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Abstract
Deficiency in vitamin E, a natural antioxidant, participates in abnormal erythrocyte membrane lipids, structural alterations and hemolysis in advanced cirrhosis. Poor absorption of fat-soluble vitamins limits full correction of deficiency with standard formulations in cirrhosis with cholestasis. The aim of the present study was to examine safety and effects of tocofersolan, a water-soluble derivative of vitamin E, on erythrocyte membrane lipids and anemia in patients with biopsy-proven advanced cirrhosis, vitamin E deficiency and hemolysis.

Reference

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Effect of hydrosoluble vitamin E on erythrocyte membrane lipid composition in patients with advanced cirrhosis: An open-label pilot trial

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Aim: Deficiency in vitamin E, a natural antioxidant, participates in abnormal erythrocyte membrane lipids, structural alterations and hemolysis in advanced cirrhosis. Poor absorption of fat-soluble vitamins limits full correction of deficiency with standard formulations in cirrhosis with cholestasis. The aim of the present study was to examine safety and effects of tocofersolan, a water-soluble derivative of vitamin E, on erythrocyte membrane lipids and anemia in patients with biopsy-proven advanced cirrhosis, vitamin E deficiency and hemolysis.

Methods: Twenty patients (age, 53 ± 10 years; Child class B/C, 8/12), with low plasma vitamin E, chronic anemia and hemolysis, received oral tocofersolan 700 mg/day for 4 weeks. Erythrocyte membrane lipid composition (cholesterol [Chol], phospholipids [Phosph]) was determined by enzymatic assays. Total and conjugated bilirubin, hemoglobin and vitamin E were measured.

Results: Abdominal pain occurred in one patient. Five patients received blood transfusions due to severe anemia. After 4 weeks, both Chol and Phosph decreased, but changes were not significant. Both plasma vitamin E (P < 0.05) and hemoglobin (P < 0.05) increased, together with a decrease in total (P < 0.05) and conjugated (P < 0.05) bilirubin.

Conclusion: In patients with advanced cirrhosis, low vitamin E plasma levels and chronic anemia with hemolysis, oral tocofersolan was overall well tolerated, but did not affect erythrocyte membrane lipid composition.

Key words: acanthocytosis, anemia, cirrhosis, erythrocyte membrane, hemolysis, vitamin E

INTRODUCTION

Anemia in patients with cirrhosis is a common finding, reported in approximately 50% of cases, and results from several causes including gastrointestinal bleeding, hypersplenism, as well as iron and folate deficiencies. In addition, chronic hemolysis aggravates anemia and participates in hyperbilirubinemia, with a negative impact on prognosis. Examination of blood smear in these patients may reveal populations of erythrocytes with abnormal shape, including echinocytes and acanthocytes. These red blood cells present both morphological and functional alterations. An excess in membrane cholesterol relative to phospholipids and a dysregulated polyunsaturated fatty acid content results in a decreased cellular ability to replace peroxidized fatty acids in their membrane. As a consequence, this acquired defect in phospholipid repair affects microviscosity of the membrane and contributes to diminished erythrocyte lifespan and chronic spontaneous hemolysis. Cirrhosis is recognized as a state of increased oxidative stress. Considering the role of oxidative stress in the dysregulated erythrocyte membrane composition, a benefit from antioxidant strategies...
could be anticipated. Patients with cirrhosis and cholestasis have a poor intestinal absorption of fat-soluble vitamins including vitamin E, a natural antioxidant. As vitamin E deficiency has been reported to play a role in spur cell hemolytic anemia, there is a rationale to supplement patients with low vitamin E levels and chronic hemolysis. However, fat malabsorption considerably limits the correction of deficiencies with standard formulations of fat-soluble vitamins. Tocofersolan, a water-soluble derivative of RRR-α-tocopherol, is a commercially available vitamin E supplement that requires neither bile salts nor pancreatic secretion for intestinal absorption. Thus, it is an orally bioavailable source of vitamin E in lipid malabsorption syndromes including pediatric syndromes of cholestasis. Due to its antioxidant properties, vitamin E supplement should improve oxidative stress. However, such studies in liver diseases yielded conflicting results, with only some trials showing improvement in the redox status and in red blood cell survival. Apart from an in vitro study showing that the addition of α-tocopherol markedly reduced erythrocyte membrane peroxidation, changes induced by vitamin E on red blood cell have not been studied in detail. Therefore, the aim of this study was to explore the safety and efficacy of the supplementation with a hydrosoluble vitamin E derivative on red blood cell membrane lipid composition, anemia and hemolysis in patients with advanced cirrhosis and liver insufficiency.

METHODS

At the University Hospitals of Geneva, between November 2011 and April 2013, we included 20 patients who met the following eligibility criteria: (i) histologically proven cirrhosis in an advanced stage (Child B or C); (ii) hemoglobin level of less than 120 g/L; (iii) serum vitamin E level of less than 23 μmol/L; (iv) chronic hemolytic anemia manifested as total serum bilirubin of more than 60 μmol/L with less than 50% of the conjugated form and presence of at least one acanthocyte visible on the peripheral blood smear; and (v) age of more than 18 years, and able to provide a written informed consent to participate. Exclusion criteria included: (i) recent (<2 weeks) gastrointestinal bleeding; (ii) active alcohol consumption; (iii) parenteral nutrition; (iv) exogenous supply in vitamin E; (v) clinical situation of chronic malabsorption that might interfere with oral α-tocopherol absorption including surgical procedures, exposure to drugs such as orlistat or known mucosal intestinal disease; (vi) renal failure (creatinine clearance <60 mL/min); and (vii) inability to provide consent. The flowchart of patients’ selection appears in Figure 1.

This was an open-label single arm phase II clinical trial in patients with cirrhosis (www.ClinicalTrials.gov no. NCT01463735) treated for 4 weeks with tocofersolan (Vedrop; Orphan Europe, Paris, France), a water-soluble derivative of α-tocopherol, and thus an efficient orally bioavailable source of vitamin E. Patients were considered deficient if plasma vitamin E level was below 23 μmol/L, a threshold that we determined based on the mean value measured in a group of patients admitted with decompensated cirrhosis or for an evaluation for liver transplantation. Tocofersolan is well absorbed in pediatric patients with chronic cholestasis and has a good bioavailability in healthy subjects, but data on efficacy and safety are lacking in adults with...
cirrhosis. It has a good safety profile, although some concerns have been raised about a negative influence on renal function. Thus, considering a recommended dose of Vedrop of 17 mg/kg in pediatric patients and no recommended dose for adults, we decided to administer with caution a daily dose of 700 mg daily in patients with cirrhosis and poor liver function. Patients were asked to take the drug (pale solution in 60-mL bottles) twice a day with meals over 4 weeks. Follow-up visits were scheduled to monitor safety and efficacy parameters as well as to assess compliance (by counting returned empty bottles and measuring the remaining volume of Vedrop, if any). The primary end-point of the study was the effect of tocofersolan on red blood cell membrane lipid composition, and secondary end-points consisted of assessing safety and measuring the effects of vitamin E supplementation on anemia and hemolysis.

**Blood studies**

Plasma levels of α-tocopherol (vitamin E) were measured by quantitative high-performance liquid chromatography at baseline, and 2 and 4 weeks of treatment with Vedrop. Presence of erythrocytes with abnormal shape (acanthocytes, echinocytes) on blood smear was assessed at baseline but not counted. Routine laboratory parameters, including hemoglobin, hematocrit, serum bilirubin (total and conjugated) and serum creatinine were measured at each visit.

**Red blood cell membrane analysis**

Antecubital venous blood was drawn during the baseline visit and after 28 days of treatment. Whole blood in a spray-coated ethylenediaminetetraacetic acid tube (BD Vacutainer, Oakville, ON, Canada) was immediately centrifuged at 1500 g for 15 min at 4°C. Plasma and buffy coat were removed and the erythrocyte pellet was washed twice using 14 mL of 0.9% NaCl to remove hemoglobin. Red blood cells were then resuspended with 250 μL of distilled water, and lipids were extracted from the red blood cells using 2.5 mL of methanol and 2.5 mL of chloroform (Sigma-Aldrich, Buchs, Switzerland) after centrifugation for 10 min. After a phase separation was obtained, the chloroform under-phase was evaporated under nitrogen and 1.5 mL of a chloroform/methanol (2:1) solution with 0.005% butylated hydroxytoluene was added. Aliquots of this extract were then stored in a cryotube at −20°C for later analysis. Cholesterol and phospholipids in erythrocyte membranes were determined by enzymatic colorimetric assays using the manufacturer’s recommendations (Sentinel Diagnostics, Milan, Italy). In brief, 200 μL of Triton X-100 detergent solution was added to red blood cell membrane extracts. The samples were then evaporated on a thermoblock at 60°C, and finally resuspended in 100 μL of bi-distilled water and centrifuged prior to transferring the supernatant into the reagent-containing wells for optical density reading. The concentrations of cholesterol and phospholipids in the samples were then measured spectrophotometrically against a blank sample. The optical densities in the samples at 510 nm (cholesterol) and 520 nm (phospholipids) are directly proportional to the cholesterol and phospholipid concentrations, respectively. Results are given as mg/dL.

**Statistical analysis**

Assuming a difference of 20% between the cholesterol/phospholipid (C/P) ratio measured at baseline and after 4 weeks of treatment, we calculated that 15 paired observations would be required to provide a statistical power of 80%, with a two-tailed significance level of 5%, and assuming a standard deviation of the mean difference of 0.02. We estimated a 50% dropout rate due to various reasons including death in relation to the patient’s condition. Thus, we planned to recruit 27 patients.

Data were expressed as mean ± standard error of the mean. We used the non-parametric Wilcoxon rank sum test and paired samples Student’s t-test as appropriate. A two-sided P-value of less than 0.05 was considered statistically significant. Statistical tests were performed using SPSS version 10.0 (SPSS, Chicago, IL, USA).

**Ethical considerations**

The study protocol was approved by the ethics committee of the University Hospitals of Geneva and by the Swiss Agency for Therapeutic Products. The study was conducted in accordance with the ethical principles of the Declaration of Helsinki. All patients gave written, informed consent to participate.

**RESULTS**

**Patients**

Patients’ characteristics are given in Table 1. The vast majority of patients presented with alcoholic cirrhosis, with liver insufficiency as assessed by the Child–Pugh classification and an elevated Model for End-Stage Liver Disease (MELD) score. All patients had chronic anemia together with elevated total serum bilirubin and low plasma vitamin E levels.
Compliance and safety

Except for two patients who were lost to follow up, compliance to the study drug exceeded 80%. One patient presented diffuse abdominal pain without associated biological abnormalities starting a few days after the initiation of tocofersolan. In this patient, symptoms were considered to be related to the study drug, and they completely resolved after discontinuation of the drug. Severe adverse events were reported in six patients (including death in three patients), in relation with portal hypertension (gastrointestinal bleeding, hepatorenal syndrome) and pulmonary complications. These severe life-threatening events are frequent complications of advanced cirrhosis and were not considered to be related to tocofersolan. Serum creatinine values remained stable over time (see Table 2). Physicians in charge decided to administrate blood transfusions in five patients due to worsening of anemia during the study period.

Erythrocyte membrane lipids

Patients who received blood transfusions were excluded from the erythrocyte membrane lipid composition analyses. Thus, cholesterol, phospholipids and C/P ratio in red blood cells were determined in 10 patients at baseline and after 4 weeks of treatment with tocofersolan. We observed a trend towards a decrease in cholesterol, phospholipids and C/P ratio in the composition of erythrocyte membranes (see Fig. 2).

Biological values

Table 2 shows the values of biological parameters measured during the study period and at follow-up visits. The liver function, as measured by the MELD score, remained unchanged. There was a statistically significant decrease in transaminases and International Normalized Ratio after 4 weeks of treatment. Figure 3 shows the increase in hemoglobin and hematocrit values. There was an increase in plasma vitamin E at week-2 and week-4 visits as compared with baseline values (see Fig. 3), but values at 4 weeks remained low as compared with healthy subjects. Measurements at week 4 were limited to six patients due to premature stop of tocofersolan for adverse event (n = 1), loss to follow up (n = 2), complications of portal hypertension with blood product administration (n = 3) and technical problems (n = 3). Both total and conjugated serum bilirubin, considered as indirect markers of hemolysis, were significantly decreased at week 4 (Fig. 4).

DISCUSSION

The aim of this pilot study was to investigate whether supplementation with tocofersolan would improve erythrocyte membrane lipid composition and

Table 1 Patient characteristics at baseline

<table>
<thead>
<tr>
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</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>53 ± 10</td>
</tr>
<tr>
<td>Sex (M/F)</td>
<td>14/6</td>
</tr>
<tr>
<td>Etiology of cirrhosis</td>
<td>17</td>
</tr>
<tr>
<td>Alcohol</td>
<td>3</td>
</tr>
<tr>
<td>Child class (B/C)</td>
<td>9/11</td>
</tr>
<tr>
<td>MELD score</td>
<td>21 ± 1.5</td>
</tr>
<tr>
<td>Hematocrit (%)</td>
<td>27.7 ± 1.1</td>
</tr>
<tr>
<td>Hemoglobin (g/L)</td>
<td>91.8 ± 0.9</td>
</tr>
<tr>
<td>Platelets (g/L)</td>
<td>48 ± 2</td>
</tr>
<tr>
<td>Total bilirubin (μmol/L)</td>
<td>216 ± 3.8</td>
</tr>
<tr>
<td>Conjugated bilirubin (μmol/L)</td>
<td>98 ± 1.6</td>
</tr>
<tr>
<td>Serum creatinine (μmol/L)</td>
<td>74 ± 1.7</td>
</tr>
<tr>
<td>Plasma vitamin E (μmol/L)</td>
<td>15.1 ± 2.9</td>
</tr>
<tr>
<td>Plasma cholesterol (mmol/L)</td>
<td>2.5 ± 1.3</td>
</tr>
<tr>
<td>Plasma triglycerides (mmol/L)</td>
<td>0.63 ± 0.45</td>
</tr>
</tbody>
</table>

MELD, Model for End-Stage Liver Disease.

Table 2 Evolution of biological values under tocofersolan

<table>
<thead>
<tr>
<th>Variable</th>
<th>Baseline</th>
<th>Week 1</th>
<th>Week 2</th>
<th>Week 4</th>
<th>Follow up</th>
<th>P (baseline vs week 4)</th>
</tr>
</thead>
<tbody>
<tr>
<td>MELD</td>
<td>21 ± 1.5</td>
<td>NA</td>
<td>NA</td>
<td>19 ± 0.2</td>
<td>NA</td>
<td>NS</td>
</tr>
<tr>
<td>AST (IU/L)</td>
<td>83 ± 53.1</td>
<td>72.8 ± 29.9</td>
<td>67.6 ± 26.8</td>
<td>61 ± 15.7</td>
<td>NA</td>
<td>P &lt; 0.001</td>
</tr>
<tr>
<td>ALT (IU/L)</td>
<td>43.9 ± 25.6</td>
<td>55.7 ± 4.9</td>
<td>44.4 ± 32</td>
<td>35.8 ± 30.7</td>
<td>NA</td>
<td>P &lt; 0.001</td>
</tr>
<tr>
<td>Serum creatinine (μmol/L)</td>
<td>74 ± 1.7</td>
<td>79 ± 1.7</td>
<td>81.6 ± 2.6</td>
<td>76.5 ± 2.4</td>
<td>73.7 ± 2.1</td>
<td>NS</td>
</tr>
<tr>
<td>INR</td>
<td>1.84 ± 0.6</td>
<td>1.86 ± 0.6</td>
<td>1.56 ± 0.5</td>
<td>1.57 ± 0.5</td>
<td>NA</td>
<td>P &lt; 0.001</td>
</tr>
</tbody>
</table>

ALT, alanine aminotransferase; AST, aspartate aminotransferase; INR, International Normalized Ratio; NA, not available; NS, not significant.
associated chronic anemia in adult patients with advanced cirrhosis. The data demonstrate safety and efficacy of tocofersolan in improving vitamin E plasma levels. This was associated with a concomitant increase in hemoglobin concentration and a progressive reduction in both total and conjugated serum bilirubin levels, consistent with an improved chronic hemolytic anemia. However, changes in red blood cell lipid composition were not significant.

Chronic hemolytic anemia with acanthocytosis is a severe manifestation of advanced cirrhosis with a poor prognosis. Reduced erythrocyte lifespan in this situation results from both dysregulated membrane lipids and oxidative damage. Thus, there is a rationale to administer vitamin E in order to improve red blood cell membrane alterations and to reduce hemolysis. Accordingly, α-tocopherol administrated to a group of eight vitamin E-deficient subjects with hemolysis improved erythrocyte survival. Although we did not

Figure 2 Individual changes in red blood cell membrane (RBCM) cholesterol content (upper panel), phospholipids (middle panel) and cholesterol/phospholipid (C/P) ratio in 10 patients after 4 weeks of tocofersolan.

Figure 3 Changes in hematocrit (Ht, upper panel) and hemoglobin (Hb, lower panel) after 4 weeks of tocofersolan in the subgroup of 10 patients free from any blood transfusions. *P < 0.05 versus baseline.
address the specific point of red blood cell survival per se, our data suggest that hemolysis, as assessed by low hemoglobin and elevated total bilirubin, is reduced by tocofersolan in this small group of cirrhotic patients. This observation could be beneficial to these patients, as liver transplantation, the only curative treatment, is not an option for the majority of advanced cirrhotics, and repeated blood transfusions may induce severe iron overload, which has been associated with an increased risk of liver cancer. The absence of significant changes in red cell membrane lipids raises two hypotheses. First, as the paired analysis of membrane cholesterol and phospholipids was limited to 10 patients as compared with the initial assessment of 27, we cannot exclude a type II error. We may have underestimated the extreme vulnerability of these patients exposed to a number of complications including manifestations of portal hypertension, infections, as well as the clinical consequences of severe anemia. Thus, five patients received one or more blood transfusions during the study period due to a very low level of hemoglobin, which prevented any analysis in red blood cell composition. Second, tocofersolan improved vitamin E plasma levels and tended to improve erythrocyte membrane lipid abnormalities, but this may not be sufficient to correct severe oxidative stress that is reported in decompensated cirrhotics. The prolonged administration of vitamin E in diabetics (who also suffer from erythrocyte membrane alterations) only moderately affected red blood cell membrane lipid peroxidation. Overall, as the benefits of antioxidant to treat liver diseases in general remains unclear, a significant improvement in the redox status and normalization of transaminases have been reported in 50 patients with cirrhosis after 6 months of 900 IU vitamin E daily. Based on this, it can be speculated that the improvement in total and conjugated serum bilirubin in our patients treated with tocofersolan could be related to some mechanisms not strictly limited to erythrocyte membrane composition but to some general improvement in oxidative stress. Accordingly, patients with alcoholic liver disease are particularly exposed to a severe imbalance in the antioxidant defense system which participates in several aspects of liver injury. Thus, hepatocellular membrane peroxidation, inflammation and fibrosis are all negatively affected by poor antioxidant status and low vitamin E plasma levels. For these reasons, we speculate that hydrosoluble vitamin E supplementation in this group of patients might have participated in the improvement in serum bilirubin, a good biological integrator of hepatocellular function. However, we must admit that we have no data on oxidative stress markers in our patients to support this hypothesis.

Whether vitamin E supplementation is safe and beneficial is still an open question. Large studies demonstrated either marginally positive effects on histology in well-compensated non-alcoholic steatohepatitis, but possible negative outcomes such as increased risk of hemorrhagic stroke or prostate cancer in middle aged individuals without liver disease. Tolerance to 500 mg vitamin E daily for a year was good in 33 patients with cirrhosis, but biological and clinical end-points were not improved as compared with placebo. Based on our
small pilot trial, vitamin E supplement appears safe in patients with advanced cirrhosis, poor liver function and low plasma tocopherol level. A larger study with a longer treatment period and a similar end-point (red blood cell membrane lipids) would be informative but difficult to perform in such vulnerable patients exposed to many severe hemorrhagic complications necessitating blood product administration. In addition, the optimal dose and duration of tocofersolan should be determined, as well as the threshold of vitamin E plasma level below which supplementation should be considered. According to recent recommendations, vitamin E levels should range 12–46 μmol/L, but this may differ in patients with advanced cirrhosis. In our patients, the increase of vitamin E plasma levels under treatment was evident at 2 weeks without further accumulation at the 4-week visit. We hypothesize that the small number of doses available at this visit might have influenced these results, thus precluding any conclusion.

Our study has several limitations, including the small number of patients, the absence of a control group, the relatively short study period, the absence of pharmacological or dose-finding studies to determine the optimal treatment duration, the limited number of parameters used to detect and monitor hemolysis, and the lack of measurement of oxidative stress indicators at the cellular level. On the other hand, we provide a detailed paired analysis of red blood cell membrane lipid composition and information regarding safety of tocofersolan in liver insufficiency. Further studies are encouraged to explore the potential benefit of tocofersolan on these clinical and biological end-points.

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REFERENCES


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