oxide species (NOS) [26,27]. Alternatively, it could be argued that the results of this therapy such as induced tumor cell death and improved immune function are a result of decreased ROS and NOS in cancer patients, leading to the possible improvement in the quality of life (QOL) of these patients. To confirm this, some of the patients continued to receive the therapy for more than 100 days. The result suggests that changes in plasma alpha and gamma tocopherol levels are associated with QOL in cancer patients. Because of other researchers already indicate about the relation effect of hyperthermia therapy with the scores of QOL in cancer patients [28,29]. The overall results of our experiments suggest that long-term local hyperthermia therapy induces an increase in plasma vitamin E level, and consequently vitamin E influences other beneficial functions in cancer patients.

Basal plasma alpha and gamma tocopherol levels did not significantly differ between the cancer and normal healthy subjects. However, we would like to emphasize the wide difference in the basal plasma tocopherol levels among the cancer subjects. As shown in Figure 1, the highest and lowest basal levels of plasma alpha and gamma tocopherols showed an approximately five-fold difference. The reason for the significant differences between the tocopherol levels before and after the therapy may be the large increases in the tocopherol levels in the subjects with low basal plasma tocopherol levels (Table III). We also would like to clarify the wide interindividual difference in the tocopherol levels observed in the cancer patients. In this study, we could not find any correlation between basal plasma alpha and gamma tocopherol levels and other parameters in the cancer patients; for instance, characteristics of the patients, difference of mechanical conditions during the therapy among others. It may be presumed that some of the patients may have taken some types of supplement by themselves, such as multivitamins, extracts of natural plants, and probiotics. But we did not check whether the patients took supplements before the start of the therapy. Had all of them not taken any supplements, their basal plasma vitamin E level may have been significantly lower than that of the healthy subjects.

Table III. Low basal plasma alpha tocopherol levels were improved after long-term local hyperthermia therapy in cancer patients. Seven cancer subjects with low basal plasma alpha tocopherol levels ($< 10\ \mu g$/ml plasma) were selected. The levels of plasma alpha tocopherol gradually increased after the therapy. In the case of plasma gamma tocopherol level, the same tendency was obserbed (data not shown).

| | α-Tocopherol level* | | | |
|---|---|---|---|---|
| Case | Basal | After therapy | Day† | Increase rate (%) |
| 1 | 8.682 | 7.459 | 53 | 85.9 |
| 2 | 6.732 | 6.172 | 54 | 91.7 |
| 3 | 8.668 | 4.202 | 54 | 48.5 |
| 4 | 5.464 | 8.838 | 57 | 161.7 |
| 5 | 8.463 | 14.733 | 79 | 174.6 |
| 6 | 5.916 | 13.353 | 105 | 225.7 |
| 7 | 9.989 | 11.382 | 133 | 114.0 |

* (μg/ml plasma). † Day = Day after first therapy.

The results of the five patients examined showed that both plasma alpha and gamma tocopherol levels were slightly elevated (Table II). Thus, it is suggested that the absorption of alpha tocopherol has been enhanced after the long-term therapy in the cancer subjects. The reason for this suggestion is that blood flow changed after heating up the tumor area. Furthermore, *in vivo*, alpha tocopherol binds to specific transfer proteins and is transported from the liver to peripheral cells by very low density lipoproteins (VLDLs) [30,31]. Van der Logt and coworkers reported that cancer patients had elevated plasma levels of isoprostane, an index of lipid peroxide [26]. Oldenburg et al. showed that whole-blood ROS production is significantly higher in inflammatory bowel disease patients. This disease has been strong connected with colon cancer [32]. Before the start of the local hyperthermia therapy, plasma ROS level might be higher compared to healthy subjects and large amounts of antioxidant substances were

continuously consumption in whole-blood of cancer patients.

In this study, we found that continuous long-term local hyperthermia therapy (at least more than 70 days) significantly increases the levels of plasma alpha and gamma tocopherols in cancer patients. Unfortunately, we could not examine the direct mechanisms and pathways underlying the effects of this therapy and changes in plasma tocopherol levels. This is the first report on the associations of long-term local hyperthermia therapy with plasma alpha and gamma tocopherol levels in cancer patients. However, it was not clarified what the role of elevated plasma vitamin E level in the therapy. Other researchers have already indicated that vitamin E has some beneficial functions in transcription and protein levels in living tissues [10,33,34]. Further study will be conducted to determine the detailed mechanisms of this result. In summary, our study suggests that long-term local hyperthermia therapy markedly affects plasma vitamin E level. However, further investigations are necessary to elucidate the mechanisms underlying the effects of this therapy on plasma vitamin E level.



## Acknowledgements

This work was supported by the Vitamin E Information and Technology Section, Eisai Co., Ltd, Tokyo, Japan and Department of Third Internal Medicine, Wakayama Medical University, Wakayama, Japan. We thank Dr Tanaka Yoshimasa of the Shouseikai Nishide Hospital for helpful suggestions.

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