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Treatment of nonalcoholic steatohepatitis with vitamins E and C: a pilot study

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Background: Nonalcoholic steatohepatitis (NASH) is a common liver disease that can progress to cirrhosis. Oxidative stress is one of the central mechanisms causing hepatocellular injury in the disease. In this study, antioxidant therapy using both vitamins C and E was conducted in patients with NASH.

Methods: Vitamin E 300 mg/day and vitamin C 300 mg/day were administered orally to 23 patients with NASH for 12 months. Body mass index was measured during therapy. Serum levels of alanine aminotransferase, thioredoxin (an oxidative stress marker), and high-sensitivity C-reactive protein were measured before treatment and after 12 months in all patients. Ten of the 23 patients underwent liver biopsy before and after treatment.

Results: Body mass index remained unchanged during treatment with vitamins C and E. Serum alanine aminotransferase, thioredoxin, and high-sensitivity C-reactive protein levels were decreased significantly at 12 months compared with pretreatment. Liver biopsies showed improved necroinflammatory activity in eight cases and improved fibrosis staging in 4.

Conclusion: Serum alanine aminotransferase, thioredoxin, and high-sensitivity C-reactive protein levels, and liver histology were clearly improved with vitamin C and E therapy. These findings suggest that combination therapy using these vitamins may be useful in patients with NASH to minimize damage from oxidative stress and slow the processes leading to cirrhosis.

Keywords: vitamin E, vitamin C, nonalcoholic steatohepatitis, oxidative stress

Introduction

Nonalcoholic steatohepatitis (NASH) is a very common chronic liver disease that resembles alcoholic liver disease clinically and histologically, but occurs in individuals in the absence of a history of significant alcohol consumption. NASH is frequently associated with clinical conditions such as obesity, type 2 diabetes mellitus, hyperlipidemia, and hypertension. These background characteristics indicate that NASH may be part of the spectrum of "metabolic syndrome". More recently, NASH has been proposed as a possible cause of cryptogenic cirrhosis.[1] The pathogenesis of NASH is multifactorial, involving abnormal lipid metabolism, production of reactive oxygen species, increased hepatic lipid peroxidation, activated stellate cells, and abnormal patterns of cytokine production. Oxidative stress appears to be a key factor in the progression from steatosis to NASH and potentially to cirrhosis.[2–5]

No universally effective treatment for NASH has been identified. Vitamin E refers to a group of naturally occurring compounds with antioxidant properties. A pilot trial of vitamin E aimed at decreasing oxidative stress in pediatric patients with presumed

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NASH showed that serum alanine aminotransferase and aspartate aminotransferase levels normalized during this treatment.[6] We have previously evaluated the efficacy of vitamin E therapy in patients with NASH.[7] Significant improvements in serum alanine aminotransferase and gamma glutamyl transferase were observed during treatment. At the same time, levels of the oxidative stress markers, thioredoxin and thiobarbituric acid, were significantly decreased. Furthermore, Harrison et al recently reported that treatment with vitamins C and E improves liver fibrosis in patients with NASH.[8] In the present study, vitamins C and E were administered for 12 months at lower doses than those used by Harrison et al, and changes in levels of alanine aminotransferase, oxidative stress markers, and high-sensitivity C-reactive protein were measured before and after treatment to clarify the efficacy and mechanisms of action underlying vitamin C and E therapy. Thioredoxin is a stress-inducible thiol-containing protein that has been shown to be an indicator of oxidative stress in a variety of diseases. Weight reduction is reported to improve liver enzyme abnormalities and liver histology in obese patients with NASH.[9,10] However, many patients find it virtually impossible to maintain body weight loss. Therefore, this study evaluated the effects of vitamin C and E on serum alanine aminotransferase, thioredoxin, high-sensitivity C-reactive protein, and liver histology in patients with NASH in association with changes in body mass index during treatment.

Materials and methods

Patients

Twenty-three patients with NASH (ten men and 13 women, Matteoni classification 3 or 4, mean age 53.1 ± 14.9 years) were included in this study. Twenty patients had a body mass index > 25 kg/m^2. Nineteen patients had hypertension and 18 patients had hyperinsulinemia and dyslipidemia (Table 1). Before pretreatment, all patients had been diagnosed as having NASH by liver biopsy.

Table 1 Demographic feature of patients at baseline

Feature	n
N	23
Age	53.1 ± 14.9
Male/female	10/13
BMI (kg/m^2)	20
Hypertension	19
Hyperinsulinemia	18
Dyslipidemia	18
Stage 0/1/2/3	0/14/6/3
Grade 0/1/2/3	0/15/5/3

Diagnostic criteria

NASH was diagnosed based on the following criteria: negative results for hepatitis B surface antigen and hepatitis C virus RNA, and exclusion of autoimmune liver disease, drug-induced hepatic disorder, or metabolic liver disease (eg, Wilson's disease, hemochromatosis); alcohol intake ≤ 30 g/week; and presence of steatosis (>30%) or steatohepatitis. The pathological classification proposed by Matteoni et al[11] was used to diagnose histological types 1 and 2 as simple steatosis and types 3 and 4 as NASH. Fibrosis was graded from stage 0 to stage 4 in accordance with the staging system, and inflammatory activity in the liver was graded from grade 0 to grade 3, also according to the grading system proposed by Brunt et al.[12] Steatosis was defined according to the percent of hepatocytes affected, and divided into four grades: grade 0, 0%; grade 1, >0% but <33%; grade 2, 33%–66%; and grade 3, >66%. Some pathologists read the liver biopsy blinded. Diabetes mellitus was diagnosed based on the following criteria proposed by the Japan Diabetic Society: fasting blood glucose ≥ 126 mg/dL; two-hour post-75 g oral glucose tolerance test result ≥ 200 mg/dL; casual blood glucose ≥ 200 mg/dL; and glycosylated hemoglobin ≥ 6.1 or treatment with one or more antidiabetic agents. Dyslipidemia was defined as triglyceride > 150 mg/dL and/or a low-density lipoprotein cholesterol level > 140 mg/dL or treatment with one or more lipid-lowering drugs. Hypertension was defined as systolic/diastolic blood pressure ≥ 140/90 mmHg or treatment with one or more antihypertensives.

Changes in body mass index were observed monthly in all patients during vitamin C and E therapy. Serum thioredoxin[13] and high-sensitivity C-reactive protein[14] levels were determined using enzyme-linked immunosorbent assays (Mitsubishi Kagaku Yatoron, Tokyo, Japan). Serum thioredoxin levels were also estimated using an enzyme-linked immunosorbent assay kit (Ledox BioScience, Kyoto, Japan). Vitamin E (α-tocopherol, Eisai, Tokyo, Japan) and vitamin C (ascorbic acid, Sionogi, Tokyo, Japan) were each administered orally at 300 mg/day for 12 months.

During therapy, the lifestyle of all patients remained unchanged. Body mass index and serum alanine aminotransferase, thioredoxin, and high-sensitivity C-reactive protein levels were measured before and after treatment. Liver biopsy was performed for all patients before treatment, and ten patients from whom consent was able to be obtained underwent liver biopsy after treatment. Liver histology was evaluated by staging of fibrosis and grading of necroinflammatory activity and steatosis.

Immunostaining for 8-hydroxydeoxyguanosine, an oxidative stress marker, was also done before and after treatment.

The study was approved by the ethics committee at our institution and complied with the Declaration of Helsinki and internationally recognized guidelines. Informed consent was obtained.

Statistical analysis

The analysis was performed using JMP version 9.0.1 software (SAS Institute Japan, Tokyo, Japan). Univariate and multivariate analyses of predictors of survival were assessed using the Cox proportional hazards model. A P value less than 0.05 was considered to be statistically significant.

Results

During the 12 months of treatment with vitamins C and E, body mass index remained unchanged (26.6 ± 3.1 kg/m^2 at baseline and 26.8 ± 3.1 kg/m^2 after treatment). Serum alanine aminotransferase and high-sensitivity C-reactive protein levels decreased significantly during the 12 months, respectively, from 96.5 ± 45.9 IU/L to 40.3 ± 17.6 IU/L ($P < 0.0001$, Figure 1) and from 133.5 ± 59.8 mg/L to 67.5 ± 47.5 mg/L ($P < 0.005$, Figure 1). Before therapy, serum thioredoxin levels were 43.5 ± 17.2 ng/mL, but remained below 35.7 ± 11.5 ng/mL by 12 months following vitamin C and E therapy ($P = 0.08$, Figure 1). In the liver, staging of fibrosis, grading of necroinflammatory activity, and steatosis were compared before and after treatment. Staging of fibrosis improved in four of 10 cases, grade of necroinflammatory activity decreased in eight of 10 cases, and grade of steatosis decreased in six of 10 cases. In four patients, the grade of steatosis remained unchanged, but the grade of necroinflammatory activity improved in all cases, and staging of fibrosis also improved in two cases (Table 2). Table 3 compares histological data for case 3 following treatment with vitamins C and E. Fibrosis stage improved from F3 to F1, while the grading of necroinflammatory activity decreased from A3 to A1. However, no change in fatty deposition was evident. Treatment with vitamins C and E also resulted in a decrease in 8-hydroxydeoxyguanosine levels (Table 4).

Discussion

The pathogenesis of NASH remains poorly understood. Obesity, type 2 diabetes mellitus, hyperinsulinemia, increased triglyceride levels, toxins, and medical conditions can lead to increased serum fatty acid levels, which are then presented to the liver. The multihit theory suggests that the first hit involves accumulation of excess fat in the hepatic parenchyma. The second hit involves oxidative stress resulting from an imbalance between pro-oxidant and antioxidant processes in the liver, which may result from induction of microsomal cytochrome P450 2E1, mitochondrial release of reactive oxygen species, release of hydrogen peroxide from peroxisomal-beta oxidation of fatty acids, release of cytokines from activated inflammatory cells, and insulin resistance.[1–5]

Optimal therapies for NASH have yet to be established. Maintenance of weight loss results in significant clinical and histological improvement.[9,10] However, adverse effects on liver histology, such as progression of fibrosis, have also been noted.[15]

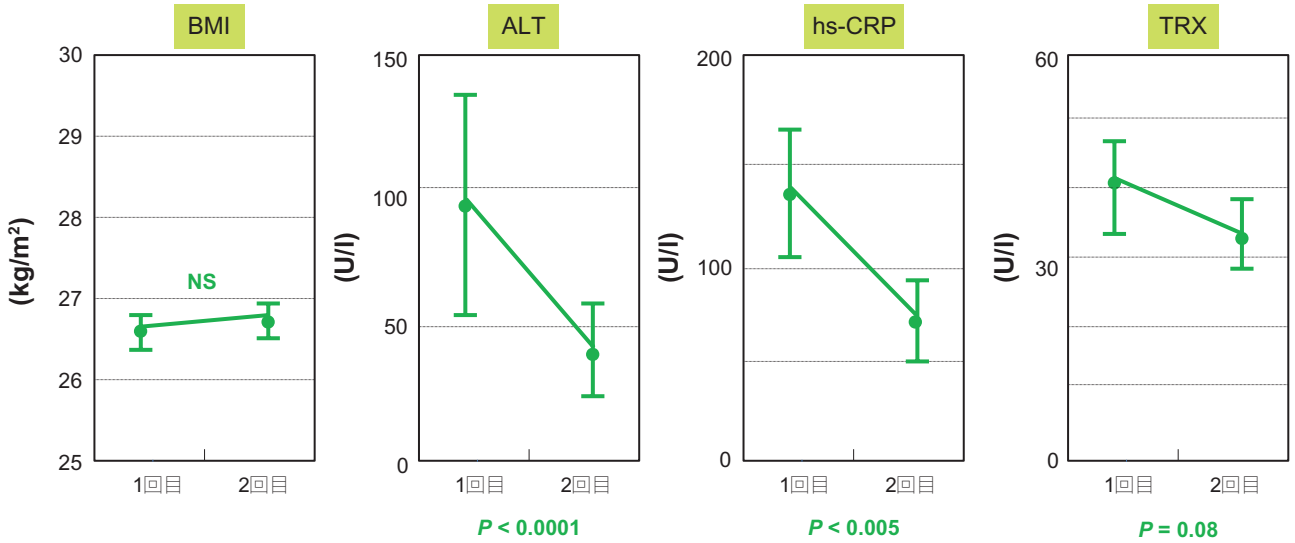


Figure 1 Body mass index remained unchanged, but changes were seen in serum alanine aminotransferase, thioredoxin, and high-sensitivity C-reactive protein levels in patients with nonalcoholic steatohepatitis before and 12 months following treatment with vitamins C and E.
Abbreviations: BMI, body mass index; ALT, alanine aminotransferase; hs-CRP, high-sensitivity C-reactive protein; TRX, thioredoxin.

Table 2 Steatosis, grading and fibrosis score in NASH patients before and after 12 months of vitamin E and C (n = 10)

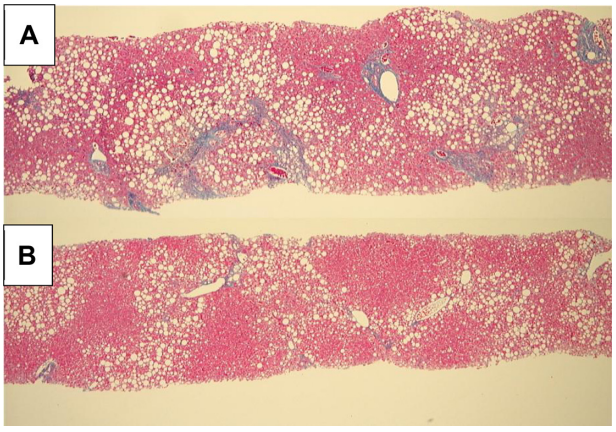
Case	Age/ gender	Grading of steatosis	Grading of activity	Grading of fibrosis
		Before/after	Before/after	Before/after
1	57/F	2→2	2→1	2→2
2	50/M	3→3	2→1	2→1
3	57/M	2→2	3→1	3→1
4	61/F	1→1	2→0	3→1
5	65/F	3→2	2→1	1→1
6	46/M	3→1	2→1	1→0
7	27/M	3→1	1→1	1→1
8	59/M	1→0	1→0	0→0
9	35/M	2→1	2→1	2→1
10	60/F	2→1	2→1	0→0

Abbreviation: NASH, Nonalcoholic steatohepatitis.

Furthermore, most obese patients find it virtually impossible to maintain weight loss. Other treatments include modification of the clinical conditions associated with NASH, in particular type 2 diabetes mellitus, hyperlipidemia, and obesity.

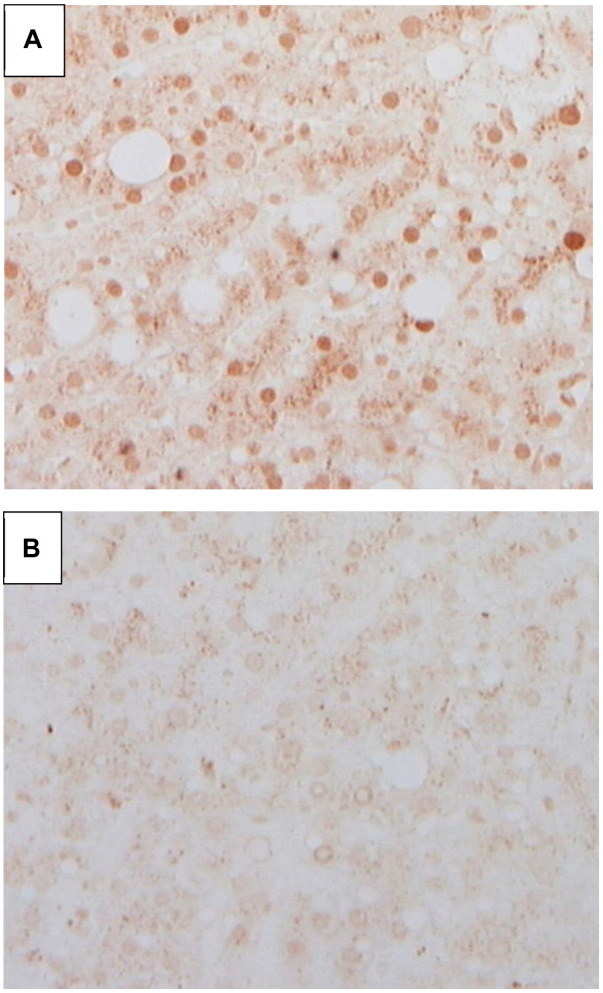
Medications that minimize oxidative stress may prove useful in the treatment of NASH. Vitamin E is a well known and potent antioxidant. A pilot study of vitamin E therapy has been done in a small, open-label study of 11 pediatric patients with presumed NASH.[6] These children were given 400–1200 IU of vitamin E for 4–10 months. The vitamin E was well tolerated and resulted in normalization of liver function tests during treatment. However, biochemical improvement was not sustained when vitamin E was discontinued. Hasegawa et al[16] did a study in patients with NASH receiving vitamin E 300 mg/day for one year. Serum alanine aminotransferase levels decreased, accompanied by decreases in plasma levels of transforming growth factor β1. We have also reported a pilot study of vitamin E treatment in NASH.[7] In this study, significant improvements were observed in both serum thioredoxin and thiobarbituric acid-reactive substance levels, which are believed to be clinically useful indicators of oxidative stress, along with a decrease in alanine aminotransferase. We have previously reported that liver damage induced by oxidative stress is reduced by vitamin E in patients with chronic hepatitis C.[17] A report investigating the effects of aerobic exercise in patients with NASH revealed no differences according to administration or nonadministration of vitamin E,[18] but this lack of apparent effect was probably due to the efficacy of treatment with diet and exercise alone. In a recent report, Sanyal reported that vitamin E (n = 84) and pioglitazone (n = 80) in patients with NASH significantly improved serum alanine aminotransferase levels, hepatic steatosis, and lobular inflammation, as compared with placebo, but with no improvement in fibrosis.[19]

Table 3 Histological interpretation of the effect of vitamin E and C on NASH (**A**) pretreatment (**B**) post treatment



Abbreviation: NASH, Nonalcoholic steatohepatitis.

Table 4 Change of 8OHdG expression of the effect of vitamin E and C on NASH (**A**) pretreatment (**B**) post treatment



Abbreviation: 8OHdG, 8-hydroxydeoxyguanosine.

Furthermore, significant improvements in liver fibrosis were reported in patients with NASH treated using a combination of vitamins C and E. When administered together with vitamin C, vitamin E can actively combat oxidative stress. Harrison et al reported that 21 patients with NASH were given vitamin E 1000 IU and vitamin C 1000 mg or placebo daily for six months, yielding a significant improvement in fibrosis score among treated patients.[8] In Japan, the approved daily doses of vitamin E and vitamin C (300 mg/day and 600 mg/day, respectively) are lower than those used by Harrison et al. Therefore, the present study investigated the utility of 300 mg vitamin E and 600 mg vitamin C for the treatment of NASH in Japanese patients. Furthermore, because previous studies of treatment with vitamin E for NASH have not evaluated the antioxidant potential of vitamin E, we also conducted immunostaining for the oxidative stress markers, thioredoxin and 8-hydroxydeoxyguanosine, in serum and hepatic tissue, respectively.

In the present study, serum alanine aminotransferase levels improved, accompanied by decreases in serum thioredoxin, high-sensitivity C-reactive protein, and 8-hydroxydeoxyguanosine levels, on combination therapy using vitamins C and E. In both arteriosclerosis and metabolic syndrome, high-sensitivity C-reactive protein offers a sensitive marker of chronic low-grade inflammation. Targher et al reported that the severity of liver histology in patients with NASH was strongly associated with increasing plasma levels of high-sensitivity C-reactive protein, plasminogen activator inhibitor-1, and fibrinogen, and decreasing plasma adiponectin concentrations. In addition, NASH and visceral adiposity predicted cardiovascular risk biomarkers independent of potential confounders, and NASH can predict a more atherogenic risk profile in a manner that is partly independent of the contribution of visceral adiposity in adult men.[20]

Our histological study found that grade of necroinflammatory activity improved in eight of ten treated patients, and fibrosis stage in four treated patients. The grade of steatosis improved in five of ten cases, indicating that the effects of combination therapy are not associated with weight loss. Although the mechanism is not clear, it may be that if liver inflammation is improved for a long time, liver fibrosis may also be improved.

Based on our experience in this pilot study, we conclude that treatment of NASH for 12 months with vitamins C and E results in significant improvement in alanine aminotransferase, thioredoxin, and high-sensitivity C-reactive protein levels, as well as in liver cell damage and inflammation. Given that no proven treatments for NASH are known, the possible benefits of vitamins C and E should be investigated further in a randomized controlled trial.

Disclosure

The authors report no conflicts of interest in this work.

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