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Vitamin E and C supplementation prevents decrease of eicosapentaenoic acid in mononuclear cells in chronic hepatitis C patients during combination therapy of interferon α-2b and ribavirin

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Abstract

Objective: We investigated the effects of vitamin E and C supplementation on the fatty acid composition of mononuclear cells and on the clinical observations in patients who had chronic hepatitis C and received interferon-α-2b (IFN-α-2b) and ribavirin combination therapy.

Methods: Patients were randomly allocated to receive daily 500 mg of vitamin E and 750 mg of vitamin C (vitamin group, $n = 14$) or no supplement (non-vitamin group, $n = 16$) in addition to IFN-α-2b and ribavirin therapy. The fatty acid composition of mononuclear cell phospholipids was analyzed before and at 2, 4, and 8 wk after treatment.

Results: After vitamin supplementation, plasma and red blood cell α-tocopherol and plasma ascorbic acid levels increased in the vitamin group. Serum levels of alanine aminotransferase decreased significantly after 2 wk of treatment in both groups. At the start of treatment, a lower level of eicosapentaenoic acid (EPA) and a higher level of the molar ratio of arachidonic acid to EPA in mononuclear cells were observed in the present patients compared with healthy volunteers, and a significant correlation between the molar ratio and serum alanine aminotransferase level was found. The EPA level of mononuclear cells was maintained in the vitamin group during treatment, whereas a significant decrease was observed in the non-vitamin group at 4 and 8 wk after treatment.

Conclusions: Antioxidant vitamin supplementation during IFN-α-2b and ribavirin therapy prevented a decrease in EPA of mononuclear cell phospholipids. If a further decrease in the ratio of arachidonic acid to EPA can be achieved by using oral EPA supplementation, the efficacy of IFN-α-2b and ribavirin therapy may be improved.

Keywords: Vitamin E; Vitamin C; Eicosapentaenoic acid; Arachidonic acid; Interferon; Ribavirin; Hepatitis C patients; Mononuclear cell

Introduction

Antiviral therapy using interferon-α-2b (IFN-α-2b) is an important component in the treatment of patients who have hepatitis C virus (HCV). IFN-α-2b therapy combined with ribavirin, a purine nucleoside analog, is reported to induce a higher sustained viral response compared with IFN-α-2b monotherapy [1]. The principal mechanism of action of ribavirin in the combination therapy has been postulated to be that ribavirin serves as an immunomodulator to enhance the production of type 1 cytokines, including interleukin-2, IFN-γ, and tumor necrosis factor-α (TNF- α) [2]. However, hemolytic anemia is a known side effect of ribavirin therapy [3]. The mechanism by which ribavirin causes hemolysis remains uncertain but may be related to its accumulation in erythrocytes,

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which results in oxidative stress [4]. A moderate decrease in leukocyte counts has also been reported in patients who receive combination therapy and those who received IFN monotherapy.

Increased oxidative stress has been shown to accompany chronic liver disease [5–8]. Various factors, including hepatocellular iron accumulation [9,10] and the direct effect of the HCV core protein [11,12], are currently considered to be the causes of oxidative stress, in addition to reactive oxygen species production that is related to activated phagocytes and inflammatory cytokines such as TNF-α and interleukin-1β during the immune response [13,14].

In a previous study [15], we observed a decreased level of plasma α-tocopherol and an increase in plasma thiobarbituric acid-reactive substances (TBARS) in patients who had HCV and cirrhosis and showed an abnormal fatty acid pattern, with low levels of polyunsaturated fatty acids (PUFAs) in phospholipids of mononuclear cells. We also reported that vitamin E supplementation induced a significant increase in ω 6 and ω 3 PUFAs in the phospholipids of erythrocyte membranes of HCV-infected patients, which was accompanied by an improvement in the alanine aminotransferase (ALT) level [16]. Previously, von Herbay et al. [17] observed that high vitamin E supplementation improves the aminotransferase status in patients who have chronic HCV. Vitamin E is one of the safest antioxidants [18], but the mechanisms of its physiologic functions are not thoroughly understood [19]. Vitamin C can regenerate α-tocopherol from the α-tocopherol radical and, hence, cooperates with α-tocopherol in inhibiting lipid peroxidation [20].

Peripheral blood mononuclear cells are involved in the antiviral immune response. Some reports have shown a change of immune state, such as the functional impairment of HCV-specific $CD4^+$ T cells, in patients with chronic hepatitis C [21]. Several human studies have suggested that PUFAs have immunomodulatory actions [22]. The PUFAs, especially arachidonic acid (AA) and eicosapentaenoic acid (EPA), in cell membrane phospholipids are precursors of the eicosanoids and are involved in lipid-mediated intracellular signaling.

In the present study, we investigated the effects of vitamin E and C supplementation on the fatty acid composition of mononuclear cell phospholipids and on the clinical observations in patients who had chronic hepatitis C and received IFN-α-2b and ribavirin combination therapy.

Materials and methods

Subjects

Thirty patients who had chronic hepatitis C (16 men and 14 women, average age 52.4 $\pm$ 2.0 y) were enrolled in this study. The criteria for enrollment were a persistently increased ALT level for longer than 6 mo before enrollment, positive results for HCV RNA in serum, and a high viral load ($>10^5$ IU/mL). Patients with decompensated cirrhosis, detectable hepatitis B virus surface antigen, other potential causes of chronic liver disease, hemoglobin (Hb) level lower than 12 g/dL, platelet count lower than 70 000 /mm^3, human immunodeficiency virus infection, poorly controlled diabetes mellitus, or cardiovascular disease were excluded.

The baseline characteristics of the patients in both groups were similar (Table 1). HCV genotypes were as follows: 1a/1b/2a/2b: 1/9/4/0 in the vitamin group and 0/10/5/1 in the non-vitamin group. No significant differences were observed in the distribution of the histologic diagnosis for grading inflammation (stages 1 to 3; not done: 3/4/2/5 in the vitamin group and 2/11/2/1 in the non-vitamin group) or for staging fibrosis (grades 1 to 3, not done: 4/4/1/5 in the vitamin group and 4/7/4/1 in the non-vitamin group) at baseline.

The control subjects were healthy volunteers (four men and four women, average age 54.3 $\pm$ 2.8 y).

Study design

We obtained informed consent from all patients and randomized them to receive daily 500 mg of vitamin E (Juvela, one tablet contains 50 mg of tocopherol acetate; Eisai, Tokyo, Japan) and 750 mg of vitamin C (HICEE, one packet contains 250 mg of ascorbic acid; Takeda Chemical Industries, Osaka, Japan) orally after all three meals (vitamin E: morning 150 mg, afternoon 150 mg, and evening 200 mg; vitamin C: morning 250 mg, afternoon 250 mg, and evening 250 mg; vitamin group: nine men and five women, average age 53.9 $\pm$ 2.6 y) or no supplementation (non-vitamin group: seven men and nine women, average age 51.1 $\pm$ 3.0 y), in addition to injections of 6 million units of IFN-α-2b (Intron A, Schering-Plough K.K., Osaka, Japan) six times weekly for 2 wk, followed by three times weekly for 24 additional wk, plus daily oral 600 mg (body weight $\leq$60 kg) or 800 mg (body weight $>$60 kg) of ribavirin (Revetol, Schering-Plough K.K.) for 24 wk. During treatment, the dose of ribavirin was decreased by 200 mg/d in patients whose Hb level decreased below 10 g/dL, and ribavirin was discontinued when the level decreased below 8.5 g/dL. Patients were required not to take iron or other antioxidant supplements for the treatment period.

Blood samples were obtained from each patient immediately before the initiation of therapy (0 wk) and at 2, 4, and 8 wk after the start of the therapy and were analyzed for the amount of α-tocopherol in red blood cells (RBCs) and plasma, ascorbic acid and TBARS in plasma, and the fatty acid composition in mononuclear cell phospholipids.

This experiment was designed in accordance with the principles of the Declaration of Helsinki of the World Medical Association and was approved by the ethics committee of Yamaguchi University Hospital.