Archives of Biochemistry and Biophysics 538 (2013) 95–102

Contents lists available at ScienceDirect

Archives of Biochemistry and Biophysics

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

Remodeling of liver phospholipidomic profile in streptozotocin-induced diabetic rats

Cláudia Simões [a], Pedro Domingues [a], Rita Ferreira [a], Francisco Amado [a], José Alberto Duarte [b], Rui Vitorino [a], Maria João Neuparth [b,c], Cláudia Nunes [a], Cláudia Rocha [d], Iola Duarte [d], M. Rosário Domingues [a,*]

[a] Mass Spectrometry Center, QOPNA, Department of Chemistry, University of Aveiro, 3810-193 Aveiro, Portugal
[b] CIAFEL, Faculty of Sport, University of Porto, Portugal
[c] CITS, IPSN, CESPU CRL, Famalicão, Portugal
[d] CICECO, Department of Chemistry, University of Aveiro, 3810-193 Aveiro, Portugal

ARTICLE INFO

Article history:
Received 24 May 2013
and in revised form 10 July 2013
Available online 13 August 2013

Keywords:
Phospholipid classes
Fatty acid remodeling
Type 1 diabetes mellitus
Mass spectrometry
Nuclear magnetic resonance
Liquid chromatography

ABSTRACT

Lipid homeostasis in liver is known to be altered with diabetes mellitus, ultimately leading to liver damage and related complications. The present work aimed to evaluate changes in the liver phospholipid profile after 4 months of uncontrolled hyperglycemia. Twenty Wistar rats were divided into two groups: control and streptozotocin-treated (T1DM). After 4 months, animals were sacrificed and morphological characterization of liver was performed and related with serum markers of hepatic damage. Lipid extracts were obtained from liver and phospholipid (PL) classes were quantified. Lipid molecular species were determined by LC–MS and LC–MS/MS, and fatty acids by GC–MS. Concomitantly with signs of hepatic damage we found variations in the relative amount of phospholipid classes in T1DM, characterized by a decrease in PLs with choline head group, and by an increase in the relative content of other PL classes. A remodeling in PL fatty acyl chains was observed in T1DM liver, with a similar pattern to all the PL classes, and consisting in the reduction of 16:0 and an increase of 18:0 and 18:2 acyl chains. The observed changes in T1DM lipid profile may contribute to the altered membrane properties underlying hepatic damage, worsening the metabolic alterations that characterize T1DM.

© 2013 Elsevier Inc. All rights reserved.

Introduction

The complex and bi-directional relationship linking liver dysfunction and diabetes mellitus (DM)[1] has recently gained new interest [1] with different studies highlighting the deleterious impact of uncontrolled hyperglycemia in liver homeostasis [2–4]. In fact, clinical observations support that life expectancy of patients with type 2 diabetes mellitus (T2DM) is compromised not only by vascular complications but also by hepatic dysfunction associated with non-alcoholic fatty liver disease (NAFLD) and hepatocellular carcinoma [1,5,6]. These patients usually present high plasmatic levels of alanine aminotransferase (ALT) and aspartate aminotransferase (AST), which is also a common feature in type 1 diabetes mellitus (T1DM) [5,7,8]. This rise of liver enzymes in the plasma correlates with elevated levels of glycated hemoglobin, triglycerides and total cholesterol, supporting the hypothesis that liver injury can occur with the time course of T1DM, and impact the regulation of glucose and lipid metabolism [1,7,8].

DM-related liver late biochemical changes are characterized by an altered profile of metabolites like fatty acyl CoAs, amino acids and acylcarnitines, increased formation of advanced glycation end products (AGEs) and reactive oxygen species (ROS), DNA fragmentation, increased expression of inflammatory markers, and apoptotic cell death [2,9]. Furthermore, changes in the phospholipid profile could be responsible for disturbances in cellular membrane properties [10] with consequences in organ homeostasis. Phospholipid alterations were already reported in heart tissue [11,12] and retina [13] from rats with STZ-induced diabetes. For instance, the significant decrease in total retinal docosahexaenoic acid (22:6), as well as the decreased incorporation of very-long-

* Corresponding author. Address: Department of Chemistry, University of Aveiro, 3810-193 Aveiro, Portugal. Fax: +351 234 370084.
E-mail address: mrd@ua.pt (M.R. Domingues).

[1] *Abbreviations used:* DM, diabetes mellitus; T2DM, type 2 diabetes mellitus; NAFLD, non-alcoholic fatty liver disease; ALT, alanine aminotransferase; AST, aspartate aminotransferase; T1DM, type 1 diabetes mellitus; AGEs, advanced glycation end products; ROS, reactive oxygen species; PUFAs, polyunsaturated fatty acids; dMPC, 1,2-dimyristoyl-*sn*-glycero-3-phosphocholine; LPC, 1-nonadecanoyl-2-hydroxy-*sn*-glycero-3-phosphocholine; dMPE, 1,2-dimyristoyl-*sn*-glycero-3-phosphoethanolamine; dMPA, 1,2-dimyristoyl-*sn*-glycero-3-phosphate; dMPG, 1,2-dimyristoyl-*sn*-glycero-3-phospho-(1′-rac-glycerol); dMPS, 1,2-dimyristoyl-*sn*-glycero-3-phospho-L-serine; dPPI, 1,2-dipalmitoyl-*sn*-glycero-3-phospho-(1′-myo-inositol); TLC, thin layer chromatography; CONT, control group.

0003-9861/$ - see front matter © 2013 Elsevier Inc. All rights reserved.
http://dx.doi.org/10.1016/j.abb.2013.07.029

chain polyunsaturated fatty acids (PUFAs), observed into retinal phosphatidylcholine was related with an inflammatory status in diabetic retina [13]. In liver, lipid disturbances were studied in pathological conditions as non-alcoholic fatty liver and non-alcoholic steatohepatitis [14], and alcoholic fatty liver diseases [15]. In non-alcoholic steatohepatitis, lipid remodeling was characterized by a decrease of arachidonic acid in free fatty acids, triacylglycerol and in phosphatidylcholine, and by a reduction of eicosapentanoic acid and docosahexanoic acid in triacylglycerol [14]. Little attention has been given to DM-related lipid alterations in liver, and some discrepancies are found on data published so far. While some authors reported the increase of cholesterol, free fatty acids, triacylglycerols and phospholipid content in diabetic liver [16,17] and in liver microssomes [18,19], others observed a decrease in such lipids [20]. Moreover, in none of these studies was performed an in-deep analysis of phospholipid molecular species in T1DM liver.

So, the goal of this work was to determine the changes in phospholipid profile in the liver of rats after 4-months of streptozotocin (STZ) administration, which mimics the effect of insulin depletion as in type 1 diabetes mellitus. Phospholipid classes and their composition in molecular species were determined by LC–MS and LC–MS/MS. Using a lipidomic approach we demonstrated a T1DM-related decrease in PL classes with choline head group, and a remodeling of PL fatty acyl chains characterized by a reduction in the amount of 16:0 and an increase of 18:0 and 18:2 acyl chains.

Material and methods

Chemicals

Streptozotocin [N-(methylnitrosocarbamoyl)-a-D-glucosamine] was obtained from Sigma Chemical Co. (St Louis, MO, USA), and prepared prior to use in 100 mM citrate, pH 4.5. Phospholipid internal standards (1′,3′-bis[1,2-dimyristoyl-*sn*-glycero-3-phospho]-*sn*-glycerol (CL), 1,2-dimyristoyl-*sn*-glycero-3-phosphocholine (dMPC), 1-nonadecanoyl-2-hydroxy-*sn*-glycero-3-phosphocholine (LPC), 1,2-dimyristoyl-*sn*-glycero-3-phosphoethanolamine (dMPE), 1, 2- dimyristoyl-*sn*-glycero-3-phosphate (dMPA), 1,2-dimyristoyl-*sn*-glycero-3-phospho-(1′-rac-glycerol) (dMPG), 1,2-dimyristoyl-*sn*-glycero-3-phospho-L-serine (dMPS) and 1,2-dipalmitoyl-*sn*-glycero-3-phospho-(1′-myo-inositol) (dPPI)) were purchased to Avanti Polar Lipids, Inc. (Alabaster, AL, USA). Malachite green and primuline were purchased from Sigma (St Louis, MO, USA), triethylamine and potassium hydroxide were purchased from Merck (Darmstadt, Germany) and Perchloric acid from panreac (Barcelona, Spain). Acetonitrile; chloroform, methanol and hexane from Fisher scientific (Leicestershire, UK) were of HPLC grade and were used without further purification. All other reagents and chemicals used were of highest grade of purity commercially available. The water was of MilliQ purity filtered through a 0.22-mm filter (Millipore, USA).

Animals

Male Wistar rats weighing 150–200 g at 6–8 weeks of age were used at this study ($n = 20$). During the experimental protocol, the animals were housed in collective cages (4 rats *per* cage) in a room at normal environment (21–24 °C; ~50–60% humidity) receiving food (standard diet) and water *ad libitum* in 12 h light/dark cycles. Housing and experimental treatment were in accordance with Guide for the Care and Use of Laboratory Animals from the Institute for Laboratory Animal Research [21]. The experimental procedures were complied with the current national laws and were approved by local Ethics Committee.

Induction of experimental diabetes

The animals were randomly divided into two groups ($n = 10$ *per* group): type 1 diabetic group (T1DM) and control group (CONT). In T1DM, hyperglycemia was induced with a single intraperitoneal injection of streptozotocin (STZ; 60 mg/kg), after a 16-h fasting period. CONT animals were injected with citrate solution. Values of weight and blood glucose (measured with Glucocard (A. Menarini Diagnostics, Florence, Italy)) were taken just before STZ administration and weekly until the end of the protocol. Animals were considered hyperglycemic when blood glucose exceeded 250 mg/dL. After 4 months, animals were sacrificed with an anesthetic overdose of pentobarbital (10 mg/100 g ip), the liver was dissected out and divided in several portions for light microscopy and lipidomic analysis. Serum levels of transaminases, glucose, cholesterol and triglycerides were determined in duplicate on an AutoAnalyzer (PRESTIGE 24i, Cormay PZ). Glycated hemoglobin HbA1c was measured in whole blood samples using a commercial kit (code 11045, Biosystems Reagents and Instruments, Barcelona, Spain).

Light microscopy

After excision, several pieces (2–4 mm^3) from each hepatic lobe were fixed in 4% formaldehyde, during 24 h, dehydrated with graded ethanol and further included in paraffin blocks. Five μm thick sections were cut and individually collected to silane-prep slides. After deparaffinization with xylene, sections were rehydrated and stained with hematoxylin-eosin following standard procedures. Slides were observed with a light photomicroscope (Axio Imager A1, Carl Zeiss) and a qualitative analysis of tissue structure organization, including the sinusoidal dimensions, the hepatocyte morphology and arrangement, as well as the presence of necrotic areas or cellular infiltration was made.

Lipidomic analysis

Phospholipid quantitation by phosphorous assays

Liver tissue was homogenized in phosphate buffer saline (PBS) pH 7.4. Total lipids were then extracted by Folch method [22] using a chloroform:methanol (2:1 v/v) solution. After extraction, total phospholipids (PL) were calculated by colorimetric determination of phosphorus, after digestion with perchloric acid at 180 °C, as described before [23]. The amount of PL within each class was measured by the same method after thin layer chromatography (TLC) separation. Liver total phospholipid content was normalized to protein concentration [12], which was determined by the biuret method using bovine serum as standard [24]. Liver DNA content was determined with Qubit® dsDNA BR assay using a Qubit® 2.0 Fluorometer.

Separation of phospholipids classes by thin layer chromatography

TLC was used for separate PL classes present in the total lipid extract (approximately 60 μg of total PL). Silica gel plates with a concentrating zone of 2.5 × 20 cm (Merck, Darmstadt, Germany) were developed with chloroform/ethanol/water/triethylamine solvent mixture (35:30:7:35, v/v/v/v) [25]. Lipid spots were visualized with UV ($\lambda = 254$ nm) after detection with primuline and PL classes were identified by means of their *Rf* values.

Phospholipid quantitation by ^{31}P NMR spectroscopy

Relative quantitation of PC, PE and SM classes was also carried out by ^{31}P NMR. Extracts were dissolved in a ternary mixture of chloroform:methanol:water (5:4:1), forming a single homogeneous phase. ^{31}P NMR spectra were acquired on a Bruker Avance DRX-500 spectrometer (Bruker, Rheinstetten, Germany) operating at 202.5 MHz for ^{31}P observation, using a 5-mm QNP probe. ^{31}P

Download English Version:

https://daneshyari.com/en/article/8290611

Download Persian Version:

https://daneshyari.com/article/8290611

Daneshyari.com