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Inflammatory markers in dependence on the plasma concentration of 37 fatty acids after the coronary stent implantation

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ABSTRACT

Using the regression model building the relationships between the concentration of 37 fatty acids of blood plasma phospholipids of 41 patients with coronary artery disease after coronary stent implantation, the inflammatory response and oxidative stress markers were estimated. The dynamics of the inflammatory response and the oxidative stress was indicated by measuring plasma concentrations of highly sensitive C-reactive protein, interleukin-6, serum amyloid A and malondialdehyde before, 24 h after stent implantation. The multiple linear regression analysis was preceded by an exploratory data analysis, principal component analysis, factor analysis and cluster analysis, which proved a hidden internal relation of 37 fatty acids. The concentration of cerotic acid (C26:0) has been positively associated with an increase of malondialdehyde concentration after stent implantation, while the concentrations of tetracosatetraenoic (C24:4 N6) and nonadecanoic (C19:0) acids were associated with decrease of lipoperoxidation. The increase of interleukin-6 during the 24 h after implantation was associated with higher levels of pentadecanoic acid (C15:0) and lower levels of α-linolenic acid (C18:3 N3). Regression models found several significant fatty acids at which the strength of the parameter β for each fatty acid on selected markers of C-reactive protein, malondialdehyde, interleukin-6 and serum amyloid A was estimated. Parameter β testifies to the power of the positive or negative relationship of the fatty acid concentration on the concentration of selected markers. The influencing effect of the cerotic acid (C26:0) concentration in plasma phospholipids exhibiting parameter $\beta = 140.4$ is, for example, 3.5 times higher than this effect of *n*-3 tetracosapentaenoic acid (C24:5 N3) with $\beta = 40.0$. Composition of fatty acids in plasma phospholipids shows spectrum of fatty acids available for intercellular communication in systemic inflammatory response of organism and should affect clinical outcomes.

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1. Introduction

Percutaneous coronary intervention (PCI) with stent is currently a common way to treat significant coronary heart disease. Coronary stent implantation elicits inflammatory response, which negatively affects clinical outcomes. The higher content of some fatty acids, e.g. *n*-3 fatty acids, oleic acid, in plasma or in erythrocyte membranes is supposed to be associated with beneficial cardiovascular effects and a low inflammatory state, whereas *n*-6 or saturated fatty acids and oxidative stress contribute to inflammation.

Fatty acids, which are of the primary interest in relation to coronary heart disease (CHD) include the *n*-6 fatty acids (linoleic, dihomo-γ-linolenic and arachidonic acids), and the *n*-3 fatty acids (α-linolenic, eicosapentaenoic and docosahexaenoic acids). Three highly unsaturated fatty acids – arachidonic (C20:4 N6), dihomo-γ-linolenic (C20:3 N6) and eicosapentaenoic (C20:5 N3) acids – are the original source of a number of eicosanoids (prostaglandins, leukotrienes, prostacyclins, thromboxanes, lipoxins, etc.) [1]. The *n*-3 polyunsaturated fatty acids have shown to exhibit many effects beneficial to cardiovascular health including anti-arrhythmic, improvement of endothelial function, and down-regulation of blood pressure [2]. Docosahexaenoic (C22:6 N3) and eicosapentaenoic (C20:5 N3) acids' enrichment of membrane phospholipids can increase arrhythmic thresholds [3], electrically stabilize cardiac myocytes by inhibiting sodium and calcium channels [4], and favorably affect autonomic tone [5,6]. Eicosanoids made from arachidonic acid are generally more potent mediators of inflammation, vasoconstriction, and platelet aggregation than those made from eicosapentaenoic acid [7], the ratio of arachidonic to eicosapentaenoic acid in membrane polyunsaturated fatty acids can theoretically influence biochemical and physiological responses to stress [8]. Further saturated fatty acids, such as myristic or palmitic

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acid, have been demonstrated to induce inflammatory signaling, e.g. stimulating NFκB [9].

Systemic inflammation is a key component in the development and progression of atherosclerosis and also determines the occurrence of complications after PCI. A serum fatty acid pattern exhibiting a high content of monounsaturated fatty acids and a low level of linoleic acid has been linked to elevated CRP [10]. In a Spanish study, CRP was inversely associated with linoleic acid and *n*-3 fatty acids whereas IL-6 correlated positively with myristic (C14:0) and palmitic (C16:0) acids [11]. The Italian study showed that lower proportions of arachidonic, eicosapentaenoic and docosahexaenoic acids were associated with higher IL-6 concentrations and that α-linolenic acid was inversely related to CRP [12]. Also Farzaneh-Far et al. [13] found that CRP and IL-6 were inversely associated with docosahexaenoic and eicosapentaenoic acid, in a large cross-sectional study of patients with stable coronary artery disease.

The induction of the pro-inflammatory cytokine IL-6 is regulated with several pathways. An important role in the regulation of expression of cytokines, such as IL-6, may be played by reactive oxygen species, generated by vascular enzymes and by other sources. Elevated reactive oxygen species contribute significantly to oxidative stress, which further induces the inflammation pathways [14]. Oxidative stress and inflammation were reported to create a self-perpetuating cycles of oxidation and inflammation. Čermák et al. [15] found a correlation between fatty acid profile of erythrocyte membranes and an increase in inflammatory reactions after PCI with stenting. The aim of our study was to decide if inflammatory response after PCI, which is highly individual, is affected by plasma phospholipid fatty acid profile, and quantitatively express to what extent by individual fatty acids in plasma phospholipids.

2. Material and methods

2.1. Study subjects

This cross-sectional study was approved by the Ethical Committee on Human Research of the Regional Hospital of Pardubice, Czech Republic, and all participants provided written informed consent. The study included 41 patients referred to the PCI with coronary stent implantation for significant coronary stenosis, *i.e.* at least 50% stenosis of the left main coronary artery or 70% stenosis of the epicardial coronary artery according to coronarographic examination. Excluded from the study were patients with an initial level of hs CRP > 10 mg/l, patients with serious health complications, ST Segment Elevation Myocardial Infarction (STEMI), heart failure according to New York Heart Association (NYHA) II–IV, renal failure, thyroid dysfunction, hepatic or oncology disease or patients that regularly consume alcohol. The appropriate institutional approval of the review board was obtained as well as the principles outlined in the Declaration of Helsinki for all human or animal experimental investigations have been followed. Participants received a description of the study and signed an informed participation consent that included permission to conduct analyses on the biological specimens collected and stored. All intervention was performed with a standard technique, and all patients received drug-eluting stents (Everolimus). Before intervention, all patients received weight-adjusted intravenous heparin with a target activated clotting time of 250–350 s.

2.2. Blood sample collection

Venous blood samples were collected in tubes with EDTA (The Vacuette Detection Tube, No. 455036, Greiner Bio-One GmbH, Kremsmünster, Austria) before and 24 h after stent implantation. After 20 min centrifugation of samples at 1500*g*, plasma was stored at −80 °C.

2.3. Determination of inflammatory markers

Inflammatory markers (high sensitivity C-reactive protein, interleukin-6 and serum amyloid A) were determined by standard procedures in the Regional Hospital of Pardubice, Czech Republic. High sensitivity C-reactive protein was measured with the analytical system VISTA, interleukin-6 with an immunochemistry analyzer Immulite and serum amyloid A with a BN ProSpec laser nephelometer (Siemens Healthcare Diagnostics Inc., USA).

2.4. Determination of malondialdehyde

Malondialdehyde was assessed by HPLC as previously described [16]. Plasma malondialdehyde was quantified as the malondialdehyde-thiobarbituric acid complex which was accomplished using an isocratic elution on a LiChroCart 250 × 4 mm, Purospher Star RP-18e, 5 μm, analytical column fitted with a LiChroCart 4 × 4 mm, Purospher Star RP-18e, 5 μm, guard column (Merck, Darmstadt, Germany).

2.5. Determination of plasma phospholipid fatty acids

Plasma fatty acids were determined using the thin layer chromatography (TLC Silica Gel 60 Glass Plates 20 × 20 cm) technique with a subsequent analysis of individual lipid fractions containing phospholipids, free fatty acids, cholesterol esters, diacylglycerols and triacylglycerols by gas chromatography with flame ionization detection (Agilent Technologies 7890 GC System, USA). The plasma samples were briefly deproteinized with 2-propanol, *n*-heptane and *ortho*-phosphoric acid (40:20:1, v/v/v) mixture. Then a mixture of methanol and toluene (4:1, v/v) and water were added and, after centrifugation (1700*g*, room temperature, 5 min), the upper organic phase was evaporated under nitrogen to dryness.

After adding chloroform-methanol mixture (2:1, v/v) to the residue, the content of the tube was transferred on the silica gel chromatography plate. Lipids were separated using a mobile phase composed of *n*-hexane, diethyl ether and acetic acid (8:2:0.3, v/v/v). The phospholipid fraction were scraped off the TLC plate, transferred to screw-capped tube, and dissolved in a mixture of methanol and toluene (4:1, v/v) containing *cis*-13,16,19-docosatrienoic acid as an internal standard. *trans*-esterification and the gas chromatographic separation of the fatty acid methyl esters have been described in a publication of Čermák et al. [15].

We used HP-88 capillary column (100 m in length, 250 μm in id, 0.25 μm in film thickness). The injector temperature was set at 250 °C, the flame ionization detector at 280 °C, and a programmed temperature ramp was used. The analysis time was 75 min. The flow rate of helium as the carrier gas was 3 mL/min and the inlet split ratio was set at 10:1. The injection volume of the sample was 1 μL.

2.6. Multivariate data analysis

At first, it was necessary to verify some assumptions made on the data. The dependent variable formed successively selected markers CRP, IL-6, SAA and MDA which represented the function on the phospholipid plasma concentration of 37 fatty acids. Matrix of the independent variables contained concentrations of 37 fatty acids in columns for 41 examined patients P1 to P41 placed in rows. Fatty acids in a regression model were labelled x_1 through x_{37} (Table 1). Mean Value (Median) of analyzed concentration [μmol/l] of 37 fatty acids in group of 41 patients is in Table 2.

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