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Original article

Effects of docosahexanoic acid on metabolic and fat parameters in HIV-infected patients on cART: A randomized, double-blind, placebo-controlled study

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SUMMARY

Background: Hypertriglyceridemia is common in HIV-infected patients. Polyunsaturated fatty acids reduce fasting serum triglyceride (TG) levels in HIV-infected patients. It is not known whether docosahexanoic acid (DHA) supplementation can reduce hypertriglyceridemia and modify fat distribution in HIV-infected patients.

Methods: We conducted a randomized, double-blind, placebo-controlled trial with 84 antiretroviral-treated patients who had fasting TG levels from 2.26 to 5.65 mmol/l and were randomized to receive DHA or placebo for 48 weeks. TG levels were assessed at baseline, week 4 and every 12 weeks. Body composition was assessed at baseline and at week 48. Registered under ClinicalTrials.gov Identifier no. NCT02005900.

Results: Patients receiving DHA had a 43.9% median decline in fasting TG levels at week 4 (IQR: −31% to −56%), compared with −2.9% (−18.6% to 16.5%) in the placebo group ($P < 0.0001$). DHA levels and decrease in TG at week 4 in the DHA arm correlated significantly ($r = 0.7110$, $P < 0.0001$). The median reduction in TG levels in the DHA arm was −43.7% (−32.4% to −57.5%), and in the placebo arm +2.9% (−21.3% to +30.1%) at week 12. The difference remained statistically significant at week 48 ($P = 0.0253$). LDL cholesterol levels significantly increased at week 4 by 7.1% (IQR: −4.8% to +35.3%) in the DHA arm but not in the placebo group. No significant changes were observed in HDL cholesterol, insulin, and HOMA-IR during the study. Limb fat significantly increased in both arms, without statistically significant differences between groups ($P = 0.3889$). DHA was well tolerated; only 3 patients experienced treatment-limiting toxicity.

Conclusions: Supplementation with DHA reduced fasting TG levels in antiretroviral-treated HIV-infected patients with mild hypertriglyceridemia. DHA was well tolerated with minor GI symptoms. Peripheral fat significantly increased in the DHA group but did not increase significantly compared with placebo.

1. Introduction

Among lipid disorders associated with HIV infection and cART, the usual phenotype is that of increased triglycerides (TG), decreased HDL-cholesterol and increased LDL-cholesterol, sometimes accompanied by increased fasting glucose levels [1]. These abnormalities are even more frequent and quantitatively more important in patients with HIV/HAART-associated lipodystrophy syndromes (HALS) [2]. They are partly related to HIV infection itself and mostly to certain HIV protease inhibitors (PI) [3]. In particular, hypertriglyceridemia is often due to ritonavir boosting of most PI, with thymidine analogues also contributing to the increase in TG levels [4]. Lipid abnormalities whether or not coupled with body fat

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redistribution, especially visceral adipose tissue hypertrophy, depict a scenario of high cardiovascular risk for these patients [1].

Polyunsaturated fatty acids (PUFA), the so-called omega-3 fatty acids, are extremely variable molecules with a range of claimed beneficial effects [5]. Among them, they decrease serum TG levels, increase HDL-cholesterol, decrease blood pressure, have anti-inflammatory effects, and in patients with a past myocardial infarction, have been associated with prevention of sudden death due to arrhythmias [5]. In the HIV setting, a number of clinical trials have demonstrated that hypertriglyceridemia can be at least partially corrected by diet supplementation with a mixture of omega-3 fatty acids of fish oil origin [6,7].

Eicosapentanoic acid (EPA) and docosahexanoic acid (DHA) are the two principal PUFA found in marine oils. Data in humans have shown that these two fatty acids have differential effects on serum lipids and lipoproteins [8], serum glucose [8], blood pressure [9], heart rate [10], and endothelial function [11]. DHA ($C_{22}H_{32}O_2$) is primarily a structural component of many organs and tissues including brain, skin, testes and retina [12]. It is usually obtained from maternal milk or fish oil although it can also be synthesized in mild amounts from alpha-linolenic acid. Dietary DHA may reduce the risk of heart disease in humans and below-normal levels have been associated with Alzheimer's disease and retinitis pigmentosa [13].

Because of its lipid effects, its claimed anti-inflammatory properties, and low levels of DHA found in HIV-infected patients [14], we implemented a randomized, double-blind, placebo-controlled clinical trial to assess DHA supplementation in cART-treated HIV-infected patients with mild hypertriglyceridemia. DHA effects on cholesterol fractions and body fat distribution measured by dual X-ray absorptiometry (DEXA) after 48 weeks were considered as secondary endpoints.

2. Patients and methods

2.1. Study population

All patients were recruited through the *Hospital de la Santa Creu i Sant Pau* HIV-1 infection clinic between July 2010 and June 2011. This clinic serves a population of 1570 adult HIV-1-infected patients on active follow-up. Inclusion criteria for screening were having an established diagnosis of HIV-1 infection, under stable cART for the prior 6 months and throughout the study period, and having TG levels between 2.26 and 5.65 mmol/l on two consecutive determinations within a 15-day interval. Since pharmacological therapy was indicated for patients with a TG level >5.65 mmol/l, these were excluded [15]. These limits were chosen because they are the upper limit of normality and the threshold for pharmacological intervention, respectively.

Exclusion criteria included known hypersensitivity to the active compound or product excipients, BMI >30 kg/m^2, pregnancy, breastfeeding, anticoagulant treatment, oral antidiabetics and hormonal treatments. Discontinuation of lipid-lowering drugs for more than 3 months before the selection visit was required to be screened for the study. This was done to dissect the actual effect of DHA on lipid fractions. Consumption of high levels of alcohol (>20 g/d), diabetes or an abnormal fasting blood glucose level (glycemia >6.6 mmol/l) were exclusion criteria. Additional exclusion criteria were: serum creatinine >150 μmol/l and alanine aminotransferase or aspartate aminotransferase >5 × upper limit of normal, anemia, >10% loss in body weight in the preceding 6 months, and any active AIDS-defining disease. There were 3 protocol violations, two in the DHA arm (positive pregnancy test and start of lipid-lowering therapy) and one in the placebo arm (fasting glycemia >6.6 mmol/l). The diagnosis of AIDS was based on the 1993 revised case definition of the Centers for Disease Control and Prevention CDC [16]. All participants were instructed not to make any changes to their lifestyle throughout the intervention period to assess the effect of DHA on circumstances close to "real-world" conditions. Written informed consent was obtained from the patients at study entry. The study was approved by the Ethics Committee of the *Hospital de la Santa Creu i Sant Pau* on March 23, 2010, and its amended version on April 22, 2010. Registered under ClinicalTrials.gov Identifier no. NCT02005900. The first patient was randomized on July 14, 2010 and the last one on June 7, 2011 (Fig. 1). The last follow-up visit for a randomized patient was on July 7, 2011. The authors confirm that all ongoing and related trials for this drug are registered.

2.2. Study design

A double-blind, phase 4, randomized, 2-arm, placebo-controlled study was performed. After a 4-week screening, eligible patients were randomized to DHA 4 g a day (in ochre single-serving drinkable vials containing 7 g of DHA oil) or placebo, during a 48-week period. The AHA recommends a total dose of EPA and DHA of 2–4 g per day for patients who need to lower TG levels [17]. Placebo ochre vials containing 7 g of olive oil were similar to DHA ones. The formulations were liquid vials but, since DHA is not tasty and has a heavy fish smell, both interventional and placebo oils were masked with lemon flavor. DHA was obtained by enzymatic synthesis and incorporated in the TG form at a 70% concentration of total fatty acid content and was provided by Brudy Technologies® (Barcelona, Spain).

Strategies to lower lipid levels have demonstrated a maximum effect within the first 4–6 weeks, and cholesterol guidelines suggest that response to lipid-lowering therapy be assessed after 4 weeks, too [18]. Consequently, the primary efficacy endpoint of the intervention was chosen to be the percent change in TG levels at week 4 after randomization. An additional 44 weeks of study follow-up was included to permit fuller characterization of the tolerability and safety of DHA, as well as the collection of fat data. Adverse clinical and laboratory events were graded according to the National Institutes of Health Division of AIDS toxicity grading table [19].

HIV infection history and demographic data were recorded, and anthropometric, blood pressure, viro-immunological, and metabolic parameters were measured at study entry. They were randomized 1:1 to receive DHA 4 g/day or a placebo of olive oil daily. The primary endpoint of the study was the percent change in TG level at 4 weeks, whereas percent change in TG level at 12, 24, 36, and 48 weeks and change in limb fat mass measured by DEXA from baseline to 48 weeks were secondary endpoints.

2.3. Randomization

The randomization process was centrally managed. A randomization list was generated by means of the PROC PLAN of the SAS software with a 1:1 ratio of assignment in blocks of 4 elements for two arms, using an "A" or "B" blinded codes format. The list was sent to the medication manufacturer, Brudy Technologies® (Barcelona, Spain), which was in charge of assigning the arm codes to either DHA or placebo, the medication conditioning and the blinded delivery to the hospital pharmacy. The files and programs used for randomization were deleted from the computer system of the statistical team once the list was generated whereas a sealed copy of the list and individual, numbered, opaque, sealed and stapled envelopes were centrally retained, and kept closed until the end of the study. After informed consent, patients were screened, and only once eligibility was confirmed were patients strictly assigned using

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