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Development of lipidomic platform and phosphatidylcholine retention time index for lipid profiling of rosuvastatin treated human plasma

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ABSTRACT

A simple and fast methodology to detect and identify multiple classes of lipid from human plasma is developed utilizing ultra-performance liquid chromatography quadrupole time-of-flight mass spectrometry (UPLC–QTOF) as lipidomics platform. All the conditions for the sample preparation and analytical instruments were optimized in detail to detect nine lipid classes (phosphatidylserine (PS), phosphatidylglycerol (PG), phosphatidylethanolamine (PE), phosphatidylcholine (PC), triacylglyceride (TG), phosphatidylinositol (PI), lysophosphatidylcholine (LysoPC), lysophosphatidic acid (LysoPA), and sphingomyelin (SM)), which are the most important biologically active lipids but have different characteristics. Finally, the plasma was prepared after a liquid–liquid extraction with a mixture of chloroform/methanol (1:2 v/v) including salting out by adding 0.15 M of NaCl and the residue after evaporation was reconstituted with a mixture of chloroform/methanol (1:1 v/v) to dissolve all lipids which have different polarity. The chromatographic conditions were set up such that mobile phase (A) comprised 10 mM ammonium acetate in 40% acetonitrile and mobile phase (B) comprised 10 mM ammonium acetate in acetonitrile:isopropanol = 10:90 (v/v) with ACQUITY BEH C_{18} as the stationary phase. In particular, a retention time index of PC was constructed by analyzing known standards to confirm each variant of PC without the use of any additional standards in every experiment. The lipidomic methodology and the retention time index of PC were applied to analyze the lipidomic profiling of human plasma from rosuvastatin (lipid lowering drug) treated subjects.

In the developed lipidomic platform, all lipids were successfully analyzed within 16 min and PCs could be confirmed with the PC retention time index. In rosuvastatin treatment, the lipid profiling was changed in all the eight lipid classes. The level of SM, TG, PI and PE decrease significantly but LysoPCs and PCs were whether decreased or increased. Those results indicated that the plasma level of overall lipids decreased by drug response, however, the changes in the lipids which are important components for biological membrane such as LysoPC and PC were more complicated, and it could be related to the side effect of rousuvastatin.

In conclusion, it was found that our lipidomic methodology and the PC retention time index provided not only overall lipidomic information but also profiled specific information of drug response.

Abbreviations: C_{max}, the peak plasma concentration; DG, Diacylglyceride; ESI, Electrospray ionization; FA, Fatty acid; HDL, High density lipoprotein; HMG-CoA, 3-hydroxy-3-methyl-glutaryl-CoA; LC-MS, Liquid chromatography–mass spectrometry; LDL, Low density lipoprotein; LLE, Liquid–liquid extraction; LysoPA, Lysophosphatidic acid; LysoPC, Lysophosphatidylcholine; PC, Phosphatidylcholine; PE, Phosphatidylethanolamine; PG, Phosphatidylglycerol; PI, Phosphatidylinositol; PLS-DA, Partial least squares discriminant analysis; PS, Phosphatidylserine; SM, Sphingomyelin; TG, Triacylglyceride; UPLC–QTOF, Ultra-performance liquid chromatography-quadrupole time-of-flight mass spectrometry.

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1. Introduction

Lipids play multiple roles in cells and organisms. They not only organize membrane components but also regulate cell signaling, energy production and storage and act as ligands or mediators of both cell–cell and protein-protein interaction. Dysregulated lipid metabolism has been related to human diseases, such as diabetes, atherosclerosis, obesity, Alzheimer's disease, Parkinson's disease, cancer and cardiovascular disease [1–5]. Lipidomics, a critical subset of metabolomics, can be defined as a system-wide characterization of lipids and their interaction with cells and other biochemical compounds; it has been expected to assume a notable role in systems biology and has recently garnered greater interest [6].

The lipids in blood are mostly transported in a protein particle known as lipoprotein. These circulating lipoproteins contain triacylglyceride (TG), free cholesterol, cholesterol ester and phospholipids. The structure of a lipid molecule consists of a polar head group attached to a lipophilic backbone consisting of various fatty acid chains; there are estimated to be hundreds of different lipid molecules in body due to the presence of diverse of fatty acid chain constituents (e.g., chain length, degrees of unsaturation, position of double bonds, etc.) and the combinatorial possibilities of these backbone structures. Furthermore, the concentration of each lipid within different lipid classes is extremely variable depending on both cell types and location [7,8]. Therefore, the identification of individual lipids within a biological system is regarded as a difficult and complex undertaking.

Mass spectrometry (MS) analysis has become the method of choice for qualitative and quantitative analysis of the lipidome [2,8,9]. One approach to lipidomic analysis, so-called "shotgun lipidomics," is performed via direct infusion of the organic extracts of tissues, cells and biological fluids. This infusion-MS-based approach generally allows for the rapid and efficient analysis of crude lipid extracts; this approach also enables us to identify metabolites with MS/MS spectral analysis using precursor ion and neutral loss scans [6,10–13]. However, some limitations, such as ion suppression/enhancement effects caused by lipid–lipid interaction [7,8,14], may induce false positive identification of lipids. For instance, when a lipid extract contains relatively abundant amounts of triacylglyceride (TG) species, the neutral losses of fatty acids from TGs overshadow the identification of phosphatidylcholine (PC) molecules [10,15]. Therefore, an additional confirmation step is necessary to verify the lipidomic study.

In this study a simple and fast methodology using ultra-performance liquid chromatography-triple quadrupole time-of-flight mass spectrometry (UPLC–QTOF) to detect and identify several classes of lipid from human plasma is introduced. Specifically, nine commercially available lipid standards representing major lipid groups such as, phosphatidylserine (PS), phosphatidylglycerol (PG), phosphatidylethanolamine (PE), phosphatidylcholine (PC), triacylglyceride (TG), phosphatidylinositol (PI), lysophosphatidylcholine (LysoPC), lysophosphatidic acid (LysoPA) and sphingomyelin (SM), were used to optimize LC parameters and sample preparation conditions to analyze lipids which have different polarity. The extraction recovery was evaluated to detect all the lipid classes we selected, and the conditions for LC analysis were controlled to have a short run time. Especially, for the confirmation of PC, a retention time index of PC was constructed and utilized to confirm the identities of the biomarkers for drug response.

Phospholipids are biologically active lipids and are the principal constituents of biological membranes [16]. They are also involved in cell signaling and the maintenance of lipid homeostasis. In most eukaryotic membranes, PC as well as PE are most prevalent, accounting collectively for approximately 75 mol% of the total phospholipid mass [7]. PCs, in particular, are one of the common phospholipids in mammals bearing a polar phosphocholine as a head group and two nonpolar fatty acyl hydrocarbon chains [7,17]. PC can be hydrolyzed to form LysoPC enzymatically by phospholipase A_2 or spontaneously. Both of these lipids are ubiquitous in nature and are involved in metabolism, inflammation and cell signaling in addition being the major source of membrane-forming phospholipids in mammalian cells [17–21]. PC and LysoPC are also implicated in various diseases, such as type-2 diabetes [22], cancer [23] and systemic immune disease [24], making it necessary to be able to monitor PCs in plasma.

The optimized lipidomics analytical platform and retention time index of PC were applied to profile lipids in the plasma of rosuvastatin-treated healthy subjects and used to investigate the drug effect. Rosuvastatin is a cholesterol-lowering agent which reduces cholesterol level in plasma and affects global lipid metabolism. We tried to discover a biomarker candidate for the drug response using exact mass and MS/MS fragment pattern in lipidomic analysis. Additionally, a retention time index of PC was utilized to confirm the identities of the biomarkers for drug response.

2. Materials and methods

2.1. Materials

HPLC grade acetonitrile, isopropanol, chloroform and methanol were purchased from Burdick & Jackson (SK Chemicals, Ulsan, Korea). Ammonium acetate, formic acid and leucine enkephalin was purchased from Sigma-Aldrich Chemical Co. (St. Louis, MO, USA). Ultrapure water (18.2 MΩ) was acquired using a Milli-Q apparatus from Millipore (Milford, USA). Lipid standards such as 1-heneicosanoyl-2-(4Z,7Z,10Z,13Z,16Z,19Z-docosahexaenoyl)-*sn*-glycero-3-phospho-L-serine (PS(21:0/22:6)), 1-heneicosanoyl-2-(4Z,7Z,10Z,13Z,16Z,19Z-docosahexaenoyl)-*sn*-glycero-3-phospho-L-serine (PG(17:0/20:4)), 1-heptadecanoyl-2-(9Z-tetradecenoyl)-*sn*-glycero-3-phosphoethanolamine (PE(17:0/14:1)), 1-dodecanoyl-2-tridecanoyl-*sn*-glycero-3-phosphocholine (PC(12:0/13:0)), 1,3-di-(6Z-octadecenoyl)-2-(9Z-octadecenoyl)-glycerol (TG(18:1/18:1/18:1)), 1-heptadecanoyl-2-(9Z-tetradecenoyl)-*sn*-glycero-3-phospho-(1'-myo-inositol) (PI(17:0/15:1)), 1-(10Z-heptadecenoyl)-*sn*-glycero-3-sphocholine (LysoPC(17:1)), 1-(10Z-heptadecenoyl)-*sn*-glycero-3-phosphate (LysoPA(17:1)) and *N*-(dodecanoyl)-sphing-4-enine-1-phosphocholine (SM(d18:0/12:0)), and deuterium-labeled lipid standards of 1-hexadecanoyl(d_{31})-2-(9Z-octadecenoyl)-*sn*-glycero-3-phosphocholine (PC(16:0/18:1)-d_{31}), 1-hexadecanoyl(d_{31})-2-(9Z-octadecenoyl)-*sn*-glycero-3-phospho-myo-inositol (PI(16:0/18:1)-d_{31}) and 1-deoxymethylsphinganine-d_5 were obtained from Avanti Polar Lipids (Alabaster, AL, USA). The phosphatidylcholine (PC) standards used for lipid index were 1-stearoyl-2-myristoyl-*sn*-glycero-3-phosphocholine (PC(32:0)), 1-oleoyl-2-myristoyl-*sn*-glycero-3-phosphocholine (PC(32:1)), 1-palmitoyl-2-oleoyl-*sn*-glycero-3-phosphocholine (PC(34:1)), 1-palmitoyl-2-linoleoyl-*sn*-glycero-3-phosphocholine (PC(34:2)), 1-stearoyl-2-oleoyl-*sn*-glycero-3-phosphocholine (PC(36:1)), 1-stearoyl-2-linoleoyl-*sn*-glycero-3-phosphocholine (PC(36:2)), 1-palmitoyl-2-arachidonoyl-*sn*-glycero-3-phosphocholine (PC(36:4)), 1-stearoyl-2-arachidonoyl-*sn*-glycero-3-phosphocholine (PC(38:4)), 1-palmitoyl-2-docosahexaenoyl-*sn*-glycero-3-phosphocholine (PC(38:6)) and 1-stearoyl-2-docosahexaenoyl-*sn*-glycero-3-phosphocholine (PC(40:6)), and they were also purchased from Avanti Polar Lipids (Alabaster, AL, USA).