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Vitamin E as treatment for chronic hepatitis B: results of a randomized controlled pilot trial☆

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Abstract

Background and aims: Interferon-α treatment has been the treatment of choice for chronic hepatitis with unpredictable results. Recently, Lamivudine has been licensed for use against HBV infection with good results. Unfortunately, recurrence of viremia after lamivudine withdrawal is common and prolonged treatment can induce the emergence of resistant mutant strains. It has been shown that vitamin E can increase the host immune response, and this may provide protection against infectious diseases. *Methods:* We evaluated vitamin E supplementation as therapy for chronic hepatitis B in a pilot study including 32 patients. Patients were randomly allocated to receive vitamin E at the dose of 300 mg twice daily for 3 months (15 patients) or no treatment (17 patients). They were seen monthly during the first 3 months and thereafter quarterly for additional 12 months. *Results:* The two groups were comparable at enrollment. At the end of the study period, alanine aminotransferase (ALT) normalization was observed in 7 (47%) patients in vitamin E group and only in 1 (6%) of the controls ($P = 0.011$); HBV-DNA negativization was observed in 8 (53%) patients in the vitamin E group as compared to 3 (18%) in the control group, respectively ($P = 0.039$). A complete response (normal ALT and negative HBV-DNA) was obtained in 7 (47%) patients taking vitamin E and in none of the controls ($P = 0.0019$). *Conclusion:* Vitamin E supplementation might be effective in the treatment of chronic hepatitis B.

1. Introduction

Approximately 350 million people worldwide are chronically infected by hepatitis B virus (HBV). The clinical course is extremely variable, ranging from self-limited liver disease to severe chronic active hepatitis potentially evolving to

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cirrhosis and hepatocellular carcinoma (Lee, 1997). HBV is generally believed not to be a direct cytophatic virus and both viral persistence and liver damage can be related to the weak and poorly effective host's immune response (Lee, 1997). Interferon-α (IFNα), an immunomodulatory and antiviral agent, has been the first choice treatment for this infection but the results are unpredictable. Patients with wild type infection (HBeAg-positive), low serum HBV-DNA concentration and high alanine aminotransferase (ALT) levels are the best candidates to benefit from IFN treatment (about 40% of sustained response) (Perrillo et al., 1990), while those with high HBV-DNA and low ALT levels or those infected by e-minus HBV type (HBeAg-negative) tend to show a poor response to IFN treatment. In addition, IFN-α therapy is burdened with relevant side effects.

In the last years several nucleoside analogues have been evaluated as treatment for HBV infection but only lamivudine has been licensed worldwide. The results of several controlled trials demonstrated that this drug is able to induce ALT normalization, HBV-DNA reduction to undetectable levels and improvement of histological scores of inflammation both in HBeAg-positive and -negative patients (Lai et al., 1998; Dienstag et al., 1999; Tassopoulos et al., 1999; Santantonio et al., 2000; Schalm et al., 2000). Lamivudine has been successfully used in liver transplant recipients (Grellier et al., 1996; Andreone et al., 1998a; Perrillo et al., 1999). In this setting, the best results were obtained when the drug was utilized as prophylaxis of HBV reinfection both as a single agent (Grellier et al., 1996) or in combination with hepatitis B immune globulin (Markowitz et al., 1998; Andreone et al., 2000). Unfortunately, recurrence of viremia after therapy withdrawal is common, and prolonged treatment can induce the emergence of drug-resistant mutant strains.

Due to the likely involvement of defective immune system response in the pathogenesis of HBV-induced liver damage, the therapeutic efficacy of several immunomodulatory substances has been tested, but the results are conflicting (Fattovich et al., 1986; Mutchnick et al., 1991; Fattovich et al., 1994; Farhat et al., 1995; Andreone et al., 1996; Chien et al., 1998; Mutchnick et al., 1999).

Vitamin E (α-tocopherol) is an essential vitamin with anti-oxidant properties (Meydani, 1995) that is also able to enhance the cell-mediated immunity (Gogu and Bloomberg, 1993; Meydani, 1995). Some recent preliminary trials showed its possible beneficial role in the treatment of both chronic hepatitis B (Andreone et al., 1998b) and chronic hepatitis C (Von Herbay et al., 1997).

Here, we report the results of a controlled pilot study aimed at assessing the efficacy of vitamin E as treatment for chronic HBV infection.

2. Patients and methods

Thirty-two patients with histologically proven chronic hepatitis B, positive serum HBV-DNA and raised ALT levels (>1.5-fold the upper normal limit) for at least 6 months were included in the study. Twelve were HBeAg-positive and 20 HBeAb-positive. Twenty-three had unsuccessfully been treated with IFNα and the treatment was stopped at least 1 yr before enrollment in this trial. No patient had received any other active drug against HBV. Patients with decompensated liver disease, history of hepatic encephalopathy or ascites, esophageal or gastric varices at risk of bleeding, hepatitis delta or C virus and human immunodeficiency virus infections, causes of liver disease other than HBV, intravenous drug abuse, pregnancy, malignancy, chronic renal failure and immunosuppressive or antiviral therapy in the preceding 6 months were excluded. Patients were randomly assigned by a computer-generated program to receive either vitamin E (Ephynal, Roche, Milano, Italy) at a dose of 300 mg twice a day for 3 months or no treatment. They were seen monthly during the first 3 months and thereafter quarterly for an additional 12 months. Clinical and laboratory assessments were performed at each visit and included routine serum chemistry tests, complete blood counts and HBV serology. A complete response was defined as serum HBV-DNA clearance and normal ALT. Commercial assays were utilized to determine: markers of

HBV (Abbott Laboratories, North Chicago, IL), anti-hepatitis D virus (Sorin Biomedical, Saluggia, Italy), antibodies to hepatitis C virus (Anti-HCV Assay kit, Ortho Diagnostic Systems, Milano), antibody to human immunodeficiency virus (Recombigen human immunodeficiency virus 1 EIA, Ortho Diagnostic Systems). Serum HBV-DNA was measured quantitatively by liquid hybridization (HBV-DNA, Abbott Laboratories; cut-off = 1 pg/ml).

Results are expressed as mean ± standard deviation and analyzed by Mann–Whitney test for unpaired data, Wilcoxon rank sum test, Fisher's exact test and Friedman two-way ANOVA test when appropriate. A P value < 0.05 was considered to be statistically significant. The computations were performed by SOLO (BMDP Statistical Software, Los Angeles, CA).

The study was approved by the Ethical Committee of our Center and oral consent was obtained from all patients.

3. Results

Thirty-two patients were enrolled in this study: 15 were randomized to receive vitamin E and 17 no treatment. Four patients (all HBeAb positive at enrollment) discontinued vitamin E (2 after the first and 2 after the second month) because of a severe ALT flare (more than 10-fold the normal upper limit). A similar finding was also observed in two controls. All patients ended the 15 months of observation except for one of the vitamin E group who dropped out 9 months after the start of therapy and was considered as non-responder.

At inclusion, the 2 groups were comparable for age, sex, source of infection, ALT and HBV-DNA levels and histological features (Table 1). No difference was found between treated and control cases concerning the time elapsed since IFN withdrawn (16.7 ± 2.8 vs. 15.9 ± 3.1 months, respectively). Table 2 reports the biochemical and virological responses during the observation period subdivided quarterly. After 3 months, in the vitamin E group 4 patients (27%) showed normalized ALT levels and 6 (40%) became HBV-DNA negative, while the corresponding figures in the control group were 1 (6%) and 4 (24%), respectively. At the end of the study period, 7 patients (47%) in the vitamin E group and 1 (6%) of the controls had normal ALT levels ($P = 0.011$), while HBV-DNA was negative in 8 (53%) and in 3 (18%), respectively ($P = 0.039$). A complete (ALT and HBV-DNA) response at the end of the study period was observed in 7 patients (47%) in the vitamin E group and in no one of the controls ($P = 0.0019$). All patients treated with vitamin E, except for one, maintained the complete response once achieved, while none of the control showed a similar finding. In 2 patients only (both HBeAb-positive) the complete response was preceded by an ALT flare. Among the 7 complete responders 3 were HBeAg-positive at enrollment and seroconverted to HBeAb (two after 3 months, and one after 9 months) and 5 were non-responders to a previous IFNα treatment. None of the HBeAg-positive patients in the control group serocon-

Table 1
Characteristics of patients at enrollment[a]

	Vitamin E (15 patients)	Controls (17 patients)	P
Age (yr)	37 ± 15	42 ± 14	NS
M/F	10/5	14/3	NS
Source of infection: intrafamilial/unknown	9/6	11/6	NS
ALT (U/l) (range)	85 ± 39 (43–163)	91 ± 41 (47–186)	NS
Serum HBV-DNA (pg/ml) (range)	61 ± 85 (5–253)	40 ± 65 (5–229)	NS
HBeAg positive	7	5	NS
Histology: CAH/CAH + cirrhosis	10/5	15/2	NS
Previous IFNα therapy	13	10	NS

[a] Data expressed as mean ± S.D. CAH – chronic active hepatitis; normal ALT values <40 U/l; NS – non-significant.

Table 2
Biological and virological response in the two patient groups[a]

	Vitamin E (15 patients)	Controls (17 patients)	*P*
After 3 months			
ALT normalization	4 (27%)	1 (6%)	NS
HBV-DNA-negative	6 (40%)	4 (24%)	NS
Complete response	2 (13%)	0 (0%)	NS
After 6 months			
ALT normalization	6 (40%)	4 (24%)	NS
HBV-DNA-negative	8 (53%)	7 (41%)	NS
Complete response	5 (33%)	2 (12%)	NS
After 9 months			
ALT normalization	9 (60%)	4 (24%)	0.04
HBV-DNA-negative	8 (53%)	4 (24%)	NS
Complete response	6 (40%)	2 (12%)	NS
After 12 months			
ALT normalization	9 (60%)	2 (12%)	0.0057
HBV-DNA-negative	9 (60%)	5 (30%)	NS
Complete response	8 (53%)	2 (12%)	0.0149
After 15 months			
ALT normalization	7 (47%)	1 (6%)	0.011
HBV-DNA-negative	8 (53%)	3 (18%)	0.039
Complete response	7 (47%)	0 (0%)	0.0019

[a] Data analyzed by Fisher's exact test. NS – non-significant.

verted to HBeAb. No patient in either groups lost HBsAg or seroconverted to HBsAb.

Baseline ALT and HBV-DNA levels were comparable between the 2 groups (Table 1). At the end of the study, ALT and HBV-DNA serum levels were significantly lower in patients receiving vitamin E, as compared to untreated (ALT: 52 ± 53 vs. 84 ± 63 U/l, $P = 0.011$; HBV-DNA: 4 ± 7 vs. 38 ± 80 pg/ml, $P = 0.034$). During the 15 month observation period, ALT and HBV-DNA trend was statistically significant (Friedman two-way ANOVA test) only in the vitamin E-treated group ($P = 0.05$ and 0.008, respectively). The mean percentages of change of HBV-DNA values are shown in Fig. 1.

When the treated patients were subdivided on the basis of the response (complete or not), no differences were found concerning demographic, biochemical, virological and histological characteristics at enrollment (Table 3). No side effects were recorded during the treatment with vitamin E.

4. Discussion

The results of this study indicate that vitamin E in doses of 600 mg daily was effective in suppressing HBV replication and normalizing ALT in a significant proportion of the patients. In fact, a complete response was seen in 47% of patients treated with vitamin E and 0% in the untreated

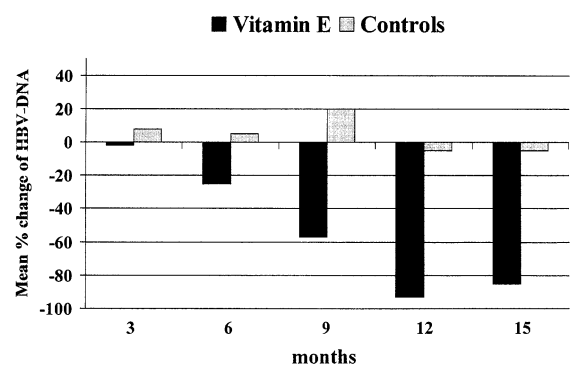

Fig. 1. Median percentage change of HBV-DNA levels during the 15-month study period.

Table 3
Differences at enrollment in the characteristics of patients treated with vitamin E subdivided on the basis of a complete response[a]

	Responders (7 patients)	Non-responders (8 patients)	*P*
Age	36 ± 16	38 ± 16	NS
M/F	4/3	6/2	NS
Source of infection: intrafamilial/unknown	6/1	3/5	NS
HBeAg-positive	3	4	NS
ALT (U/l)	74 ± 28	94 ± 46	NS
Serum HBV-DNA (pg/ml)	78 ± 107	47 ± 66	NS
Histology: CAH/CAH + cirrhosis	5/2	5/3	NS

[a] Data expressed as mean ± S.D. CAH – chronic active hepatitis; ALT normal values <40 U/l; NS – non-significant.

patients after 15 months of observation. This response rate is similar to that obtained in trial utilizing IFNα (Perrillo et al., 1990) or Thymosin-α1 (Andreone et al., 1996; Chien et al., 1998)

Interestingly, in the group treated with vitamin E, complete response (ALT normalization and HBV-DNA negativization) increased gradually after the end of treatment, and was particularly evident at 9 and 12 months after stopping treatment. This delayed response was never observed during treatment with interferon or nucleoside analogues (Perrillo et al., 1990; Nevens et al., 1997; Lai et al., 1998), which usually induce responses during the first months of treatment. On the contrary, it was well demonstrated in patients treated with Thymosin-α1 (Andreone et al., 1996; Chien et al., 1998), an immunomodulating agent of thymic origin. The reasons for this delayed effect of vitamin E are not clear. Since vitamin E is not known to possess a direct antiviral activity, the delayed response is likely due to an immunostimulating effect as postulated for Thymosin-α1 (Andreone et al., 1996; Chien et al., 1998; Mutchnick et al., 1999).

In this regard, it has been demonstrated that vitamin E is able to increase the proliferative response of lymphocytes and the natural killer cell activity and to polarize the T helper cells towards the T helper1-type (Th1) phenotype (Meydani et al., 1990; Wang et al., 1994). These effects seem to be crucial for cellular protection against viral spreading (Paul and Seder, 1994). In particular, in chronic hepatitis B, it has been demonstrated that the predominant intrahepatic T cell population is represented by Th0 cells mostly secreting IL-4 and IL-5, while the Th1 phenotype is poorly represented (Bertoletti et al., 1997). Therefore, it can be hypothesized that vitamin E helps restoring an adequate immune response to HBV antigens. Support to this hypothesis stems from the gradual serum HBV-DNA decrease and HBeAb seroconversion achieved in patients treated with vitamin E.

In this study we administered a 600 mg daily dose based on the evidence of previous reports by Meydani et al. (1990) and Meydani (1995), who demonstrated that a dosage ranging from 400 to 800 mg was able to improve immune responsiveness in healthy elderly individuals. The tolerability of vitamin E was excellent and without side effects.

In conclusion, the results of this pilot trial indicate that, at the dosage tested, vitamin E is of potential interest in the treatment of chronic hepatitis B. Considering its good tolerability and low price, it might represent an alternative or additional treatment to other therapies. Further studies are needed to confirm these interesting results in a larger number of patients and to evaluate different schedules of treatment and the effect of combination with antiviral drugs.

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