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Original Research Article

Decreased eicosapentaenoic acid levels in acne vulgaris reveals the presence of a proinflammatory state

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ABSTRACT

This study aimed to determine circulating levels of polyunsaturated fatty acids (PUFAs), secretory phospholipase A2 (sPLA2), lipoprotein lipase (LPL) and measure circulating protein levels of angiopoietin-like protein 3 (ANGPTL3), ANGPTL4, cyclooxygenase-2 (COX-2) and prostaglandin E2 (PGE2) in patients with acne vulgaris. Serum from 21 control subjects and 31 acne vulgaris patients were evaluated for levels of arachidonic acid (AA, C20:4n- 6), dihomo-gamma-linolenic acid (DGLA, C20:3n-6), eicosapentaenoic acid (EPA, C20:5n-3) and docosahexaenoic acid (DHA, C22:6n-3). PUFA levels were determined by an optimized multiple reaction monitoring (MRM) method using ultra fast-liquid chromatography (UFLC) coupled with tandem mass spectrometry (MS/MS). Lipid profile, routine biochemical and hormone parameters were assayed by standard kit methods Serum EPA levels were significantly decreased while AA/EPA and DGLA/EPA ratio were significantly increased in acne vulgaris patients compared to controls. Serum levels of AA, DGLA and DHA showed no significant difference while activity of sPLA2 and LPL were significantly increased in acne vulgaris compared to controls. Results of this study reveal the presence of a proinflammatory state in acne vulgaris as shown by significantly decreased serum EPA levels and increased activity of sPLA2, AA/EPA and DGLA/EPA ratio. Increased LPL activity in the serum of acne vulgaris patients can be protective through its anti-dyslipidemic actions. This is the first study reporting altered EPA levels and increased sPLA2 activity in acne vulgaris and supports the use of omega-3 fatty acids as adjuvant treatment for acne patients.

1. Introduction

Acne vulgaris is a common skin disease characterized by inflammation of the follicular portion of the pilosebaceous unit [1]. Mild cases are associated with open and closed comedones of the face while severe disease is characterized by cysts, nodules, folliculopustules, and folliculopapules occuring in the face and chest [2].

Earlier studies considered inflammation as a secondary event in acne vulgaris and reported that inflamed papules and pustules developed from open and closed comedones [3]. Later studies demonstrated that vascular endothelial cells were activated and inflammatory responses occurred in the very early stages of acne lesion development [4]. Although current evidence supports the crucial role of cellular inflammation at all stages of acne lesion development, the sequence of events which triggers initiation of the inflammatory cascade remain speculative [5].

Secretory phospholipase A2 (sPLA2), hydrolyses phospholipids, generating free fatty acids (FFAs) and lisophospholipides, which lead to an increase of proinflammatory mediators [6]. Generation of free polyunsaturated fatty acids (PUFAs) regulate inflammatory responses through the production of eicosanoids including prostaglandins (PGs), thromboxanes (TXs) and leukotrienes (LTs) [7]. Eicosanoids derived from n-6 PUFAs such as arachidonic acid (AA, C20:4n-6) have proinflammatory and immunoactive functions, whereas eicosanoids derived from n-3 PUFAs such as eicosapentaenoic acid (EPA, C20:5n-3) have anti-inflammatory properties, attributed to their ability to inhibit the formation of n-6 PUFA-derived eicosanoids [8]. Resolvins and protectins generated from EPA and docosahexaenoic acid (DHA, C22:6n-3) display potent anti-inflammatory properties and are recognized in the resolution of inflammation [9]. A randomised double-blinded controlled study was conducted to evaluate the efficacy of n-3 fatty acids for the treatment of mild to moderate facial acne. It was shown

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that moderate doses of n-3 fatty acids (DHA, EPA) improved acne lesions [10]. Although studies have documented that dietary supplementation with n-3 fatty acid ameliorates acne vulgaris serum PUFA levels have not been evaluated in patients with acne vulgaris.

Free fatty acids can also be generated for tissue utilization via lipoprotein lipase (LPL). This enzyme catalyzes the hydrolysis of triglycerides (TG) in plasma TG-rich lipoproteins at the capillary endothelial cell (EC) surface [11]. Lipoprotein lipase mRNA expression has been detected in skin appendages showing that apocrine and sebaceous glands have the capacity to sequester fatty acids that may have important implications for the understanding of acne vulgaris [12]. The angiopoietin-like proteins (ANGPTLs) 3 and 4 are key regulators of plasma lipid metabolism by serving as potent inhibitors LPL [13]. Although LPL, ANGPTL 3 and 4 are important modulators of lipid metabolism, their serum concentrations in acne vulgaris patients have not been determined.

The present study was undertaken to evaluate serum PUFA levels, determine serum activity of sPLA2, LPL and measure circulating protein levels of COX-2, PGE2, ANGPTL3 and ANGPTL4 in patients with acne vulgaris.

2. Materials and methods

2.1. Study groups

The patient group involved 31 female patients aged between 18 and 40 years, who were admitted to the Dermatology Clinic. Disease activity was established by the Global Acne Grading System (GAGS) criteria [14]. The GAGS score was determined using the disease activity and the patients were diagnosed with either moderate or severe acne. The control group included 21 age and gender matched healthy subjects between 19 and 40 years. Patients and controls included in the study did not receive any systemic or topical drug therapy for the last three months, had no history of systemic medication use, and had no smoking habits. This study was approved by the Institutional Review Board and was performed in accordance with the Declaration of Helsinki. Venous blood samples from the control subjects and acne patients were collected, and serum from individual subjects was stored at −80 °C until analyzed.

2.2. Laboratory measurements

Lipid profile, routine biochemical and hormone parameters were assayed by standard kit methods using autoanalyzers (Beckman Coulter AU5800 Clinical Chemistry and UniCel DxI 800 immunoassay systems). Insulin sensitivity was evaluated using homeostatic model assessment for insulin resistance (HOMA IR) [15].

2.3. Electrospray ionization mass spectrometry

Standards for AA (C20:4n6), DGLA (C20:3n6), EPA (C20:5n3) and DHA (C22:6n3) were purchased from Sigma-Aldrich (St. Louis MO, USA). Deuterium labeled AA-d8 internal standard (5,6,8,9,11,12,14,15-AA-d8) was obtained from Santa Cruz Biotechnology (Santa Cruz, CA, USA). Solutions of AA, DGLA, EPA, DHA and AA-d8 standards were prepared in analytical grade methanol (Merck, Darmstadt, Germany). An optimized multiple reaction monitoring (MRM) method was developed using ultra-fast liquid chromatography (UFLC) coupled with tandem mass spectrometry (MS/MS) as previously described [16]. A UFLC system (LC-20 AD UFLC XR, Shimadzu Corporation, Japan) was coupled to a LCMS-8040 triple quadrupole mass spectrometer (Shimadzu Corporation, Japan). Chromatographic separations were carried out using Inertsil HPLC column (ODS-4, 2.1 × 100 mm, 3 μm; GL Sciences Inc. Tokyo, Japan) maintained at 40 °C. DHA, EPA, AA and DGLA were separated using a gradient elution with a flow rate of 0.45 ml/min. Mobile phase solvent A was 10 mM ammonium acetate (Sigma-Aldrich, St. Louis, MO, USA) in water and solvent B was acetonitrile (Sigma-Aldrich, St. Louis, MO, USA). Gradient program was solvent B, 70% (0 min), 90% (3 min), 100% (3.01–4 min) and 70% (4.01–8 min). MRM transitions and responses were automatically optimized for individual compounds in negative electrospray ionization (ESI). In the negative ESI–MS mode the precursor and product m/z values for analyzed polyunsaturated fatty acids were as follows: DGLA (C20:3n6), precursor m/z: 304.80, product m/z: 59.00, 260.70; AA (C20:4n6), precursor m/z: 303.10, product m/z: 59.00, 258.90; EPA (C20:5n3), precursor m/z: 301.10, product m/z: 59.10, 256.70; DHA (C22:6n3), precursor m/z: 327.10, product m/z: 59.10, 283.20; AAd8, precursor m/z: 311.10, product m/z: 59.10, 97.90, 267.10. Responses to AA, DHA, EPA and DGLA were optimized to a linear calibration range from 100 ng/ml to 30 μg/ml and a sample analysis time of 8 min.

2.4. Sample preparation for LC–MS/MS

Samples were prepared for LC–MS/MS analysis as previously described [17]. Briefly, in a glass test tube, 200 μl serum was added to 200 μl AA-d8 internal standard solution. 1 ml of acetonitril/37% hydrochloric acid (Cayman, Ann Arbor, MI, USA) was added to the mixture in a 4:1 v/v. Tubes were capped with reusable teflon liner screw caps and samples were hydrolyzed by incubating at 90 °C for 2 h in a heating block (VLM, Bielefeld, Germany). After cooling down to room temperature, fatty acids were extracted with 2 ml of hexane. Samples were vortex-mixed for 20 s, left at room temperature for 5 min and centrifuged at 3000 rpm for 1 min. The upper phase containing free fatty acids were transferred to glass tubes and evaporated at room temperature under a constant stream of nitrogen with height adjustable gas distribution unit (VLM, Bielefeld, Germany). Fatty acids were dissolved in 200 μl methanol-water (180:20, v/v) filtered via 0,2 μm polytetrafluoroethylene (PTFE) syringe filters (Whatman, GE Healthcare Bio-Sciences, Pittsburgh, USA) and transferred to autosampler vials (Vertical Chromatography, Nonthaburi, Thailand).

2.5. Measurement of secretory phospholipase A2 activity

Serum activity of sPLA2 was measured via a sPLA2 assay kit (Abcam, Cat No: ab133089, Cambridge, MA, USA). Before performing the assay, low molecular weight contaminants were removed from the samples using an ultrafiltration unit via centrifugation through a 10-kDa molecular mass cut-off filter (Amicon, Millipore Corporation, Bedford, MA, USA) for 30 min at 25 °C. The 1,2-dithio analog of diheptanoyl phosphatidylcholine was used as a substrate to detect sPLA2 activity. Upon hydrolysis of the thio ester bond at the sn-2 position by sPLA2, free thiols were detected by 5,5′-dithiobis-2-nitrobenzoic acid (DTNB). One unit of enzyme activity was defined as the amount of enzyme that hydrolyzes one μmol of diheptanoyl thio-PC per minute at 25 °C.

2.6. Measurement of cyclooxygenase-2 and prostaglandin E_2

Quantitative determination of COX-2 concentrations in serum was done using a COX-2 enzyme immunoassay (ELISA) test kit (Elabscience, Cat No: E-EL-H1846, WuHan, China) according to manufacturer's instructions. Prostaglandin E_2 was measured in serum by a commercial ELISA kit (R&D Systems, Cat No: KGE004B Minneapolis, MN, USA) according to manufacturer's instructions. To inhibit PG synthesis by COX-2, indomethacin (10 μg/ml) was added to serum collection tubes immediately following draw. A standard curve of absorbance values of known COX-2 and PGE2 standards were plotted as a function of the standard concentra-