Vitamin E is an effective treatment for nonalcoholic steatohepatitis in HIV mono-infected patients

Giada Sebastiani\textsuperscript{a,b}, Sahar Saeed\textsuperscript{c}, Bertrand Lebouche\textsuperscript{a}, Alexandra de Pokomandy\textsuperscript{a}, Jason Szabo\textsuperscript{a}, Louis-Patrick Haraoui\textsuperscript{a}, Jean-Pierre Routy\textsuperscript{a,d}, Philip Wong\textsuperscript{b}, Marc Deschenes\textsuperscript{b}, Peter Ghali\textsuperscript{b}, Marina Klein\textsuperscript{a}, for the LIVEHIV Study Group

Objective: HIV-infected patients are at increased risk of nonalcoholic steatohepatitis (NASH). Vitamin E is recommended for treatment of NASH in the general population. However, its safety and efficacy among HIV-infected patients remain unknown.

Design: Single-centre, phase IV, open-label, single arm clinical trial.

Methods: HIV mono-infected patients without significant alcohol intake or viral hepatitis coinfection were included. The diagnosis of NASH was based on the coexistence of fatty liver, diagnosed by controlled attenuation parameter (CAP) at least 248 dB/m and significant hepatocyte apoptosis, defined by the serum biomarker cytokeratin 18 (CK-18) greater than 130.5 U/L. Participants were treated with 800 IU daily of oral vitamin E (alpha-tocopherol) for 24 weeks, and followed for an additional 24 weeks postdiscontinuation. Generalized linear mixed effects models were used to evaluate changes in alanine aminotransferase (ALT), CAP and CK-18 at the completion of treatment and end of follow-up, controlling for pretreatment trends.

Results: A total of 27 patients were included. Four (15%) had a pretreatment liver biopsy, which confirmed the diagnosis of NASH in all cases. Compared with baseline, 24 weeks of vitamin E treatment improved ALT [−27 units/l; 95% confidence interval (CI) −37 to −17], CAP scores (−22 dB/m; 95% CI −42 to −1) and CK-18 (−123 units/l; 95% CI −201 to −46). Conversely, there was no change in BMI. No serious adverse event was reported and no patient was lost to follow-up.

Conclusion: In this first clinical trial, we showed that vitamin E is an effective and well tolerated treatment for NASH in HIV-infected patients.

Keywords: alanine aminotransferase, controlled attenuation parameter, HIV mono-infection, nonalcoholic steatohepatitis, vitamin E
nonalcoholic steatohepatitis (NASH), a progressive disease characterized by inflammation leading to liver fibrosis and cirrhosis. NASH affects 3–7% of the general population [6]. Higher rates of the disease are reported in HIV-infected patients, ranging from 7.3 to 57.1% [7]. This is likely because of both high frequency of metabolic conditions underlying the pathogenesis of NASH and ART-related hepatotoxicity [3,8,9]. Despite the disease burden, therapeutic options for NASH are limited in the general population and there are no proven treatments to reduce HIV-associated NASH.

In addition to insulin resistance, oxidative stress plays a key role in the pathogenesis of NASH [6,10]. Vitamin E is a lipophilic antioxidant essential for human health, which protects cell membranes from oxidation and regulates apoptotic pathways. Two randomized controlled trials, PIVENS in adults [11] and TONIC in children [12], demonstrated that vitamin E effectively reduces transaminases and ameliorates liver histology in HIV-negative patients with NASH. As a result, vitamin E at daily dose of 800 IU/day is recommended as first-line pharmacotherapy of NASH in the general population by guidelines of the European Association for the Study of the Liver and of the American Association for the Study of Liver Diseases (AASLD) [6,13].

There are several reasons why an intervention proved to be effective in the general NASH population requires ad hoc validation in HIV-infected patients. First, the pathogenesis of HIV-associated NASH is more complex. Elevated oxidative stress is a hallmark of HIV-infected patients and may predict all-cause mortality [14,15]. This could be both a consequence of HIV itself and of ART, which interferes into the redox system, mainly by reducing the antioxidant defenses [16]. Second, because of its pathogenetic complexity, HIV-associated NASH might be less responsive to interventions. Finally, vitamin E may be not well tolerated in all settings. An increased risk of cardiovascular events, bleeding and prostate cancer has been suggested [17,18].

Given the high prevalence of NASH among HIV-infected patients and the lack of therapeutic options, this clinical trial was aimed to investigate the effect of vitamin E treatment on NASH diagnosed through noninvasive tools.

Patients and methods

Trial design

This was a phase 4, single-centre, open-label, single-arm study to evaluate changes in alanine aminotransferase (ALT), controlled attenuation parameter (CAP) and cytokeratin (CK)-18 with a 24-week treatment of vitamin E (soft-gelatin capsules of alpha-tocopherol, 800 IU, natural form, once daily) in HIV mono-infected patients with a noninvasive diagnosis of NASH. The diagnosis of NASH was based on CAP measurement at least 248 dB/m, indicative of the presence of hepatic steatosis involving more than 10% of hepatocytes and on serum biomarker CK-18 >130.5 U/l, indicative of significant hepatocyte apoptosis [4,19,20]. The estimated sample size for this trial was 30 patients. This size would have been sufficient to achieve a statistical power of 87% to detect a change in ALT of −35 IU/l between baseline and end of treatment, with a bilateral 95% confidence interval (CI) [21]. As an intermediate analysis, planned for when two-thirds of the patients were included, proved differences in the primary outcome, the inclusion of patients was stopped before reaching the estimated sample size.

Patients

Between February 2015 and September 2018, HIV mono-infected patients were enrolled at a single clinical site in Canada, the Chronic Viral Illness Service of the McGill University Health Centre (MUHC). Inclusion criteria were age at least 18 years; receiving ART for the last at least 6 months; plasma HIV RNA less than 50 copies/ml for at least 24 weeks documented in at least two clinical visits; presence of both CAP at least 248 dB/m and CK-18 greater than 130.5 U/l [4,19,20]. Exclusion criteria were any of the following: hepatitis C virus antibody positive; hepatitis B surface antigen positive; significant alcohol intake, defined as self-reported average weekly intake of more than 21 drinks in men and more than 14 drinks in women, as per AASLD guidelines on NAFLD [6]; pregnancy; an AIDS-defining opportunistic disease in the 24 weeks before recruitment; history of hepatocellular carcinoma or liver transplantation; active illicit drug use or any other condition that might compromise the study drug adherence in the opinion of the investigators; failure of transient elastography with CAP examination or unreliable measurement. Standardized dietary and physical activity recommendations, as well as a nutritional consultation, were provided to all patients at baseline.

Ethics

The study was approved by the Research Ethics Board of the Research Institute of MUHC (code 2014–1192) and was registered at ClinicalTrials.gov (NCT03988725). The study was conducted according to the Declaration of Helsinki and Good Clinical Practice guidelines. All patients provided their informed written consent prior to participation.

Study assessments

Clinical visits were scheduled at baseline, week 4, week 12, week 24 and week 48 (Fig. 1). The following parameters were collected at each study visit: BMI, laboratory tests for hematology, blood chemistry, CD4+ cell counts, plasma HIV RNA. Insulin was determined to compute the homeostasis model for assessment of insulin resistance (HOMA-IR) index [fasting insulin...
Transient elastography with CAP measurement and plasma to measure CK-18 were also acquired at each study visit. Transient elastography examination was performed in patients fasting for at least 3 h using FibroScan 502 Touch (Echosens, Paris, France). The same two experienced operators performed all elastographic measurements. The standard M probe was used in all patients. The XL probe was used in cases of failure of transient elastography with the M probe. The following criteria were applied to define the result of transient elastography as reliable: at least 10 validated measurements and an interquartile range (IQR) less than 30% of the median liver stiffness measurement [23]. Wherever histology was available, the stage of fibrosis and degree of steatosis were reported according to the Brunt classification [24]. The threshold used to define significant liver fibrosis was histological stage 2 out of 4 by the Brunt staging system (F2–4). Hepatic steatosis was graded as follows: S1 = steatosis 10–33% (mild), S2 = steatosis 33–66% (moderate) and S3 = steatosis greater than 66% (severe). A diagnosis of NASH was made by the presence of classic histological features including steatosis, lobular inflammation and ballooning [24]. Significant liver fibrosis by transient elastography examination was defined as liver stiffness measurement at least 7.1 kPa [25]. The CAP cut-off values used for diagnosis of steatosis grades S1, S2, and S3 were 248, 268 and 280 dB/m, respectively [19]. Plasma stored at −80 °C was used for quantitative measurement of CK-18 levels by the Human cytokeratin ELISA kit (MJS Biolynx Inc, Brockville, Ontario, Canada). The severity of adverse events was evaluated according to the Division of AIDS toxicity table [26].

**Statistical analysis**

Sociodemographic, behavioral, clinical and HIV treatment regimens were summarized for all participants who met eligibility criteria at baseline. Categorical variables were summarized as percentages and continuous variables as medians and IQR. Each outcome was summarized graphically as means with 95% CI and medians with IQR at each time point. Changes in the mean response from baseline were assessed using a generalized linear mixed model. We used quasi-experimental methodology to emulate a control group, by analyzing outcome measurements 12 and 24 weeks before treatment for each participant. This availed two functions: evaluate the stability of our baseline measurement and construct a ‘counterfactual’ preexposure trend, which we used as a control to estimate the impact of vitamin E, using a segmented generalized mixed model [27]. This method allows for each participant to act as their own control, therefore time fixed covariates are controlled for by design. Time was modelled as fixed effect at each time point. Random individual intercepts allowed for the natural heterogeneity of participants baseline outcome measurements. We conducted a sensitivity analysis to evaluate the validity of our control group. Here we used ALT measurements from seven control individuals from a co-occurring clinical trial of HIV mono-infected patients diagnosed with NASH that had the same eligibility criteria as this present trial. All analyses were performed using STATA version 15/IC (StataCorp LP, College Station, Texas, USA).

**Results**

A total of 133 patients were screened for the presence of NASH; 27 (20%) of them had a CAP at least 248 dB/m, CK-18 greater than 130.5 U/l and met other eligibility criteria, therefore, were included into the study. The baseline characteristics of the study population are...
Changes in alanine aminotransferase, controlled attenuation parameter and cytokeratin 18

Changes in ALT, CAP and CK-18 are summarized at each study time point in Supplemental Figure S1a, 1b and 1c, respectively. Box plots for each of the outcomes are available in Supplemental Figure S2, http://links.lww.com/QAD/B556. Changes in ALT, CAP and CK-18 are summarized at each study time point in Supplemental Figure S1a, 1b and 1c, respectively. Box plots for each of the outcomes are available in Supplemental Figure S2, http://links.lww.com/QAD/B556. Changes in ALT, CAP and CK-18 are summarized at each study time point in Supplemental Figure S1a, 1b and 1c, respectively. Box plots for each of the outcomes are available in Supplemental Figure S2, http://links.lww.com/QAD/B556.

Table 2. Mean change of each outcome from baseline.

<table>
<thead>
<tr>
<th>Change compared with baseline (week 0)</th>
<th>ALT (units/l)</th>
<th>CAP (dB/m)</th>
<th>CK-18 (units/l)</th>
<th>BMI (kg/m²)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pre 24 weeks</td>
<td>−3 (~14 to 7)</td>
<td>17 (~8, 42)</td>
<td>No data</td>
<td>No data</td>
</tr>
<tr>
<td>Pre 12 weeks</td>
<td>−12 (~30 to 7)</td>
<td>3 (~17 to 24)</td>
<td>16 (~61 to 93)</td>
<td>−0.2 (~1.6 to 0.9)</td>
</tr>
<tr>
<td>4 weeks</td>
<td>−12 (~24 to −1)</td>
<td>−4 (~24 to 17)</td>
<td>−58 (~135 to 19)</td>
<td>−0.3 (~1.3 to 0.8)</td>
</tr>
<tr>
<td>12 weeks</td>
<td>−22 (~33 to −11)</td>
<td>−24 (~44 to −3)</td>
<td>−88 (~165 to −11)</td>
<td>−0.4 (~1.4 to 0.7)</td>
</tr>
<tr>
<td>24 weeks</td>
<td>−27 (~37 to −17)</td>
<td>−22 (~42 to −1)</td>
<td>−123 (~201 to −46)</td>
<td>−0.2 (~1.3 to 0.9)</td>
</tr>
<tr>
<td>48 weeks</td>
<td>−20 (~30 to −10)</td>
<td>−3 (~24 to 18)</td>
<td>16 (~61 to 93)</td>
<td>−0.7 (~1.7 to 0.4)</td>
</tr>
</tbody>
</table>

Variables are expressed as mean and 95% confidence interval. ALT, alanine aminotransferase; CAP, controlled attenuation parameter; CK-18, cytokeratin 18.
of normal of 45 IU/l) (Fig. 2a). This effect was carried over 24 weeks postcessation of treatment. At week 24, severe steatosis was observed in only 41% of patients, as compared with 67% at baseline. Furthermore, resolution of hepatic steatosis was observed in 22% of cases (Fig. 2b). Our sensitivity analysis of seven control participants revealed no change in ALT (Supplemental Figure S3, http://links.lww.com/QAD/B556). The proportion of patients with significant liver fibrosis by liver stiffness measurement remained the same between baseline and week 24 (41%). Among the four patients with available liver histology, normalization of ALT was observed in
100% of cases, whereas one case presented steatosis resolution, from baseline CAP of 350–235 dB/m at week 24.

Changes in metabolic parameters
In contrast to the above findings, there was no change in BMI (Supplemental Figure S1d and S2d, http://links.lww.com/QAD/B556). BMI over 24 weeks of treatment changed by 0.1 kg/m² (95% CI 0.5 to 0.7) controlling for a pretreatment trend (−1.7 kg/m², 95% CI −3.2 to −0.2 over 12 weeks). Similarly, there was no change in triglycerides, total cholesterol, HDL cholesterol and HOMA-IR between baseline and week 24 (results not shown).

Safety outcomes
Eleven patients showed clinical adverse events during the study period, including two reporting tiredness, two with nausea and abdominal cramps, two reporting blurred vision, two with headache, two reporting dizziness and one patient with itching. There were no laboratory adverse events and no serious adverse event. No patients discontinued therapy because of adverse events.

Discussion
In this first trial, we showed that 24 weeks of vitamin E treatment decreased ALT, the degree of hepatic steatosis and hepatocyte apoptosis in HIV mono-infected patients with NASH. The intervention was both effective and well tolerated, with no patient lost to follow-up and no serious adverse event reported. HIV-infected patients are currently excluded from registrational clinical trials of new antifibrotic molecules for the treatment of NASH, and this poses an ethical and clinical dilemma. Vitamin E represents an available therapeutic option.

In the ART era, liver disease is emerging as a major driver in morbidity and mortality of HIV-infected patients [1]. Increasing rates of metabolic disorders among aging HIV-infected patients are contributing to this trend [3,9,28,29]. Insulin resistance is highly prevalent among HIV-infected patients, because of inflammation, aging and specific ART regimens [29]. Dyslipidemia and hypertension are also common [28]. Other risk factors unique to this population may contribute to the epidemic of liver disease. First, ART is used lifelong and is known to elevate ALT and induce oxidative stress in hepatocytes [8]. Second, HIV viremia from ART treatment interruptions is a risk factor for elevated ALT and liver cirrhosis. HIV is believed to have immune-activating and pro-apoptotic effects on hepatocytes [8]. These multiple metabolic and HIV-related hepatotoxic hits represent pathogenetic triggers for NASH, a severe liver condition eventually leading to liver cirrhosis and related end-stage complications. Currently, NASH represents the second most common indication for liver transplantation in North America and it is projected to become the leading indication over the next 10 years [30]. NASH is more frequent in HIV-infected persons, with reported prevalence up to 57.1% in those with chronically elevated ALT and at 10% in patients attending a routine screening program [7,31,32]. Few interventions can alter the natural history of the disease and improve NASH. Life-style changes, characterized by a combination of dietary restriction and increase in aerobic exercise/resistance, are the first-line treatment and can result in significant regression of liver fibrosis and NASH [13]. However, this intervention is seldom implemented by patients and has not been tested for HIV-associated NASH [33]. Waiting until end-stage liver complications arise will result in the need for transplant, which is not a viable option being particularly challenging and not widely available for HIV-infected patients [34]. Although new antifibrotic molecules are currently being studied in HIV-negative NASH in randomized controlled trials, as HIV-infected patients are excluded, it is estimated that it will take at least 7–10 years for them to become available for the HIV-infected community [35]. As such, there is a clear need for a simple and well tolerated intervention for NASH in HIV-infected patients over the next years.

Vitamin E is a lipophilic antioxidant acting as a regulator of the activity of genes controlling apoptosis, inflammation and collagen deposition [36]. The role of vitamin E in the treatment of NASH is based on its activity as a free radical scavenger. Two large randomized controlled trials concluded that vitamin E is an effective treatment to reduce transaminases and to ameliorate liver histology in HIV-negative NASH [11,12]. Guidelines recommend vitamin E as a first-line pharmacotherapy for patients with NASH [13,37]. In this trial, we observed a rapid change in ALT, already significant at month 1, which further continued with a mean reduction of −271 U/1 at week 24. As a result, the proportion of patients with elevated ALT dropped from 74% at baseline to 15% at the end of treatment. Elevated ALT has been repeatedly associated with significant liver fibrosis, high proportions of histologic NASH and mortality in HIV-infected patients [38]. The effect of vitamin E on ALT observed in this trial is similar to that reported in the PIVENS trial, where the mean ALT change at 96 weeks was −37 IU/L [11]. Furthermore, we observed a change in both CAP and CK-18, as surrogate markers of hepatic steatosis and apoptosis. A reversal of hepatic steatosis by CAP was observed in 22% of cases. Because of the role of apoptosis in NASH pathogenetic cascade, the reduction in hepatocyte apoptosis suggests an improvement in NASH [10].

Unlike the PIVENS trial, in our study, we did not observe a significant change in insulin resistance or HDL-
Vitamin E in HIV+ patients with NASH Sebastiani et al.

cholersterol. HIV-infected patients have multiple hits for oxidative stress [16]. Supplements of vitamin E have been previously shown to reduce oxidative stress in HIV and produce a trend towards a reduction in viral load [39]. Our results with vitamin E treatment, combined with these pathogenetic considerations and the lack of metabolic changes in our study, suggest that oxidative stress may have a greater importance in the pathogenesis of NASH in the specific context of HIV infection and could be one of the drivers of the high prevalence of NASH in this setting. Importantly, the stability of BMI throughout the study period, despite counselling on lifestyle modifications, suggests that, as already observed in HIV-negative NASH, the uptake of such recommendations are suboptimal also in HIV-infected patients [33].

We wish to acknowledge several limitations of this study. First, the study period was relatively short. As such, these trial results should be regarded as exploratory. Nevertheless, we observed clinically significant changes over a 24-week course of vitamin E treatment, which also persisted at week 48, posttreatment discontinuation, suggesting a carry-over effect. It would be important to know whether continued treatment would lead to longer benefit. Second, we based the diagnosis of NASH on noninvasive tests instead of liver biopsy, which is still considered the gold standard. However, both CAP and CK-18 have been previously validated against liver biopsy in the specific setting of HIV infection [31,40]. CAP has been already employed in HIV-infected patients to study changes in hepatic steatosis during the course of an interventional trial [41]. Importantly, we previously showed a relatively high rate of patient refusal or ineligibility to liver biopsy in HIV [31]. Third, this trial was not designed to have a control arm. However, we did leverage existing historical data and quasi-experimental methodology to emulate a control group, which we confirmed with a sensitivity analysis of seven control individuals from another study. Fourth, we did observe a reduction in liver stiffness measurement as a surrogate of liver fibrosis regression. This observation mirrors the findings of the PIVENS trial, where improvements in steatosis and necroinflammation were observed, whereas there was no improvement in fibrosis. It is also possible that the short study duration was not sufficient to allow fibrosis regression. Finally, given the small numbers, we were unable to conduct a subgroup analysis on the 12 diabetic patients included in the present trial, which could have been valuable since the PIVENS trial excluded them. In an exploratory univariate analysis, we found similar changes in ALT, CAP and CK-18 between diabetic and nondiabetic patients (data not shown).

In conclusion, vitamin E treatment seems safe and reduces ALT, CAP and CK-18, leading to rapid and sustained normalization of ALT and reduction of hepatic steatosis in a significant number of HIV mono-infected patients with a noninvasive diagnosis of NASH. This effect may be related to the importance of oxidative stress in NASH pathogenesis in HIV infection. Considering the epidemic of NAFLD in HIV-infected patients, as well as their exclusion from current clinical trials of antifibrotic molecules for NASH, vitamin E should be regarded as a therapeutic option. Larger studies, also based on liver histology, are needed to confirm these promising results, define optimal treatment duration and long-term benefits.

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Contributors: G.S. contributed to conception, study design, data, interpretation of the data and first draft of the manuscript. S.S. contributed to study design, statistical analysis, interpretation of data and first draft of the manuscript. B.L., A.d.P., J.S., L.P.H., J.P.R., P.W., M.D., P.G. and M.B.K. contributed to study design, data and interpretation of the data. All authors approved the final version of the article.

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Conflicts of interest

G.S. has acted as speaker for Merck, Gilead, Abbvie, ViiV, served as an advisory board member for Merck, Gilead and Novartis has received research funding from Merck and Echosens. B.L. has acted as a consultant for ViiV, Gilead, and Merck and received research funding from Merck and Gilead. A.d.P. participated in advisory boards committees for ViiV, received speaker fees by Merck, and is site principal investigator or co-investigator for Janssen, Merck, Gilead and ViiV trials. J.S. has served as a consultant and member of a scientific advisory board for ViiV, Gilead, Merck and Teva, and received speakers fees from Gilead, Merck and Theratechnologies. P.W. has acted as consultant for BMS, Gilead, Merck, Novartis. M.D. has served as an advisory board member for Merck, Janssen, Gilead. P.G. has acted as consultant for Merck and Gilead. M.B.K. and J.P.R. have acted as consultant for ViiV, Gilead, Janssen and Merck and received research funding from Merck and ViiV. S.S. and L.P.H. have nothing to disclose.

References


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