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A rapid quantitative analysis of bile acids, lysophosphatidylcholines and polyunsaturated fatty acids in biofluids based on ultraperformance liquid chromatography coupled with triple quadrupole tandem massspectrometry

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ABSTRACT

Much evidence suggested that quantitative analysis of bile acids (BAs), lysophosphatidylcholines (LPCs), and polyunsaturated fatty acids (PUFAs) in biofluids may be very useful for diagnosis and prevention of hepatobiliary disease with a non-invasive manner. However, simultaneously fast analysis of these metabolites has been challenging for their huge differences of physicochemical properties and concentration levels in biofluids. In this study, we present a liquid chromatography–mass spectrometry method with a high throughput analytical cycle (10 min) to fast and accurately quantify fifteen potential biomarkers (eight BAs, four LPCs and three PUFAs) of hepatobiliary disease. The accuracy for the fifteen analytes in plasma and urine matrices was 80.45%–118.99% and 84.55%–112.66%, respectively. The intra- and inter- precisions for the fifteen analytes in plasma and urine matrices were all less than 20% and the lower limit of quantification (LLOQ) of analytes is up to 0.0283–8.2172 nmol/L. Therefore, this method is fast, sensitive and accurate for the quantitative analysis of BAs, LPCs and PUFAs in biofluids. Moreover, the stability and concentration differences of the analytes in plasma and serum were evaluated, and the results demonstrated that LPCs is stable, but PUFAs is very unstable in freeze and thaw cycles, and the concentrations of the analytes in serum were slightly higher than those in plasma. We suggested plasma may be a kind of better bio-sample than serum using for quantitative analysis of metabolites in blood, due to the characteristics of plasma are more close to blood than those of serum.

1. Introduction

Bile acids (BAs) are a large family of chemically related steroid acids, which are major components of bile [1]. The distinction of chemical structure between different BAs is minute and only depends on the presence or absence of hydroxyl groups at positions 3, 7, or 12 (Fig. 1A) [2]. The BAs are usually divided into two categories, primary BAs, i.e., cholic acid (CA) and chenodeoxycholic acid (CDCA), and secondary BAs, i.e., deoxycholic acid (DCA) and ursodeoxycholic acid (UDCA). The former are synthesized from cholesterol in the liver through a complex oxidative pathway, and they will be conjugated with taurine or glycine to form primary conjugated bile salts prior to being excreted into bile canaliculi. The primary conjugated bile salts are further passed into the small intestine where they act as detergents and contribute to the absorption of dietary lipids. Simultaneously, in the intestine, BAs are de-conjugated and metabolized into secondary BAs by microbiota. Most BAs in the gut are re-absorbed back to the liver and re-excreted into bile in a process known as enterohepatic circulation [2,3].

BAs play a critical role in regulating lipid, glucose and energy homeostasis, cell proliferation, and inflammatory processes through activating several nuclear receptors and signaling cascades [4–6]. Phosphatidylcholines (PCs) containing very-long-chain polyunsaturated fatty acid is a kind of very important membrane lipids, which can be cleaved at the sn-2 position to liberate lysophosphatidylcholines (LPCs; Fig. 1B) and polyunsaturated fatty acids (PUFAs; Fig. 1C) by Phospholipase A2 [7]. LPCs play important roles in physiological events including cell proliferation, survival, and cytoskeletal changes as well as pathophysiological processes that include autoimmune disease, fibrotic disease, cancer, and inflammation [8]. *n*–3 and *n*–6 PUFAs

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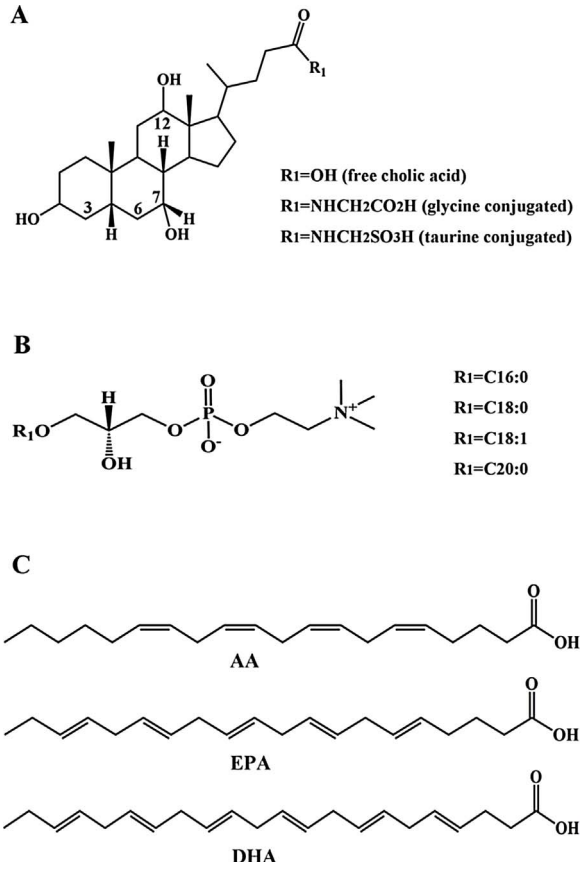

Fig. 1. Example of the structures BAs, LPCs and PUFAs.

eicosapentaenoic acid (EPA), docosahexaenoic acid (DHA), and arachidonic acid (AA) are important bioactive nutrients that exert various biological effects, including altering the composition of cellular membranes, activating gene transcription and signaling cascades, and being metabolized to a wide range of potent lipid mediators [9]. In which, AA is the most important endogenous PUFA, which can be metabolized into eicosanoids including, leukotrienes (LTs), prostaglandins (PGs) and thromboxanes (TXs) by lipoxygenases (LOXs) and cyclooxygenases (COXs) [10]. These bioactive molecules are intimately involved in inflammation and immunity [11] and the occurrence, development, transferring and other pathological process of cancer [12].

Liver is the most important organ for the metabolism of exogenous or endogenous metabolites, numerous evidences demonstrated that the liver develops a core metabolomic phenotype (CMP) that involves dysregulation of BAs, LPCs, and PUFAs homeostasis provoked by different causes of liver injury. The CMP commences at the transition among the healthy liver (Phase 0), non-alcoholic fatty liver disease (NAFLD)/non-alcoholic steatohepatitis (NASH), alcoholic liver disease (ALD) or viral hepatitis (Phase 1), cirrhosis (Phase 2), and hepatocellular carcinoma (HCC)/cholangiocarcinoma (CCA) (Phase 3). Beyoğlu and Idle summarized all metabolomics data for hepatobiliary disease, and we found that eight BAs, which are glycochenodeoxycholic acid (GCDA), taurochenodeoxycholic acid (TCDA), glycoursodeoxycholic acid (GCDCA), glycodeoxycholic acid (GDCA), deoxycholic acid (DCA), glycocholic acid (GCA), taurocholic acid (TCA) and chenodeoxycholic acid (CDCA), four LPCs, which are LPC16:0, LPC18:0, LPC18:1 and LPC20:0, and three PUFAs including AA, EPA, and DHA are differentially expressed in different hepatobiliary disease [13]. The evidence prompted that quantitative analysis of the fifteen metabolites in biofluids may be very useful for diagnosis and prevention of hepatobiliary disease with a non-invasive manner. Within the past decade, gas chromatography–mass spectrometry (GC–MS) and liquid chromatography–mass spectrometry (LC–MS) have been widely utilized for the separation and detection of BAs in human and animal model biofluid samples [14–18], in addition quantitative analysis of PUFAs [19–21] and LPCs [22] in biofluid samples by GC–MS and LC–MS also have been reported. However, simultaneously fast analysis of BAs, LPCs and PUFAs has been challenging for their huge differences of physicochemical properties and concentration levels in biofluid samples. Here we present a targeted ultra-performance liquid chromatography–mass spectrometry (UPLC–MS) multiple -reaction monitoring (MRM) method, which combines a high throughput analytical cycle (10 min) with simple and fast sample preparation, producing an analytical pipeline capable of supporting the rapid analysis of fifteen potential biomarkers (eight BAs, four LPCs and three PUFAs, shown in Supplemental material Table S1) of hepatobiliary disease. Their measurements in plasma and urine were validated according to established FDA criteria for accuracy and precision on intra/inter-day, linearity, stability, and matrix effect [14].

2. Methodology

2.1. Materials and reagents

Water was purified by a Milli-Q system (Millipore, Bedford, MA, USA). Organic solvents (HPLC grade), methanol, chloroform, isopropanol, and acetonitrile used for the precipitation were obtained from Aladdin (Shanghai, China). LC–MS grade acetonitrile and formic acid in mobile phases were obtained from Merck (Darmstadt, Germany). The eight BA standards, four LPC standards, three PUFA standards, and two internal standards (see Supplemental material Table S1) were obtained from Sigma-Aldrich (Dorset, UK).

2.2. Collection of human plasma, serum and urine for targeted analysis

Blood samples from four patients with HCC as well as from four healthy volunteers were collected into anticoagulation tubes (adding heparin lithium salt) and coagulation-promoting tubes (adding coagulant), separately. About 10 mL of blood were collected in the anticoagulation tube and then were centrifuged at 1000*g* at 4 °C for 10 min, immediately, to get about 4 mL of plasma samples. About 10 mL of blood were collected in the coagulation-promoting tube samples were stored at 4 °C for 30 min, and then were centrifuged at 1000*g* at 4 °C for 10 min to get about 4 mL of serum samples. All samples were aliquoted and stored at −80 °C. Urine samples were collected into 15 mL centrifuge tubes, and then were centrifuged at 10,000*g* at 4 °C for 10 min, 3 mL of supernatants were aliquoted and stored at −80 °C. The protocol was approved by Eastern Hepatobiliary Surgical Hospital Review Board and written consents were signed by all participants prior to the study.

2.3. Optimization of UPLC–MS/MS conditions

Quantification of targeted metabolites was performed on a Waters Acquity UPLC system (Waters® Corporation, MA, USA) using a Waters Acquity BEH C18 column (2.1 mm × 100 mm, 1.7 μm) or a Waters Acquity BEH C18 column (2.1 × 50 mm^2, 1.7 μm) coupled to an AB Sciex Triple Quad™ 6500 mass spectrometer (Applied Biosystems Corporation, MA, USA). The binary gradient elution system consisted of (A) water (containing 0.1% formic acid, v/v) and (B) acetonitrile (containing 0.1% formic acid, v/v) and separation on BEH C18 column (2.1 × 50 mm^2, 1.7 μm) was achieved under the following gradient: 10–10% B over 0–0.2 min, 10–55% B over 0.2–3.5 min, 55–80% B over 3.5–6 min, 80–100% B over 6–6.5 min, 100–100% B over 6.5–8 min, 100-10% B over 8–8.3 min,10–10% B over 8.3–10 min. Separation on BEH C18 column (2.1 × 100 mm^2, 1.7 μm) was achieved under the following gradient: 10–55% B over 0–3 min, 55–80% B over 3–6 min, 80–100% B over 6–8 min, 100–100% B over 8–10 min, 100–10% B over