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Effect of Vitamin E on Serum Aminotransferase and Thioredoxin Levels in Patients with Viral Hepatitis C

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Objectives: Oxidative stress induces cellular responses such as cell death, gene activation and cell proliferation, in the liver. Vitamin E (Vit. E) has been found to protect the liver against oxidative stress in animal experiments. Thioredoxin (TRX) is a stress inducible, multifunctional protein, secreted during oxidative stress. This study evaluated effects of Vit. E on serum TRX and aminotransferase levels in hepatitis C virus (HCV) patients, partly non-responsive to initial interferon (IFN), with higher than average level of serum alanine aminotransferase (ALT) after receiving anti-inflammatory drug treatment.

Methods: Seventeen HCV patients (male = 3; female = 14) of age 62 ± 7.65 years receiving anti-inflammatory drug therapy, at least 6 months prior to Vit. E administration, were given d-$\acute{\alpha}$-tocopherol 500 mg/day, orally, for a period of 3 months. ALT, aspartate aminotransferase (AST), TRX and Vit. E were measured at 0, 1, 2 and 3 months and 1 month after end of treatment. As controls, the same patients biochemical data, 3 months from the start of therapy were used. Patients were divided into three categories: total patients "T", low ALT group "L" (ALT $<$ 70 IU/l) and high ALT group "H"(ALT $>$ 70 IU/l), respectively.

Results: The ALT level was lowered, significantly in group H, in the 1st, 2nd, 3rd and 1-month post therapy, compared to the initial value. But group L showed little or no change in ALT. Post Vit. E therapy, in groups T and H, the TRX level was elevated but remained below initial levels, whereas in group L, TRX level remained significantly lower than the pretreatment value. Groups T and L, showed significant reduction ($p < 0.05$) in serum TRX levels in the 2nd and 3rd month. Group H showed a tendency towards TRX reduction, but not significantly. Serum Vit. E levels increased significantly ($p < 0.0001$) from the 1st to 3rd month in all three T, H and L groups.

Conclusion: Oxidative stress induced liver damage is reduced by Vit. E in patients with viral hepatitis C, particularly those with initial ALT levels $>$70 IU/l. Vit. E treatment causes reduction of oxidative stress markers as TRX and ALT in sera. Therefore, Vit. E can act as a supportive therapy to combat liver damage caused by oxidative stress, in such patients with continuously high levels of ALT even after anti-viral and anti-inflammatory drug therapy.

Keywords: Viral hepatitis C oxidative stress; Vitamin E; ALT; Thioredoxin

INTRODUCTION

Hepatitis C virus (HCV) infection rarely resolves spontaneously once chronicity is established.[1] In Japan, chronic hepatitis type C (CHC) accounts for nearly 60% of cases confirmed.[2] CHC with cirrhosis is a major risk factor for hepatocelluar carcinoma.[3] In CHC cases, interferon (IFN) therapy, particularly alpha interferon ($\acute{\alpha}$-IFN) has been effective in normalizing transaminase levels, clearing HCV from serum and improving liver histology.[4–6] Several studies have shown that an end-of-treatment response {alanine aminotransferase (ALT) normal; HCV RNA negative} is obtained in about 50% cases.[5,7] Half of these cases have a complete sustained response (ALT normal; HCV RNA negative 12 months after treatment), but others will relapse, with reappearance of viremia and

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elevation of ALT[8] or have no-response throughout. In such cases, alternative treatment with other anti-viral drugs such as Ribavirin or their combination, anti-inflammatory drugs and supplementary treatments are adopted, to decrease ALT levels, reduce inflammation and slow down disease progression. Among the liver enzymes, ALT is a well known marker of liver cell necrosis, as it represents the inflammatory necrosis of hepatocytes.

Continuous suppression of ALT is essential in CHC patients to slow down the processes leading to enhanced fibrosis and subsequent HCC. Oxidative stress has been found to cause enhancement of fibrogenesis in CHC patients.[9] Vitamin E (Vit. E) has been reported to improve aminotransferase status in CHC patients and protect against liver damage caused by oxidative stress.[10] Thioredoxin (TRX) is a stress inducible, multifunctional protein, secreted during oxidative stress.[11–14] Serum TRX levels of patients with HCV infection have been reported to be increased with their serum ferritin levels and the progression of liver fibrosis.[15] In this study, we evaluated the effects of Vit. E on serum TRX and aminotransferase levels in patients with viral hepatitis C, partly non-responsive to initial IFN therapy and receiving anti-inflammatory drug treatment.

MATERIALS AND METHODS

Patients

HCV patients with chronic and progressive fibrosis, as confirmed by liver biopsy were included in this study. Some patients were partly non-responsive to initial IFN therapy, others had no previous IFN therapy. All patients received anti-inflammatory drugs such as Stronger Neo-Minophagen (SNMC), Ursodeoxycholic acid (UDCA) or Shosaiko-to (TJ-9), for at least 6 months prior to this study. Vit. E (d-α-tocopherol) 500 mg/day, orally, was given to all patients for a period of 3 months. Clinical parameters such as ALT, AST, TRX and serum Vit. E were determined at 0, 1, 2, 3 and 1 month after cessation of Vit. E therapy. Since almost half the patients had an initial pretreatment ALT value >70 IU/l, and as ALT levels have been related to liver disease progression, we observed the changes in TRX and ALT in three categories; total patients "T", low ALT group "L" (ALT <70 IU/l) and high ALT group "H" (ALT >70 IU/l), respectively. To minimize errors due to host conditions, the biochemical data of the same patients three months consecutively, prior to the administration of Vit. E, was considered as control data.

Study Design

The study was primarily divided into three phases; pretreatment phase (3 months); treatment phase (3 months) and post-treatment phase (1-month). Serum levels of Vit. E, TRX, ALT and AST were measured monthly beginning at the pretreatment phase and ending with the post treatment phase (Fig. 1).

Biochemical Determinations

As an index of oxidative stress, serum TRX was determined using an enzyme-linked immunosorbant assay.[16,17] To assess liver function serum ALT, AST were determined using commercially available assays. A chromatographic instrument (Shimadzu, Kyoto, Japan) was used to determine serum d-α-tocopherol. HCV RNA was measured by the Branched DNA assay (Bayer Diagnostics, Emeryville, CA. USA).

Statistical Analysis

All results were expressed as mean $\pm$ standard deviation. A P value of <0.05 was considered

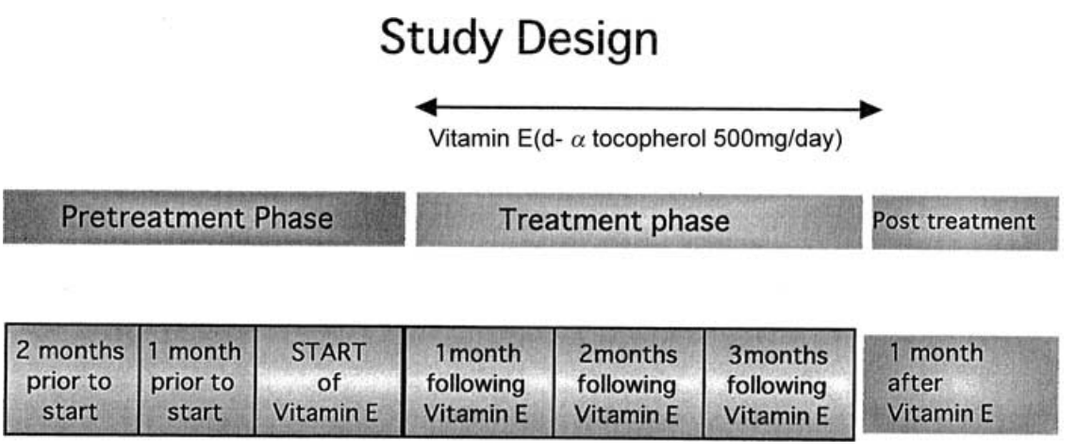

FIGURE 1 Study design of Vit. E therapy in 3 treatment phases.

TABLE I Pretreatment values of patients based on initial ALT levels

	Group A (ALT > 70 IU) ($n = 8$)	Group B (ALT < 70 IU) ($n = 9$)
Age (in years)	62.8 ± 8.31	62 ± 7.68
Sex (M/F)	3/5	(0/9)
HCV RNA (Meq/ml)		
Median:	22.5	7.2
Range	(<0.24–63)	(<0.24–37)
Fibrosis staging		
(F2/F3/F4)	(0/2/6)	(1/4/4)
IFN therapy	5/8 (62.5%)	6/9 (66.7)
PLT ($\times 10^4$ μl)	9.31 ± 3.67	10.24 ± 4.64
TRX (ng/ml)	63.5 ± 30.8	53.6 ± 23.03
Vit. E (μmol/l)	12.8 ± 4.75	10.3 ± 2.13

IFN: interferon; PLT: platelet; TRX: thioredoxin; Vit. E: vitamin E. Data Mean ± SD.

significant. Statistical analyses were performed by the statistical package SAS (version 6.0).

RESULTS

The pretreatment features of the patients based on initial ALT levels are given in Table I. Eight out of 17 patients had pretreatment ALT values above 70 IU/l and 9 had ALT value below IU/l. Changes in biochemical values in group T before, during and after Vit. E administration are shown in Table II. Compared to the initial pretreatment value, TRX reduced significantly in the 2nd and 3rd month and serum Vit. E on the 1st, 2nd and 3rd month. The levels of ALT and AST showed a tendency towards reduction in the first 2 months after the beginning of therapy. In the 3rd month both ALT and AST levels tended to rise slightly. One month after therapy, serum ALT, AST and TRX levels tended to be elevated compared to the third month of treatment but were below the initial pretreatment values. Vit. E levels decreased 2 month post therapy, but tended to be slightly elevated compared to pretreatment values. Table III shows the changes in ATL, TRX and Vitamin E levels before, during and 1-month after Vit. E therapy, according to groups H and L. In group H, the serum ALT level decreased significantly on the 1st, 2nd, 3rd and 1-month after Vit. E therapy. TRX levels in group H showed a tendency towards reduction but not significantly. In group L, there was no comparable changes in ALT levels throughout the observation period. Also in group L, the TRX level decreased significantly in the 2nd, 3rd and 1-month after Vit. E therapy. Serum Vit. E levels were significantly elevated in both H and L groups in the 1st, 2nd and 3rd months following treatment. One month after withdrawal, serum Vit. E levels fell to almost pretreatment values. Table IV shows the pretreatment values of ALT, TRX and Vit. E, used as controls. No significant changes in the above biochemical values were observed prior to Vit. E therapy.

DISCUSSION

Oxidative stress has been implicated in virus infection and also observed in peripheral blood mononuclear cells from chronic hepatitis C patients.[18] TRX is a stress inducible thiol-containing protein whose activities include scavenging of active oxygen radicals and regulation of redox-sensitive molecules.[11,15] The increased serum TRX levels in HCV patients suggests that oxidative stress may contribute to the cytopathic effects of HCV on the liver. Measurement of serum TRX level has been shown to be a useful clinical parameter when HCC is suspected.[19] The antioxidant activities of Vit. E in HCV have been demonstrated previously.[20,21] Lower levels of Vit. E in patients with acute or chronic viral hepatitis, with high activity of disease, has been suspected to be due to free radical mediated liver injury.[22] In this study, in HCV patients with chronic

TABLE II Data showing changes in serum levels ALT, AST, TRX and Vit. E, in the course of the study in all HCV patients ($n = 17$)

	Vitamin E (d α-tocopherol 500 mg/day)				
	Initial value	1st Month	2nd Month	3rd Month	Post treatment
ALT (U/l)	73.78 ± 32.48	65.24 ± 30.52	60.94 ± 27.11	62.77 ± 18.96	62.65 ± 19.13
AST (U/l)	71.72 ± 29.90	62.29 ± 19.89	59.22 ± 21.58	62.29 ± 18.01	63.47 ± 17.96
TRX (ng/ml)	59.56 ± 26.39	62.72 ± 31.87	42.93 ± 17.22*	40.21 ± 18.81*	48.03 ± 27.06
Vit. E (μmol/l)	11.38 ± 3.63	28.21 ± 6.88**	30.19 ± 8.15**	28.60 ± 7.62**	12.18 ± 6.59

ALT: alanine aminotransferase; TRX: thioredoxin; Vit. E: vitamin E; *($p < 0.05$); **($p < 0.0001$). Mean ± SD.

TABLE III Data showing changes in serum levels ALT, TRX and Vitamin E according to group H and group L

	Vitamin E (d α-tocopherol 500 mg/day)				
	Initial value	1st Month	2nd Month	3rd Month	Post treatment
Group H					
ALT (U/l)	86.50 ± 8.57	68.75 ± 11.79*	61.88 ± 7.43*	70.75 ± 13.70*	74.25 ± 19.13*
TRX (ng/ml)	63.46 ± 30.80	63.57 ± 34.46	48.33 ± 20.25	45.06 ± 22.57	60.78 ± 33.49
Vit. E (μmol/l)	12.78 ± 4.75	27.81 ± 5.92*	29.64 ± 9.13*	28.70 ± 6.75*	14.50 ± 9.49
Group L					
ALT (U/l)	51.11 ± 11.48	49.95 ± 17.97	49.78 ± 17.05	55.67 ± 20.84	52.33 ± 16.13
TRX (ng/ml)	53.60 ± 23.03	66.64 ± 28.63	37.76 ± 15.08*	35.90 ± 14.78*	36.68 ± 13.03*
Vit. E (μmol/l)	10.33 ± 2.12	30.17 ± 6.56**	32.29 ± 6.09**	30.25 ± 7.04**	10.30 ± 1.94

ALT: alanine aminotransferase; TRX: thioredoxin; Vit. E: vitamin E. *($p < 0.05$); **($p < 0.0001$). Mean ± SD.

and progressive fibrosis, TRX was used as an indicator of oxidative stress and TRX, ALT, AST and Vit. E levels were observed in serum, before, during and after a 3-month period of Vit. E therapy.

Reduction in ALT and AST levels in the subsequent months following Vit. E therapy can be also attributed to the effect of Vit. E, though continuous anti-inflammatory drugs may be responsible for most part of it.

In group T, the significant decrease in TRX in the 2nd and 3rd months after Vit. E supplementation suggested that, oxidative damage was reduced and the rise in TRX one month after cessation of Vit. E therapy showed that absence of Vit. E caused elevation of TRX. The fact that the TRX level 1-month after cessation of therapy was, below initial pretreatment values, might be attributed to the presence of Vit. E in very small amounts, still in the system. However, 2 month after cessation of Vit. E therapy, ALT and AST showed a tendency towards reduction. The significant rise in serum Vit. E levels in the 1st, 2nd and 3rd months of Vit. E therapy shows some absorption by the system and thus its presence in causing the observed effects, that is reduction of TRX and transaminase levels. Dividing the patients into two groups enabled us to observe the individual effects of Vit. E between low (L) and high (H) ALT groups.

In group H, the TRX levels reduced, though not significantly and was elevated after withdrawl of Vit. E. Pretreatment values of TRX tended to be higher in group H compared to group L. Perhaps in group H, the level of oxidative damage was much more severe.

The significant decrease in ALT in group H, in the months following Vit. E therapy and 1-month post therapy is a clear indication that Vit. E has been effective in aiding the activity of continuous anti-inflammatory drugs to lower serum ALT levels. Of course, to actually state that Vit. E is solely responsible for the reduction of ALT in this study group, is not possible, as all these patients have received anti-inflammatory drugs and some cases IFN, prior to Vit. E. Untreated cases are essential to understand the actual role of Vit. E in ALT reduction in CHC patients.

In group L, the significant reduction of TRX in the months following Vit. E therapy and one month after suggests the effectiveness of Vit. E in successfully controlling oxidative damage in this group. However, ALT levels showed little or no change at the end of the study, from pretreatment values. Longer supplementary regimes might be necessary to actually observe the effects of Vit. E if any, on the ALT status in this group.

In patients belonging to the high ALT group, even after receiving initial IFN or anti-inflammatory drugs, pretreatment transaminase levels were high. Perhaps these drugs were not fully effective. In such cases, Vit. E seems to be a good choice as a supplementary treatment, to reduce oxidative stress and control liver damage. Further combinations of Vit. E with other supplementary treatments such as Vitamin C,[23] may prove more effective in controlling

TABLE IV Data showing changes in serum levels ALT, AST, TRX and Vit. E, in first (control phase) among the total patients

	Vitamin E (d α-tocopherol 500 mg/day)		
	Two months prior to Vit. E	One month prior to Vit. E	Initial value prior to Vit. E
ALT (U/l)	69.14 ± 22.74	71.14 ± 29.18	73.78 ± 32.48
AST (U/l)	65.64 ± 27.3	65.0 ± 21.1	71.72 ± 29.90
TRX (ng/ml)	58.95 ± 30.96	49.46 ± 22.14	59.56 ± 26.39
Vit. E (μmol/l)	5.78 ± 1.18	6.25 ± 1.79	11.38 ± 3.63

ALT: alanine aminotransferase; TRX: thioredoxin; Vit. E: vitamin E.

disease progression in CHC, and in slowing down the processes leading to HCC.

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