Osong Public Health Res Perspect 2014 5(6), 345–350
http://dx.doi.org/10.1016/j.phrp.2014.10.002
pISSN 2210-9099 eISSN 2233-6052

ORIGINAL ARTICLE

Aging-related Changes in Mouse Serum Glycerophospholipid Profiles

Seungwoo Kim, Hyo-Soon Cheon, Jae-Chun Song, Sang-Moon Yun, Sang Ick Park, Jae-Pil Jeon*

Division of Brain Diseases, Korea National Institute of Health, Cheongju, Korea.

Received: August 12, 2014
Revised: September 18, 2014
Accepted: October 1, 2014

KEYWORDS:
aging,
glycerophospholipid,
serum,
targeted-metabolomics

Abstract
Objectives: Metabolic dysfunction is a common hallmark of the aging process and aging-related pathogenesis. Blood metabolites have been used as biomarkers for many diseases, including cancers, complex chronic diseases, and neurodegenerative diseases.
Methods: In order to identify aging-related biomarkers from blood metabolites, we investigated the specific metabolite profiles of mouse sera from 4-month-old and 21-month-old mice by using a combined flow injection analysis–tandem mass spectrometry and liquid chromatography–tandem mass spectrometry.
Results: Among the 156 metabolites detected, serum levels of nine individual metabolites were found to vary with aging. Specifically, lysophosphatidylcholine (LPC) acyl (a) C24:0 levels in aged mice were decreased compared to that in young mice, whereas phosphatidylcholine (PC) acyl-alkyl (ae) C38:4, PC ae C40:4, and PC ae C42:1 levels were increased. Three classes of metabolites (amino acids, LPCs, and PCs) differed in intraclass correlation patterns of the individual metabolites between sera from young and aged mice. Additionally, the ratio of LPC a C24:0 to PC ae C38:4 was decreased in the aged mice, whereas the ratio of PC ae C40:4 to LPC a C24:0 was increased, supporting the aging-related metabolic changes of glycerophospholipids.
Conclusion: The ratios of the individual metabolites PC and LPC could serve as potential biomarkers for aging and aging-related diseases.

1. Introduction

Aging is an irreversible and progressive, multidimensional, complex process that includes the accumulation of cellular and organ damage, leading to a decline in function [1,2]. Aging is also known to be a risk factor for many prevalent diseases such as diabetes, cancers, and cardiovascular and neurodegenerative diseases (e.g. Alzheimer's and Parkinson's diseases) [3]. Therefore, the discovery of aging and aging-related disease biomarkers is important for the early diagnosis and therapy of diseases.

*Corresponding author.
E-mail: jaepiljeon@hanmail.net

Recent metabolomic studies have primarily attempted to understand the underlying biological process to develop disease biomarkers [4]. In particular, metabolomic analysis is suitable for the discovery of aging-related biomarkers because it has been shown that metabolic dysfunction is a common hallmark of the aging process [5]. The utility of targeted metabolomics for the discovery and validation of disease biomarkers has been recently established [6]. Although metabolomic studies have resulted in the identification of aging-related biomarkers from human samples [7–9] and animal models [5,10,11], consistent blood biomarkers of the aging process still need to be identified.

Phospholipids can function as pathogenic indicators of many diseases such as Alzheimer's disease [12–15], ovarian endometriosis [16], and rheumatoid arthritis [17]. Phosphatidylcholine (PC) is a major component of phospholipids. It was reported that rat plasma phospholipids consist of PCs (~70% of total phospholipids), very low-density lipoproteins with sphingomyelin (SM; 11%), lysophosphatidylcholine (LPC; 3%), phosphatidylethanolamine (4%), and phosphatidylinositol (3%) [18]. LPCs are generated by the hydrolysis of PCs by phospholipase A_2, which plays an important role in inflammatory responses [19].

The purpose of this study was to examine aging-related changes in serum concentrations of metabolites in young and aged mice to further increase our understanding of the metabolite-related aging process. We found the ratios of LPC acyl (a) C24:0 to PC acyl-alkyl (ae) C38:4 and PC ae C40:4 to LPC a C24:0 were significantly different among sera from young and aged mice. These results suggest that the ratios of PC and LPC levels could provide aging-related metabolic information from blood as well as provide clues for the identification of antiaging molecular targets.

2. Materials and methods

2.1. Animals

C57BL6/J mice were purchased from The Laboratory Animal Resource Center, Korea Research Institute of Bioscience & Biotechnology (KRIBB; Ochang, Chungcheongbuk-do, Korea). All mice were maintained in a specific-pathogen free area, according to standard animal care protocols. Whole blood was collected via heart puncture following euthanasia by cervical dislocation. Collected blood was incubated for 20 minutes at room temperature in Serum Separator Tubes (BD Bioscience, Franklin Lakes, NJ, USA), and clots were then removed by centrifugation at 1500 × *g*. Sera were stored at −70°C until needed.

2.2. Metabolite quantification

Targeted metabolite concentrations were measured using the Absolute IDQ p180 Kit (Biocrates Life Sciences AG, Innsbruck, Austria). The assay procedures have been previously described in detail [20]. Serum metabolites were quantified using appropriate internal standards included in the kit. The 186 metabolites targeted included 40 acylcarnitines (ACs), 21 amino acids (AAs), 19 biogenic amines (BAs), 14 LPCs, 38 PC diacyl, 38 PC ae, 15 SMs, and the sum of hexoses (H1). In brief, AAs and BAs were measured by liquid chromatography–tandem mass spectrometry, and ACs, phospho- and sphingolipids, and hexoses were measured by flow injection analysis–tandem mass spectrometry on API 4000 QTRAP (ABSCIEX, Framingham, MA, USA). To ensure data quality, metabolites coefficient of variance (CV) that was under 20% and above the limit of detection (LOD) were selected. The CV and LOD criteria were passed by 156 metabolites. Concentrations of all detected metabolites are reported as μM.

2.3. Statistical analysis

Statistical analysis was performed using three different methods, including *t* test and additional BiomarkeR and limma packages in Bioconductor (http://www.bioconductor.org), to identify statistically powerful biomarkers. The BiomarkeR package in Bioconductor has a network-based approach to identify biomarkers in metabolomics research [21]. It provides the biomarker identifier (BI) score by measuring the change of each metabolite and connectivity of the metabolites within the network. The measured metabolite data were imported into the BiomarkeR package and analyzed with the unpaired BI method. The selected biomarkers were plotted with the unpaired BI Graph method. Next, for the linear modeling approach, the concentration of each metabolite was transformed by log and normalized with the quantile normalization method. Then, we employed the limma package in Bioconductor that is designed to stabilize the analysis with linear modeling. The *p*-values were adjusted for multiple tests. Volcano plots were constructed using an empirical Bayes method in the limma package. The metabolite serum concentrations were normally distributed as indicated by the Kolmogorov–Smirnov test. All statistical analyses were carried out using SPSS version 20 (SPSS Inc., Chicago, IL, USA) and R program (http://www.R-project.org).

3. Results

We used a targeted metabolomic analysis to identify aging-related biomarkers and specific profiles of serum metabolites in the aging process. We investigated seven classes of metabolites (ACs, BAs, AAs, LPCs, PCs, SMs, and hexoses) from young and aged mice. Of these 186 metabolites, 156 were detected in mouse sera at levels above the LOD. Via statistical analysis, we found changes in serum concentrations of 23 metabolites in the young (4-month-old) and aged (21-month-old) mice