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A lipidomic analysis approach in patients undergoing lipoprotein apheresis

C. Schmöcker [a, b], U. Kassner [a], S. Kiesler [a], M. Bismarck [a], M. Rothe [c], E. Steinhagen-Thiessen [a], K.H. Weylandt [a, d, *]

[a] Medical Department, Division of Hepatology and Gastroenterology (including Metabolic Diseases), Charité University Medicine Berlin, Campus Virchow Klinikum, Berlin, Germany
[b] Department of Gastroenterology, Sana Klinikum Lichtenberg, Berlin, Germany
[c] Lipidomix GmbH, Berlin, Germany
[d] Experimental and Clinical Research Centre (ECRC), Charité University Medicine and Max Delbrück Center for Molecular Medicine (MDC), Campus Buch, Berlin, Germany

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ABSTRACT

Lipoprotein apheresis such as heparin-induced extracorporal LowDensityLipoprotein (LDL) Cholesterol precipitation (HELP) reduces apolipoprotein B-containing lipoproteins, most importantly low-density-lipoprotein (LDL), and lipoprotein (a) [Lp(a)]. It is used in patients with atherosclerotic disease and therapy-refractory hypercholesterolemia or progressive atherosclerotic disease in patients with elevated Lp(a). While lipid-lowering effects of lipoprotein apheresis are well-established, there are only sparse data regarding the effect of apheresis on individual omega-6 and omega-3 polyunsaturated fatty acids (n-6 PUFA and n-3 PUFA), such as arachidonic acid (AA), eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), which could increase (AA) or decrease (EPA and DHA) cardiovascular risk.

Here we analyzed different omega-6 and omega-3 fatty acids in the blood of patients undergoing a single HELP apheresis procedure using gas chromatography (GC). Furthermore, we assessed the effect of HELP treatment on formation of lipid metabolites and mediators arising from these polyunsaturated fatty acids in the plasma by LC/ESI-MS/MS. Lipoprotein apheresis reduced the concentrations of fatty acids analyzed in the plasma by 40–50%. This was similar for AA, EPA and DHA. The reduction in fatty acid plasma levels was similar to the reduction of total triglycerides. However there was a trend towards an increase of PUFA metabolites associated with platelet activation, such as 12-hydroxyeicosatetraenoic acid (12-HETE) and 14-hydroxydocosahexaenoic acid (14-HDHA).

These data indicate that HELP apheresis could interfere with achieving higher levels of n-3 PUFA in the plasma. Lipid apheresis treatment might also increase the formation of potentially pro- as well as anti-inflammatory lipid mediators derived from AA or EPA and DHA.

1. Introduction

Lipoprotein apheresis is an established therapeutic method to treat patients with homozygous and heterozygous as well as heterozygous compound familial hypercholesterolemia and other forms of severe hypercholesterolemia when – in the presence of clinically significant cardiovascular disease – lipid-lowering medications alone are not successful in reducing low-density lipoprotein cholesterol (LDL-C) levels [1]. Furthermore lipoprotein (a) (Lp(a)), an established independent cardiovascular risk factor, can be lowered by lipoprotein apheresis treatment [2,3]. Extracorporeal lipoprotein apheresis has been proven to effectively reduce levels of LDL-C and Lp(a), leading to a significant decrease of the development of atherosclerosis and of the incidence of major cardiovascular events (such as myocardial infarction, cerebrovascular events, limb amputation, renal vascular involvement and death) [4–6].

Abbreviations: *n*-3-PUFA, omega-3 polyunsaturated fatty acids; *n*-6-PUFA, omega-6 polyunsaturated fatty acids; EPA, eicosapentaenoic acid; DHA, docosahexaenoic acid; AA, arachidonic acid; COX, cyclooxygenase; LOX, lipooxygenase; CYP450, cytochrome P450.

* Corresponding author. Division of Hepatology and Gastroenterology (including Metabolic Diseases), Charité, Campus Virchow Hospital, Augustenburger Platz 1, 13353, Berlin, Germany.
E-mail address: karsten.weylandt@charite.de (K.H. Weylandt).

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Presently several apheresis methods are available which all have been shown to effectively reduce LDL-C levels in the plasma, while some also decrease fibrinogen levels leading to a further decrease in plasma viscosity [7,8]. However, while effects of lipid apheresis on common lipid parameters are well established, only little is known regarding its effects on long-chain polyunsaturated fatty acids (PUFA) as well as their bioactive lipid mediators in the blood.

Numerous studies have shown that essential fatty acids and their mediators can directly influence the course of diseases. In particular the excessive consumption of omega-6 PUFA (n-6 PUFA) has been introduced as an important risk factor for the progression of cardiovascular, inflammatory and autoimmune diseases [9–11].

For cardiovascular diseases high intake of n-3 PUFA is associated with antiarrhythmic, anti-thrombotic and vasodilatory effects [12,13] whereas AA (20:4 n-6) derived 20-HETE has been described as an inducer of arterial hypertension [14]. In the field of hyperlipidemia omega-3 PUFA supplementation is established for the treatment of hypertriglyceridemia. The pharmaceutical intake of omega-3 PUFA can lower plasma triglycerides (TGs) by up to 50% [15].

The most prominent n-6 PUFA arachidonic acid (AA, 20:4 n-6) as well as the omega-3-PUFA (n-3 PUFA) eicosapentaenoic acid (EPA, 20:5 n-3) and docosahexaenoic acid (DHA, 22:6 n-3) can be processed by different enzymatic (e.g. COX, LOX, CYP 450 enzymes) and non-enzymatic pathways resulting in a wide spectrum lipid mediators [16]. These oxygenated lipid mediators, named oxylipins, have been described as highly bioactive and inflammation modulating [17–19]. Modifications in the dietary composition can change the omega-6/omega-3-ratio in tissues and organs resulting in a replacement of arachidonic acid (AA, 20:4 n-6) -derived oxylipins by their n-3 PUFA counterparts [9,12,20–22]. Moreover in vitro and in vivo studies showed increased formations of CYP–dependent n-3 PUFA mediators as response to dietary interventions [23,24].

So far the only lipidomic analysis performed in the context of lipid apheresis focused on the composition of phospholipids and membrane lipids such as lysophospatidylcholines, phosphatidylcholines, sphingomyelins and cholesterol esters. A study in serum samples from six apheresis patients demonstrated that these lipid compounds are lowered by apheresis treatment by approximately 50% [25]. However, Tselmin et al. did not assess individual PUFAs or their bioactive metabolites.

Using gas chromatography (GC) for the analysis of fatty acids and liquid chromatography coupled to tandem mass-spectrometry (LC/ESI-MS/MS) it is feasible to precisely determine PUFA and their derived hydroxy metabolites (oxylipins) in different tissues and body fluids [26–28]. Our previous studies have shown formation of many of these lipid metabolites and mediators in human blood [29]. Here we report a lipidomic characterization of patients treated with Heparin-induced Extracorporeal LDL Precipitation (HELP) lipid apheresis and the direct influence of HELP apheresis on the PUFA and lipid metabolite composition in human plasma. We show that apheresis treatment effectively reduces AA as well as the potentially cardioprotective n-3 PUFA EPA and DHA in plasma. At the same time, treatment tends to increase levels of 12-HETE, which might indicate platelet activation and could lead to vasoconstriction and arterial hypertension [30–32]. However, 18-HEPE, recently identified as cardioprotective EPA metabolite [33], was not affected by apheresis treatment.

2. Materials and methods

2.1. Patients and blood sampling

Eight male patients aged between 42 and 74 years undergoing weekly lipoprotein apheresis treatments using the HELP technology in our lipid clinic were included. Indication for lipoprotein apheresis was familial hypercholesterolemia and/or hyperlipoproteinemia (a) with clinically relevant cardiovascular disease in all cases. Most patients received lipid-lowering drugs, including statins and all patients also received low-dose aspirin treatment (Table 1). Lipid apheresis procedures were performed according to standard operating procedures using Heparin-induced LDL precipitation apheresis (HELP, Plasmat Futura; B. Braun, Melsungen, Germany).

Blood was sampled in EDTA containers directly before and after one lipid apheresis session. Plasma was then obtained by centrifugation for 10 min at 4 °C and 3500 rpm and the cell components of the blood were kept in a separate vial. Samples were then immediately stored at −80 °C until GC and LC/ESI-MS/MS analysis. For routine clinical chemistry analysis samples from each patient were taken at the same time, and serum levels of total cholesterol, LDL-cholesterol, HDL-cholesterol and triglycerides were determined at the central routine laboratory facility of the Charité University Medicine in Berlin. The study was approved by the ethical committee of the Charité University Medicine Berlin in accordance with the Declaration of Helsinki.

2.2. Determination of fatty acid profiles using gas chromatography

Fatty acids used as standards were purchased from Nu-Chek Prep (Waterville, MN, USA) or from Supelco (Supelco, Bellefonte, PA, USA). Fatty acid compositions were analyzed as described previously [34,35]. In order to transesterify and methylate total fatty acids in the samples blood cell fractions or plasma samples were mixed with 50 μl pentadecaenoic acid (1 mg/mL) as internal standard and 500 μl Borontrifluoride (BF3) in 12% methanol and 500 μl *N*-hexane, overlaid with nitrogen gas, and then tightly closed. After vortexing samples were cooked for 60 min in a preheated heating block at 100 °C. After cooling to room temperature, the mixture was added to 750 μl distilled water, vortexed and shaken out for 4 min. Then centrifugation at 3500 rpm for 5 min was performed. 100 μl of the upper hexane phase was removed into a micro-insert in a vial and tightly closed for subsequent measurement. Gas chromatography was performed using a HP5980 Gas Chromatograph (Hewlett-Packard, now Agilent, Santa Clara, CA, USA) equipped with an Omegawax column (Supelco, Bellefonte, PA, USA) using nitrogen as carrier gas. Fatty acids were identified by comparison with a standard mixture of fatty acids consisting of pentadecaenoic acid, AA, EPA and DHA, which was methylated alongside the samples as well as a predefined standard mix from Supelco.

2.3. Sample preparation and LC/ESI-MS/MS

Lipid mediators and deuterated standards used in this study were purchased from Cayman Chemical (Ann Arbor, MI, USA). Materials used for solid phase extraction (SPE), such as sodium acetate, ethylacetate, acetic acid and *n*-hexane were obtained from Carl Roth (Karlsruhe, Germany) and methanol from Merck (Darmstadt, Germany). Butylhydroxytoluol (BHT, 2,6-di-*tert*-butyl-4-methylphenol) at 99% was from Acros Organics (Geel, Belgium) and Bond Elute Certify II columns from Varian (Palo Alto, CA, USA) were used. Other solvents such as methanol (LC–MS-grade) and acetonitrile (HPLC gradient grade) were from Fisher Scientific (Loughborough, United Kingdom).

For sample preparation an internal standard consisting of 15-HETE-d8 (10 ng), LTB4-d4 (10 ng), PGE2-d2 (5 ng) and ice-cold methanol containing BHT (0.1%) was added to the plasma. The pH was adjusted with 1 M sodium acetate buffer containing 5% v/v methanol at pH 6. After centrifugation, the obtained supernatant was added to the SPE-columns, which were preconditioned with 3 mL methanol, followed by 3 mL of 0.1 mol/L sodium acetate buffer containing 5% methanol (pH6). The SPE-columns were then washed