Free Radical Biology and Medicine ∎ (∎∎∎∎) ∎∎∎–∎∎∎

Contents lists available at SciVerse ScienceDirect

Free Radical Biology and Medicine

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

Methods in Free Radical Biology and Medicine

Analytical strategies for characterization of oxysterol lipidomes: Liver X receptor ligands in plasma

William J. Griffiths [a,*], Peter J. Crick [a], Yuchen Wang [b], Michael Ogundare [a], Karin Tuschl [c], Andrew A. Morris [d], Brian W. Bigger [d], Peter T. Clayton [c], Yuqin Wang [a]

[a] Institute of Mass Spectrometry, College of Medicine, Swansea University, Swansea SA2 8PP, UK
[b] Clinical Laboratory, Jinan Infectious Disease Hospital, Shandong University, Jinan, Shandong, China
[c] Clinical and Molecular Genetics Unit, Institute of Child Health, University College London, London WC1N 1EH, UK
[d] Willink Biochemical Genetics Unit, Genetic Medicine, St Mary's Hospital, CMFT, Manchester M13 6WL, UK

A R T I C L E I N F O

Keywords:
Lipidomics
Sterol
Steroid
Oxysterol
Liquid chromatography
Mass spectrometry
Bile acid
Derivatization
Cerebrotendinous xanthomatosis
Nuclear receptor
G-protein-coupled receptor
Liver X receptor
Free radicals

A B S T R A C T

Bile acids, bile alcohols, and hormonal steroids represent the ultimate biologically active products of cholesterol metabolism in vertebrates. However, intermediates in their formation, including oxysterols and cholestenoic acids, also possess known, e.g., as ligands to nuclear and G-protein-coupled receptors, and unknown regulatory activities. The potential diversity of molecules originating from the cholesterol structure is very broad and their abundance in biological materials ranges over several orders of magnitude. Here we describe the application of enzyme-assisted derivatization for sterol analysis (EADSA) in combination with liquid chromatography–electrospray ionization–mass spectrometry to define the oxysterol and cholestenoic acid metabolomes of human plasma. Quantitative profiling of adult plasma using EADSA leads to the detection of over 30 metabolites derived from cholesterol, some of which are ligands to the nuclear receptors LXR, FXR, and pregnane X receptor or the G-protein-coupled receptor Epstein–Barr virus-induced gene 2. The potential of the EADSA technique in screening for inborn errors of cholesterol metabolism and biosynthesis is demonstrated by the unique plasma profile of patients suffering from cerebrotendinous xanthomatosis. The analytical methods described are easily adapted to the analysis of other biological fluids, including cerebrospinal fluid, and also tissues, e.g., brain, in which nuclear and G-protein-coupled receptors may have important regulatory roles.

© 2012 Elsevier Inc. All rights reserved.

Introduction

Oxysterols and their downstream metabolites, including cholestenoic acids, represent important biologically active components of plasma. These molecules are of increasing interest to bioscientists on account of their important signaling roles in the immune system [1–3], as agonists to nuclear receptors [4–7], and as markers of oxidative stress [8], atherosclerosis [9,10], and neurodegenerative disease [11]. Oxysterols can be formed from cholesterol and its sterol precursors both enzymatically and nonenzymatically. In vertebrates, the first step of all cholesterol metabolism leads to the formation of an oxysterol. 22*R*-Hydroxycholesterol and 20*R*,22*R*-dihydroxycholesterol are the precursors of pregnenolone and steroid hormones [12], whereas 7α-, 24*S*-, 25-, and (25*R*),26-hydroxycholesterols all represent precursors of bile acids [13]. Some of these oxysterols and their downstream metabolites are ligands to nuclear receptors, e.g., liver X receptors (LXRs)[1] [5,6], farnesoid X receptor (FXR) [7], pregnane X receptor [4,14], vitamin D receptor [15], and other receptors involved in lipid homeostasis, e.g., INSIG [16,17], and also G-protein-coupled receptors [2,18,19]. Oxysterols also play a role in the immune response [1,20,21], in which, e.g., 25-hydroxycholesterol is secreted by macrophages in response to Toll-like receptor activation and suppresses immunoglobulin A production, whereas its metabolite 7α,25-dihydroxycholesterol directs B-cell migration [1,3]. Clearly, the presence of these regulatory molecules in biological fluids is of major physiological importance and analytical methods are required to reliably identify and quantify such molecules.

Abbreviations: API, atmospheric pressure ionization; CDCA, chenodeoxycholic acid; CTX, cerebrotendinous xanthomatosis; EADSA, enzyme-assisted derivatization for sterol analysis; DHEA, dehydroepiandrosterone; ESI, electrospray ionization; FT, Fourier transform; FWHM, full width at half-maximum height; FXR, farnesoid X receptor; GC, gas chromatography; GP, Girard P; HPLC, high-performance liquid chromatography; HSD, hydroxysteroid dehydrogenase; LC, liquid chromatography; LIT, linear ion trap; LXR, liver X receptor; MRM, multiple reaction monitoring; MS, mass spectrometry or spectrometer; MS^n, multistage fragmentation; PQD, pulsed Q collision induced dissociation; Q-TOF, quadrupole–time-of-flight; RIC, reconstructed ion chromatogram; RP, reversed phase; SIR, selected ion recording; SPE, solid-phase extraction

* Corresponding author. Fax: +44 1792 295554.
E-mail address: w.j.griffiths@swansea.ac.uk (W.J. Griffiths).

0891-5849/$ - see front matter © 2012 Elsevier Inc. All rights reserved.
http://dx.doi.org/10.1016/j.freeradbiomed.2012.07.027

Please cite this article as: Griffiths, W.J.; et al. Analytical strategies for characterization of oxysterol lipidomes: Liver X receptor ligands in plasma. *Free Radic. Biol. Med.* (2012), http://dx.doi.org/10.1016/j.freeradbiomed.2012.07.027

ARTICLE IN PRESS

Oxysterols have traditionally been analyzed in plasma by gas chromatography (GC)–mass spectrometry (MS), after saponification and derivatization, utilizing selected ion recording (SIR) to attain the necessary sensitivity [22], although liquid chromatography (LC)–MS methods utilizing SIR or multiple reaction monitoring (MRM) are gaining popularity [23–25]. To improve MS response in LC–electrospray ionization (ESI)–MS studies a number of groups are now exploiting derivatization methods to enhance ionization [26–29]. Cholestenoic acids are usually analyzed by GC–MS in an analysis separate from oxysterols [30], this is on account of different requirements of sample preparation. However, Axelson and colleagues developed a GC–MS assay for C_{27} acids in blood and plasma [31], which was extended to include some oxysterols.

Oxysterols represent just one subgroup of the lipidome, and in recent years lipidomics has become a field of great activity in, and intense interest to, the bioscience community [32–35]. Many of the lipidomic studies performed have been on biofluids, particularly plasma or serum [25]. The dominant technology has been ESI-MS. ESI-MS analysis has been performed linked to LC, i.e., LC-ESI-MS, and also in a stand-alone direct infusion mode. Stand-alone, or shotgun, lipidomics offers the advantage of simplicity, but is unable to differentiate between isobaric metabolites. Interfacing ESI with LC separation can overcome this shortcoming, but introduces some added complexity to the analysis. GC–MS offers an alternative analytical method; however, the requirements of solvolysis and/or hydrolysis followed by derivatization, often of multiple functional groups with freshly prepared reagents and solvents, discourage many would-be analysts [36].

Any lipidomic experiment consists of essentially three key stages: (i) lipid extraction, (ii) lipid analysis, and (iii) lipid quantification. Clearly the extremely different physical properties of many lipids and their presence in cells, tissues, and body fluids at widely different levels make analysis of the global lipidome extremely challenging. An alternative to global lipidomics is to adopt a targeted approach. Here a particular class of lipid is targeted, usually based on physicochemical properties. This is the approach adopted by many lipid scientists [32,33,37]. Whereas glycerolipids, glycerophospholipids, and sphingolipids are often well represented in global ESI-MS-based lipidomic studies, this is not true of members of the other classes [38]. The explanation for this is simple; most ESI-MS methods are biased toward the most abundant and readily ionized compounds. Cholesterol and some of its metabolites are present at high levels in blood and plasma but are barely detectable in a global lipidomics experiment based on the ESI-MS analysis of these fluids. This is on account of difficulties experienced in sample handling and poor ionization characteristics of sterols. Whereas established MS methods exist for analysis of the ultimate products of cholesterol metabolism, i.e., hormonal steroids and bile acids [39], methods for the analysis of intermediates in their biosynthesis, i.e., oxysterols and cholestenoic acids, are less mature, and to date this region of the lipidome has been largely ignored.

Over the past decade we have developed targeted methods for sterol, oxysterol, and cholestenoic acid analysis, which we have exploited in the analysis of brain tissue, cerebrospinal fluid, plasma/serum, cells, and cell media [40–45]. Our methodology is based on separating oxysterols and cholestenoic acids from cholesterol in the first step of the sample preparation process. This avoids the potential problem of cholesterol autoxidation generating oxysterols nonenzymatically with structures similar to those formed endogenously [46] and allows the subsequent storage of oxysterols without the possibility of their formation via cholesterol and air. Oxysterols are then activated to allow subsequent "click chemistry" with a charge-baring group, which enhances their ESI-MS response. In our experience, unprocessed samples can be stored effectively at $-80\,^{\circ}C$ before sample preparation with minimal autoxidation. However, growing peaks corresponding to 7-oxocholesterol, 7β-hydroxycholesterol, 5,6-epoxycholesterol, and cholestane-3β,5α,6β-triol should alert the analyst of potential autoxidation problems.

Note on nomenclature

Here we regard a steroid as a molecule based on the cyclopentanoperhydrophenanthrene ring structure. In vertebrates hormonal steroids usually contain 18, 19, or 21 carbon atoms; bile acids 24 carbon atoms with a carboxylic acid group at C-24; and cholestanoic and cholestenoic acids 27 carbon atoms with an acid group at C-26 or C-27. In cholestenoic acids the stereo center at C-25 is usually 25*R*. Endogenous sterols are precursors or metabolites of cholesterol and include cholestenoic acids, which, like cholesterol, possess a hydroxy (or oxo) group at C-3 and usually contain 27 carbons. Oxysterols are a category of sterols, mostly derived from cholesterol, containing an additional oxygen function. In this article we have adopted the nomenclature recommended by the Lipid Maps Consortium [37].

Principles

In this article we describe a LC-ESI-MS method for the quantitative profiling of a wide range of oxysterols and their downstream acidic metabolites from microliter quantities of plasma. Our method is based on ethanol extraction, separation of cholesterol metabolites from cholesterol itself by reversed-phase (RP) solid-phase extraction (SPE), followed by enzyme-assisted derivatization for sterol analysis (EADSA). EADSA consists of enzymatic conversion of 3β-hydroxy-5-ene- and 3β-hydroxy-5α-hydrogen-containing sterols to 3-oxo-4-ene and 3-oxo sterols followed by tagging a positively charged quaternary nitrogen group to the resulting oxo group in a "click reaction" (Scheme 1). Analysis and quantification are performed by LC-ESI-MS. Our preference is to perform mass analysis at high mass resolution (30,000, full width at half-maximum height, FWHM) using stable-isotope or structural analogue internal standards for quantification, with compound identification achieved using exact mass measurements (<5 ppm) and multistage fragmentation (MS^n). We perform these analyses on an LTQ-Orbitrap instrument. Alternative MS formats can be used, such as quadrupole–time-of-flight (Q-TOF), tandem quadrupole, and cylindrical or linear ion trap (LIT), but none of these offers the combination of high-resolution exact mass measurements and MS^n provided by LTQ-Orbitrap or LTQ-FT-ICR instruments. We complement the EADSA LC-ESI-MS method with shotgun ESI-MS, also performed at high resolution with exact mass measurement and MS^2, to characterize oxysterols and their downstream metabolites conjugated with sulfuric and/or glucuronic acids. Shotgun ESI-MS is appropriate for analysis of cholesterol metabolites sulfated at C-3 and thus inaccessible to EADSA. Using shotgun ESI-MS we do not attempt to exactly identify or quantify conjugated oxysterols on account of an absence of authentic standards. Ongoing work is in progress to rectify this situation by the synthesis of appropriate standards.

Materials

High-performance liquid chromatography (HPLC)-grade water, absolute ethanol, and other HPLC-grade solvents were from Fisher Scientific (Loughborough, UK) or Sigma–Aldrich (Dorset, UK). Acetic acid was AnalaR NORMAPUR grade (BDH, VWR, Lutterworth, UK). Authentic sterols, steroids, bile acids, and their precursors were from Avanti Polar Lipids (Alabama, USA), Steraloids, Inc. (Rhode Island, USA), Sigma–Aldrich, or previous studies in our laboratories [41].

Please cite this article as: Griffiths, W.J.; et al. Analytical strategies for characterization of oxysterol lipidomes: Liver X receptor ligands in plasma. *Free Radic. Biol. Med.* (2012), http://dx.doi.org/10.1016/j.freeradbiomed.2012.07.027

Download English Version:

https://daneshyari.com/en/article/8271445

Download Persian Version:

https://daneshyari.com/article/8271445

Daneshyari.com