Cytokine 105 (2018) 73–79

Contents lists available at ScienceDirect

Cytokine

journal homepage: www.elsevier.com/locate/cytokine

Effects of docosahexanoic acid supplementation on inflammatory and subcutaneous adipose tissue gene expression in HIV-infected patients on combination antiretroviral therapy (cART). A sub-study of a randomized, double-blind, placebo-controlled study

Pere Domingo[a,*], José Miguel Gallego-Escuredo[b,*], Irene Fernández[a], Joan Villarroya[a], Ferran Torres[c,d], María del Mar Gutierrez[a], María Gracia Mateo[a], Francesc Villarroya[b], Francesc Vidal[e], Marta Giralt[b], Joan Carles Domingo[b]

[a] Infectious Diseases Department, Hospital de la Santa Creu i Sant Pau, Institut de Recerca del Hospital de Sant Pau, Barcelona, Spain
[b] Department of Biochemistry and Molecular Biology, Institute of Biomedicine (IBUB), University of Barcelona and CIBER Fisiopatologia de la Obesidad y Nutrición, Barcelona, Spain
[c] Biostatistics and Data Management Core Facility, IDIBAPS, Hospital Clinic, Barcelona, Spain
[d] Biostatistics Unit, School of Medicine, Universitat Autònoma de Barcelona, Barcelona, Spain
[e] Infectious Diseases Unit, Department of Internal Medicine, Hospital Universitari Joan XXIII, IISPV, Universitat Rovira i Virgili, Tarragona, Spain

ARTICLE INFO

Keywords:
Triglycerides
Docosahexanoic acid
Subcutaneous adipose tissue
IL-6
IL-8
TNF-α
PPAR-γ
MCP-1

ABSTRACT

Background: Omega-3 fatty acids have the potential to decrease inflammation and modify gene transcription. Whether docosahexanoic acid (DHA) supplementation can modify systemic inflammatory and subcutaneous adipose tissue (SAT) gene expression in HIV-infected patients is unknown.

Methods: A randomized, double-blind, placebo-controlled trial that enrolled 84 antiretroviral-treated patients who had fasting TG levels from 2.26 to 5.65 mmol/l and received DHA or placebo for 48 weeks was performed (ClinicalTrials.gov, NCT02005900). Systemic inflammatory and SAT gene expression was assessed at baseline and at week 48 in 39 patients.

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 ($P < 0.0001$). High sensitivity C reactive protein (hsCRP) and arachidonic acid levels significantly decreased in the DHA group. Adipogenesis-related and mitochondrial-related gene expression did not experience significant changes. Mitochondrial DNA (mtDNA) significantly decreased in the placebo group. SAT inflammation-related gene expression (Tumor necrosis factor alpha [TNF-α], and monocyte chemoattractant protein-1 [MCP-1]) significantly decreased in the DHA group.

Conclusions: DHA supplementation down-regulated inflammatory gene expression in SAT. DHA impact on markers of systemic inflammation was restricted to hsCRP and arachidonic acid.

1. Introduction

HIV-1 infection is associated with chronic inflammation that most likely is a pathogenic mechanism underlying the increased appearance of co-morbid conditions not directly related to HIV infection itself, such as cardiovascular disease or non-AIDS cancers, in otherwise virologically-controlled patients [1]. This inflammatory phenotype has also often been described in patients with HIV/HAART-associated lipodystrophy syndromes (HALS) in whom it is thought to be involved, together with some antiretroviral drug toxic effects, in their pathogenesis [2].

Polyunsaturated fatty acids (PUFA), the so-called omega-3 fatty acids, are pleiotropic molecules with a wide range of claimed beneficial effects [3]. Among them, they decrease serum triglyceride levels, increase high-density lipoprotein (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 [3]. In the HIV setting, a number of clinical trials have demonstrated that hypertriglyceridemia can at least be partially corrected by diet supplementation with a mixture of omega-3

* Corresponding authors at: Infectious Diseases Department, Hospital de la Santa Creu i Sant Pau, Av. Sant Antoni Mª Claret, 167, 08025 Barcelona, Spain.
E-mail address: pdomingo@santpau.cat (P. Domingo).

https://doi.org/10.1016/j.cyto.2018.02.008
Received 4 September 2017; Received in revised form 1 February 2018; Accepted 3 February 2018

fatty acids of fish oil origin [4,5].

Docosahexanoic acid (DHA) competes with arachidonic acid (ARA) as a substrate for the synthesis of pro-inflammatory factors such as leukotrienes, prostaglandins, and cytokines, and this is the basis for its anti-inflammatory properties [6]. A number of clinical trials describe the effects of PUFA on the circulating levels of inflammatory mediators. A decrease in C reactive protein (CRP) and tumor necrosis alpha (TNF-α) serum levels was observed in athletic men after DHA administration [7], whereas DHA administered to patients with dyslipidemia caused a decrease in plasminogen activator inhibitor-1, fibrinogen, and CRP serum levels [8]. Notwithstanding this, data regarding the effects of PUFA on inflammation in HIV-infected patients are discordant, some studies showing a beneficial effect by decreasing interleukin-6 (IL-6) and TNF-α circulating levels [9] while others do not show any change [10].

PUFAs have also been claimed to be gene regulators since it has been shown that they are able to bind and activate all peroxisome proliferator-activated receptor (PPAR) isoforms, including PPAR gamma [11], which are major regulators of adipocyte differentiation, whole-body insulin sensitivity, and are involved in the control of local inflammation in adipose tissue [2].

With all this information in mind, we performed a nested sub-study within a clinical, double-blind, placebo-controlled, randomized trial of DHA supplementation in virologically-controlled HIV-infected patients with mild hypertriglyceridemia [12]. Our working hypothesis was that DHA supplementation might decrease inflammation and improve adipocyte biology functional markers at subcutaneous adipose tissue level.

2. Patients and methods

2.1. Study population

Patients' recruitment was done between July 2010 and June 2011 at the *Hospital de la Santa Creu i Sant Pau* HIV infection clinic, which serves a population of 1700 adult patients on active follow-up. Candidates need to have 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 in two consecutive determinations within a 15-day interval. Exclusion criteria included a TG level > 5.65 mmol/l, since then pharmacological therapy was indicated [13]. Additional exclusion criteria were hypersensitivity to the active compound or product excipients, BMI > 30 kg/m^2, pregnancy, breastfeeding, anticoagulant treatment, oral antidiabetics and hormonal treatments. Lipid-lowering drugs were not allowed except if stopped more than 3 months before the selection visit. Consumption of high levels of alcohol (> 20 g/d), diabetes or fasting blood glucose level (glycemia > 6.6 mmol/l) were excluded as well. Other exclusion criteria were: an active AIDS-defining disease, serum creatinine > 150 μmol/l and alanine aminotransferase or aspartate aminotransferase > 5 × upper limit of normal, anemia, and > 10% loss in body weight in the preceding 6 months. The 1993 revised case definition of the Centers for Disease Control and Prevention (CDC) was used to diagnose AIDS [14]. All participants were instructed to maintain their usual diets, alcohol intakes, and physical activities, and not to make any changes to their lifestyle during the intervention period. 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* and was registered under ClinicalTrials.gov Identifier No. NCT02005900. Thirty-nine randomized patients volunteered for the inflammatory and molecular subcutaneous adipose tissue (SAT) sub-study (18 from the DHA and 21 from the placebo groups).

2.2. Study design

A placebo-controlled, double-blind, phase 4, randomized, 2-arm 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. This total dose is within the 2–4 g of EPA and DHA per day recommended by the AHA for patients who need to lower TG levels [14]. Both DHA and placebo were presented as ochre vials similar to each other. Placebo vials contained 7 g of olive oil. 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). The National Institutes of Health Division of AIDS toxicity grading table was used to grade adverse clinical and laboratory events [15].

At recruitment for the study, HIV infection history and demographic data were recorded and anthropometric, blood pressure, viro-immunological, and metabolic parameters were measured for each patient. 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 change in TG level at 12, 24, 36, and 48 weeks and change in limb fat mass, and in inflammatory and fat molecular parameters were secondary endpoints.

2.3. Laboratory measurements and plasma fatty acid concentrations

These measurements were performed at baseline and at weeks 4, 12, 24, 36 and 48. Laboratory methods used have been previously described [16]. The fatty acid composition of was determined through the Lepage and Roy method [17], which has also been described elsewhere [18].

2.4. Systemic inflammatory markers

Serum levels of interleukin-1 (IL-1), IL-6, IL-8, monocyte chemoattractant protein-1 (MCP-1), nerve growth factor (NGF), TNF-α, and hepatocyte growth factor (HGF) were measured at baseline and at week 48 through an antibody-linked, fluorescently labeled microsphere bead-based multiplex analysis system (Linco Research/Millipore, Billerica, MA) and quantified by Luminex100ISv2 equipment. The intra and inter-assay coefficient of variation for each cytokine was: IL-1: 7% and 12%; IL-6: 2% and 10%; IL-8: 3% and 14%; MCP-1: 2% and 11%; NGF: 4% and 11%, TNFα: 3% and 19% and HGF: 3% and 11%, respectively.

2.5. Gene expression profiling in subcutaneous adipose tissue

A subcutaneous adipose tissue biopsy was performed on each patient at baseline and at 48 weeks. First of all, local anesthesia was applied in periumbilical zone and biopsy was undertaken with a number 8 punch. We extracted an approximated 2 cm^3 sample. Fat samples were immediately frozen in liquid nitrogen and kept at −80 °C until processing. DNA was isolated, after homogenization in RLT (Qiagen, Hilden, Germany) buffer, using a phenol/chloroform extraction methodology. RNA was isolated using a column affinity-based methodology, including on-column DNA digestion (RNeasy; Qiagen). One microgram of RNA was transcribed into cDNA using MultiScribe reverse transcriptase and random-hexamer primers (TaqMan Reverse Transcription Reagents; Applied Biosystems, Foster City, California, USA). For quantitative mRNA expression analysis, TaqMan reverse transcriptase (RT)-polymerase chain reaction (PCR) was performed on the ABI PRISM 7700HT sequence detection system (Applied Biosystems). The TaqMan RT-PCR reaction was performed in a final volume of 25 μl using TaqMan Universal PCR Master Mix, No AmpErase UNG reagent and the following specific primer pair probes: PPARγ (Hs00234592_s1), Adiponectin (Hs00605917_s1), mitochondrial cytochrome b, cytochrome b (Cyt b) (Hs002596867_s1), MCP-1 (Hs00234140_s1) and TNFα (Hs00174128_s1). Quantification of mtDNA was performed using Cyt band referred to nuclear DNA, as determined by the amplification