Atherosclerosis 246 (2016) 130–140

Contents lists available at ScienceDirect

Atherosclerosis

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

Decreased phosphatidylcholine plasmalogens – A putative novel lipid signature in patients with stable coronary artery disease and acute myocardial infarction

Iryna Sutter [a, b, 1], Roland Klingenberg [c, 1], Alaa Othman [a, e], Lucia Rohrer [a], Ulf Landmesser [b, c, d], Dierik Heg [e], Nicolas Rodondi [f], Francois Mach [g], Stephan Windecker [h], Christian M. Matter [b, c], Thomas F. Lüscher [b, c], Arnold von Eckardstein [a, b, i], Thorsten Hornemann [a, b, i, *]

[a] Institute of Clinical Chemistry, University and University Hospital of Zurich, Zurich, Switzerland
[b] Competence Center for Integrated Human Physiology, University of Zurich, Zurich, Switzerland
[c] Department of Cardiology and Cardiovascular Research, University Heart Center, University Hospital Zurich, Zurich, University of Zurich, Switzerland
[d] Department of Cardiology, Charité Universitätsmedizin Berlin, Berlin, Germany
[e] Institute of Social and Preventive Medicine, Clinical Trials Unit, University of Bern, Bern, Switzerland
[f] Department of Internal Medicine, University Hospital Bern, Bern, Switzerland
[g] Cardiology, University Hospital Geneva, Switzerland
[h] Cardiovascular Center, University Hospital Bern, Switzerland
[i] Competence Center for Systems Physiology and Metabolic Diseases, ETH Zurich and University of Zurich, Zurich, Switzerland

ARTICLE INFO

Article history:
Received 22 May 2015
Received in revised form
30 December 2015
Accepted 3 January 2016
Available online 6 January 2016

Keywords:
Glycerophospholipids
Sphingolipids
Coronary artery disease
Acute myocardial infarction

ABSTRACT

Objective: Glycerophospholipids and sphingolipids are structurally heterogeneous due to differences in the O- and N-linked fatty acids and head groups. Sphingolipids also show a heterogeneity in their sphingoid base composition which up to now has been little appreciated. The aim of this study was to investigate the association of certain glycerophospholipid and sphingolipid species with stable coronary artery disease (CAD) and acute myocardial infarction (AMI).

Methods: The lipid profile in plasma from patients with stable CAD (n = 18) or AMI (n = 17) was compared to healthy subjects (n = 14). Sixty five glycerophospholipid and sphingolipid species were quantified by LC-MS. The relative distribution of these lipids into lipoprotein fractions was analyzed.

Results: In the CAD cohort, 45 glycerophospholipid and sphingolipid species were significantly lower compared to healthy controls. In the AMI group, 42 glycerophospholipid and sphingolipid species were reduced. Four PC plasmalogens (PC33:1, PC33:2, PC33:3 and PC35:3) showed the most significant difference. Out of eleven analyzed sphingoid bases, four were lower in the CAD and six in the AMI group. Sphingosine-1-phosphate (S1P) levels were reduced in the AMI group whereas an atypical C16:1 S1P was lower in both groups. Phosphatidylcholine and sphingomyelin species were exclusively present in lipoprotein particles, whereas lysophosphatidylcholines were mainly found in the lipoprotein-free fraction. The observed differences were not explained by the use of statins as confirmed in a second, independent cohort.

Conclusions: Reduced levels of four PC plasmalogens (PC33:1, PC33:2, PC33:3 and PC35:3) were identified as a putatively novel lipid signature for CAD and AMI.

1. Introduction

Coronary artery disease (CAD) is a major cause of death and morbidity in the western world [1]. CAD is caused by the gradual development of atherosclerotic plaques in the arterial wall. A rupture or erosion of plaques with subsequent total occlusion of an

* Corresponding author. Inst. for Clinical Chemistry, University Hospital Zurich, Raemistrasse 100, CH-8091, Zurich, Switzerland.
E-mail address: thorsten.hornemann@usz.ch (T. Hornemann).
[1] Both authors contributed equally.

http://dx.doi.org/10.1016/j.atherosclerosis.2016.01.003
0021-9150/

epicardial coronary artery may lead to acute myocardial infarction (AMI) [2,3]. Recent data suggest that beyond established lipids risk factors like cholesterol and triacylglycerols also glycerophospholipids and sphingolipids contribute to atherogenesis [4,5]. Plasma glycerophospholipids and sphingolipids are mainly associated with lipoprotein particles [6–8] and form a lipid monolayer around the hydrophobic core of triacylglycerols and cholesteryl esters. Prior work identified distinct lipid profiles of plasma, serum or lipoprotein particles in patients with CAD [9–13]. Hundreds of different glycerophospholipid and sphingolipid species were identified in plasma lipoproteins and atherosclerotic plaques [14,15]. In particular sphingolipids are structurally diverse because of a multiplicity in O-linked head groups and N-linked fatty acids. Sphingolipids also show a significant heterogeneity in the sphingoid base composition which yet has been only little appreciated. Sphingolipid de-novo formation is initiated by the conjugation of L-serine and acyl-CoA, a reaction catalyzed by the enzyme serine palmitoyltransferase (SPT). Sphingoid bases, the product of this reaction are the common structural feature for all sphingolipid species. The most abundant sphingoid bases in plasma is C_{18} sphingosine, but SPT can also form other sphingoid bases in the range of C_{14}–C_{20} [16]. Under certain conditions, SPT also metabolizes L-alanine and glycine which forms an atypical category of 1-deoxysphingolipids (1-deoxySLs) [17,18] which were shown to be elevated in several metabolic conditions.

The aim of this study was to identify novel plasma lipid signatures for patients with CAD or AMI.

2. Materials and methods

2.1. Patients

Healthy subjects (n = 14) were recruited from the local blood bank. Exclusion criteria were the use of cardiovascular medication, positive cardiovascular family history, smoking (including cessation of smoking within 2 years prior to study enrollment), history of hypertension, elevated total cholesterol (≥5.0 mmol/L), BMI > 30 kg/m2, history of diabetes mellitus, evidence of relevant vascular or structural heart disease and/or a reduced LVEF (<55%) on echocardiogramm. The stable CAD group (n = 18) was selected from patients with angiographically documented coronary artery stenosis ≥50%. Exclusion criteria comprised an ACS within the preceding 6 months, systemic infectious, inflammatory or autoimmune disease, known severe renal dysfunction (serum creatinine >220 μmol/L), known severe hepatic dysfunction (3x ULN for LFTs), neoplasm or other life-threatening disease with a life expectancy less than one year, extended surgery in the preceding 3 months and/or evidence of vascular or structural heart disease and/or a reduced systolic LV function on echocardiogram or left ventricular angiogram.

Patients with AMI (n = 17) were selected from the Inflammation in Acute Coronary Syndromes cohort (SPUM-ACS, NCT01000701). SPUM-ACS inclusion criteria comprised both genders (aged ≥18 years) presenting within five days (preferably within 72 h) after onset of chest pain and a main diagnosis of STEMI, NSTEMI or unstable angina as described previously [19].

All patients gave written informed consent and the study was conducted with approval from the local ethics committee (EK-1680).

2.2. Sampling of plasma

Blood was drawn from the antecubital vein in healthy subjects and from the femoral/radial artery at the time of diagnostic coronary angiography in patients with CAD or AMI, respectively. Samples were centrifuged at 2700 *g* for 10 min at room temperature to obtain plasma, frozen and stored in aliquots at −80 °C until serial measurement (no prior freeze-thaw cycles). To avoid inter-assay differences all samples were extracted and analyzed in a single batch. The person who performed the analysis was blinded to the patient's data by the use of numbered ID codes.

2.3. Clinical chemistry

Plasma concentrations or activities of total cholesterol, triglycerides, LDL-C and HDL-C, glucose, creatine kinase, CK-MB, and troponin levels were determined by photometric tests or immunoassay by using the Cobas 8000 autoanalyzer from Roche diagnostics (Rotkreuz, Switzerland).

2.4. Isolation of lipoproteins

Human lipoprotein fractions and the lipoprotein-free fraction (LFF) were isolated from plasma of three healthy blood donors by stepwise ultracentrifugation, as described previously [20]. Purity of lipoprotein fractions was confirmed by SDS-PAGE and Coomassie Blue staining.

2.5. Analysis of plasma glycerophospholipids and sphingolipids

Six lipid classes including phosphatidylglycerols (PGs), phosphatidic acids (PAs), phosphatidylethanolamines (PEs), phosphatidylcholines (PCs), sphingomyelins (SMs) and ceramides/hexosylceramides (Cers/HexCers) were analyzed. Lipids were quantified in relation to internal standards (200 ng) including PG(17:0/17:0), LPG(17:1/0:0), PA(14:0/14:0), LPA(17:0/0:0), PE(14:0/14:0), LPE(17:1/0:0), PC(14:0/14:0), PC(24:0/24:0), LPC(17:0/0:0), SM (d18:1/12:0) and Cer(d18:1/17:0) (Avanti Polar Lipids, Alabaster, AL, USA). 20 μl plasma was extracted with 375 μl of methanol/chloroform (2:1 v/v). After vortexing, 100 μl water and 125 μl chloroform were added, agitated (15 min), centrifuged (16,100 ×*g* for 5 min at 25 °C) and the lower phase collected. Again 250 μl chloroform was added, agitated (15 min) and centrifuged. The lower phases were combined and evaporated under a stream of nitrogen. Analysis was done on a TSQ Quantum Access triple quadrupole connected to a Rheos 2200 pump. Separation was done on a diol silica-based column (QS Uptisphere 6 OH, 150 × 2.1 mm, 5 μm). Mobile phase (A) was hexane/isopropanol/water (70:30:2 v/v) including 15 mM NH_4COOH; and (B) isopropanol/water (50:2 v/v) including 15 mM NH_4COOH at a flow rate of 0.35 ml/min. Gradient was 0–7 min A/B (%) 80/20; 8–10 min A/B (%) 60/40, 11–23 min A/B (%) 40/60 and 25–30 min A/B (%) 80/20. Dried material was reconstituted in 200 μl of mobile phases A (80%) and B (20%) an injected (10 μl) into the LC-MS. Neutral loss and precursor scans were used to detect specific glycerophospholipids and sphingolipids. A neutral loss scan of *m/z* 115 and 189 from $[M + NH_4]^+$ ions was used for the analysis of PA and PG lipids. Precursor ion scan of *m/z* 184, specific for phosphocholine was used for PC, SM and LPC lipids. Neutral loss scan of *m/z* 141 was used for PEs and precursor scanning of *m/z* 264 was applied for Cer and HexCer species. Data were analyzed in Xcalibur (version 2.0.6, Thermo Scientific). Identification of molecular species was performed by lipid mass spectrum analysis software (LIMSA) [21].

The assignment of glycerophospholipids includes total numbers of carbons and double bonds in two acyl chains. The SM assignment comprises total numbers of carbons and double bonds in the sphingoid base and N-linked fatty acid. Hexosylceramide species include isomeric glucosyl- and galactosylceramides. For identified Cers and HexCers, molecular structures are reported. Quantification was done in relation to the respective internal standards.