

A STUDY OF FACTORS RESPONSIBLE FOR THE LOW CONCENTRATION OF ERYTHROCYTE α -TOCOPHEROL IN CHRONIC RENAL FAILURE

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Summary

Erythrocyte- α -tocopherol (RBC-TOC) in haemodialysis patients showed a significantly low value compared with healthy subjects and a negative correlation with high density lipoprotein tocopherol (HDL-TOC) in contrast with healthy subjects. After oral administration of tocopherol, the transfer to RBC-TOC was poor; contrarily, HDL-TOC showed a high level and an enantiomorphism with RBC-TOC. In the experimental systems in vitro, the transfer of tocopherol from healthy subjects high density lipoprotein to patients red blood cells was found most prominent in the lecithin:cholesterol acyltransferase (LCAT)-containing system.

Introduction

Acceleration of haemolysis is frequently seen in haemodialysis patients [1]. Involvement of various factors has been considered as the cause, including an increase of red blood cell-malonyldialdehyde [2]. While tocopherol and glutathione peroxidase [3] are known to have a scavenger activity against red blood cell-malonyldialdehyde, tocopherol is more important [4]. Thus, RBC-TOC has a very important role in relation to acceleration of haemolysis or anaemia in haemodialysis patients; however, there have been few reports on tocopherol concentrations, probably because of the complexity of assay. According to the results [1,2] so far reported, RBC-TOC is significantly low in haemodialysis patients, with the cause remaining unknown. The present study was performed to elucidate the cause of this lowering of RBC-TOC both in vivo and in vitro.

Materials and methods

Fifteen haemodialysis patients and 15 healthy adult volunteers (all male) were studied. The mean age of the former was 47 (range 19-65) and the latter 40 (range 21-55). The average duration of haemodialysis was 48 months (range

15–90 months). They received haemodialysis on a regular basis of five hours three times weekly. Acetate dialysate and heparin as anticoagulant were used. Both haemodialysis patients and the healthy subjects were given ordinary meals; the oral administration of any drugs which affect the studied indices was discontinued two weeks prior to test. Heparinized blood was sampled from the arterial flow in haemodialysis patients and from the antecubital vein in healthy subjects; the sampled blood was divided into plasma and blood cells. A part of plasma was separated into high density lipoprotein by the dextran MnCl_2 method; blood cells were washed three times to remove blood cells other than red blood cells. Plasma tocopherol, high density lipoprotein and red blood cells were determined according to the method of Mino et al [5]. Plasma HDL-TOC was expressed as non-HDL-TOC. We questioned whether the high density lipoprotein fraction could be obtained accurately; therefore, using the same specimen, we compared the results obtained by an ultracentrifugation and the dextran method with the following results: $r=0.88$, $p<0.001$ ($n=15$) in healthy subjects; $r=0.86$, $p<0.001$ ($n=13$) in haemodialysis patients. In view of this very good positive correlation, we used the dextran method alone, for the sake of convenience and in view of the number of specimens. From the 15 healthy subjects and 15 haemodialysis patients, seven of each (age-matched) were selected and given orally 600mg tocopherol after a meal; blood sampling was done at 0, 3, 6, 10 and 24 hours.

As an in vitro experiment, plasma and red blood cells were sampled from age and blood type-matched healthy subjects and haemodialysis patients (three subjects in each group). A part of plasma from healthy subjects was submitted to heat inactivation (56°C , 30 minutes) or was supplemented with 2mM parachloromercuric benzoic sulphate (PCMS) to inhibit LCAT activity. Plasma (with and without LCAT activity) from healthy subjects and red blood cells from patients were mixed at 3:2, and incubated at 37°C for 30 minutes; based on decreases of plasma- and HDL-TOC before and after incubation, the transfer of tocopherol to red blood cells was estimated.

The values obtained were expressed as mean \pm SD and correlation coefficient, r =the significant difference, p was calculated with the Student's 't' test.

Results

Correlation between RBC-TOC and plasma factors

RBC-TOC levels in healthy subjects and haemodialysis patients were 1.38 ± 0.25 and $0.85\pm 0.14\mu\text{g/ml}$ packed cell, respectively; a significant decrease in haemodialysis patients ($p<0.001$). RBC-TOC was noted to have no correlation with plasma tocopherol, but showed a significant positive correlation with HDL-TOC ($r=0.66$, $p<0.01$) and a significant negative correlation with non-HDL-TOC ($r=0.57$, $p<0.05$) in healthy subjects. On the other hand, in haemodialysis patients, RBC-TOC was found to have a significant negative correlation with HDL-TOC ($r=0.60$, $p<0.02$) and a tendency to positive correlation with non-HDL-TOC ($r=0.46$, $p<0.1$).

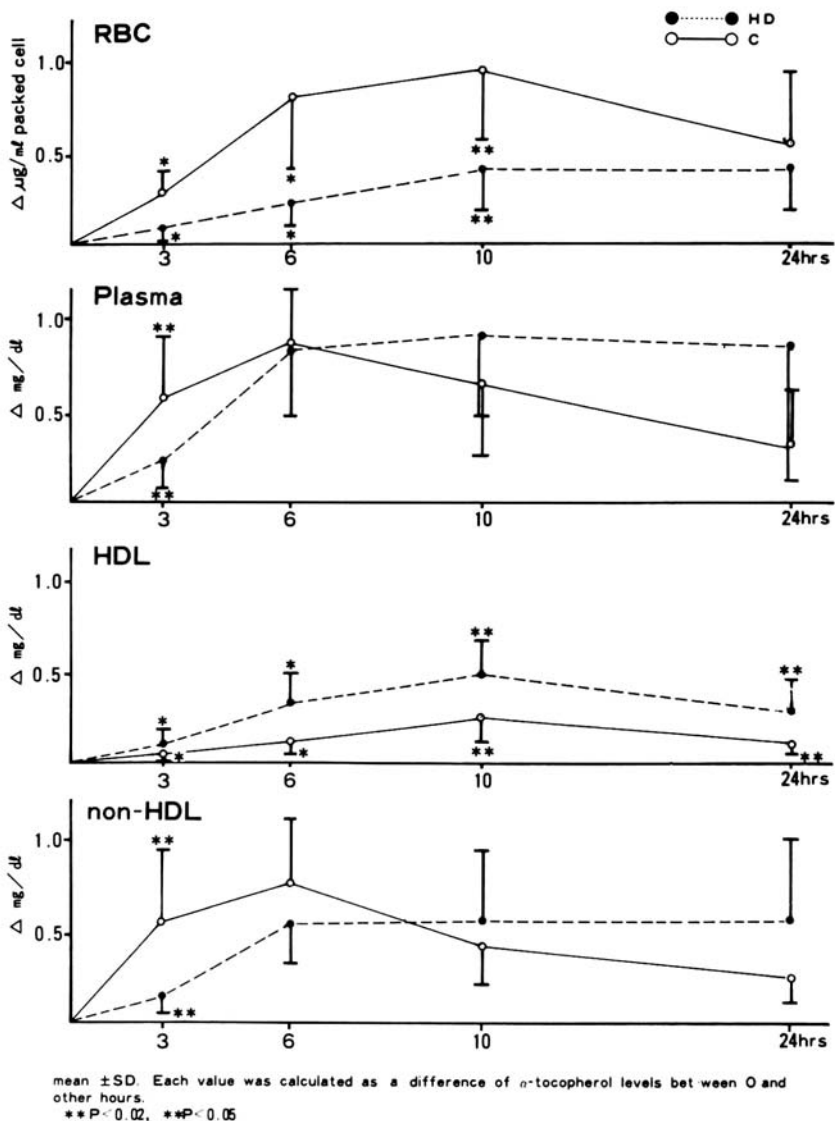
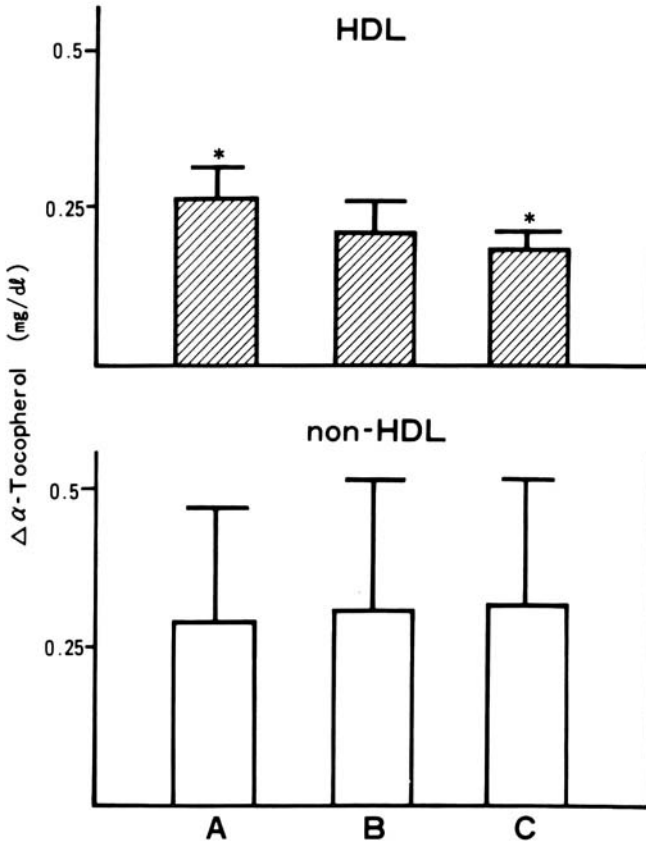


Figure 1. Time course of an increase in tocopherol levels of red blood cells (RBC), plasma, high density lipoprotein (HDL) and non-high density lipoprotein (non-HDL) after orally administered 600mg tocopherol in seven haemodialysis (HD) patients and in seven control (C) subjects

In vivo results

The time course of the increment (difference from 0 hour) of tocopherol after oral administration is shown in Figure 1. While RBC-TOC in healthy subjects

continued to increase until the tenth hour and slightly declined at 24 hours, RBC-TOC in haemodialysis patients gradually increased up to 24 hours, showing, however, significantly low levels at 3, 6 and 10 hours as compared with healthy subjects. Unlike RBC-TOC, plasma-tocopherol in healthy subjects increased until the sixth hour and gradually decreased thereafter, while in haemodialysis patients plasma-tocopherol, of which increment at hour three was significantly low, increased until hour six and thereafter continued a slight increase until hour 24. The time course of this plasma-tocopherol was similar to that of non-HDL-TOC. HDL-TOC showed a significant difference quite opposite to RBC-TOC, and its time course formed an enantiomorphism with that of RBC-TOC.



mean \pm SD (n = 3). *P < 0.01.

The experimental conditions are as follows: A: control plasma (CP) + patient (HD)-RBC, B: CP with heat inactivation + HD RBC, C: CP with 2mM PCMS + HD-RBC

Figure 2. Reduced tocopherol levels in high density lipoprotein (HDL) and non-high density lipoprotein (non-HDL) under various in vitro experimental conditions

In vitro results

Decrements of tocopherol in high density lipoprotein and non-high density lipoprotein fractions were examined in the experimental systems A (containing LCAT), B and C (both having no LCAT reaction). As a result, in high density lipoprotein, A was obviously high compared to B and C, especially a significant difference was noted between A and C. In non-high density lipoprotein, there was no difference at all among A, B and C (Figure 2).

Discussion

The RBC-TOC level in our haemodialysis patients was significantly low, and this coincides with the results obtained by Ono [1] and Giardini et al [2]. As factors responsible for this low concentration of RBC-TOC, acceleration of its utilization and insufficient supply from blood are possible. Giardini et al suggested an enhanced tocopherol utilization in view of the high value of RBC-MDA [2]. However, in their study, RBC-MDA was found to show a marked increase, and the anti-oxidation activity of tocopherol alone seems less demonstrable.

The findings obtained in our present study suggested an insufficient supply from plasma as the main factor. Kayden et al [6] have already reported that HDL-TOC is important as the source of RBC-TOC. According to our results in healthy subjects, RBC-TOC showed a significant positive correlation with HDL-TOC, but rather a reversed correlation with non-HDL-TOC. This finding suggests that under steady state in healthy subjects RBC-TOC is mainly supplied from high density lipoprotein and is hardly supplied from non-high density lipoprotein. In haemodialysis patients, RBC-TOC had a reversed correlation with HDL-TOC; thus, the increase of RBC-TOC is a phenomenon hardly to be expected. To confirm this *in vivo*, tocopherol was given to haemodialysis patients and RBC-TOC showed a poor increase compared with healthy subjects and HDL-TOC inversely showed an enantiomorphic increase. On the other hand, non-HDL-TOC showed a quite different variation from RBC-TOC. These findings demonstrate that in haemodialysis patients the transfer of tocopherol from high density lipoproteins to red blood cells was insufficient, resulting in no appreciable increase of RBC-TOC.

Since it is generally accepted that the exchange between high density lipoproteins and substances in tissues is carried out by mediation of the LCAT reaction [7] we investigated the exchange of RBC-TOC with HDL-TOC in the presence or absence of LCAT activity *in vitro*. As a result, the LCAT reaction-containing system showed a marked decrease of HDL-TOC as compared with other LCAT reaction-inhibiting systems. In this experimental system, the transfer of tocopherol from non-high density lipoproteins was also noted independently of the LCAT reaction. This is considered as so-called non-enzymatic transfer [8]. Although the factor responsible for this transfer is yet unknown, possible explanations are that since the haematocrit in this experimental system was twice that of haemodialysis patients, the patients red blood cells would require a good deal of tocopherol, or that the transfer of tocopherol

from plasma other than high density lipoproteins in healthy subjects would be easier than in haemodialysis patients. In this regard, further investigations are needed including a study on the experimental methods to be employed.

References

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