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Simultaneous quantitative profiling of 20 isoprostanoids from omega-3 and omega-6 polyunsaturated fatty acids by LC–MS/MS in various biological samples

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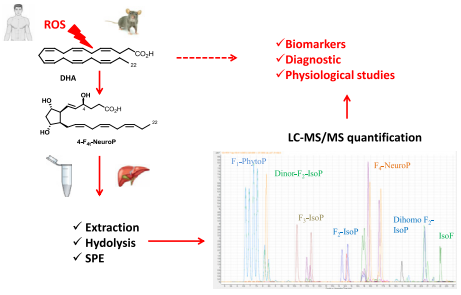
HIGHLIGHTS

- Isoprostanoids are a group of non-enzymatic oxygenated metabolites of polyunsaturated fatty acids which are key intermediates in a lot of physiological mechanisms.
- An quantitative LC–MS/MS profiling of these biomarkers was developed, validated and applied on various biological sample.
- This method will be highly useful to follow biological process dealing with ROS.

GRAPHICAL ABSTRACT



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ABSTRACT

Isoprostanoids are a group of non-enzymatic oxygenated metabolites of polyunsaturated fatty acids. It belongs to oxylipins group, which are important lipid mediators in biological processes, such as tissue repair, blood clotting, blood vessel permeability, inflammation and immunity regulation. Recently, isoprostanoids from eicosapentaenoic, docosahexaenoic, adrenic and α-linolenic namely F_3-isoprostanes, F_4-neuroprostanes, F_2-dihomo-isoprostanes and F_1-phytoprostanes, respectively have attracted attention because of their putative contribution to health. Since isoprostanoids are derived from different substrate of PUFAs and can have similar or opposing biological consequences, a total isoprostanoids profile is essential to understand the overall effect in the testing model. However, the concentration of most isoprostanoids range from picogram to nanogram, therefore a sensitive method to quantify 20 isoprostanoids simultaneously was formulated and measured by liquid chromatography-tandem mass

Abbreviations: AA, Arachidonic acid; AdA, Adrenic acid; ALA, α-Linolenic acid; BHT, Butylated hydroxytoluene; CSF, Cerebrospinal fluid; DHA, Docosahexaenoic acid; DPDS, 2,2'-dipyridyl disulfide; EGTA, Ethylene glycol tetra acetic acid; EPA, Eicosapentaenoic acid; ESI, Electrospray ionization; HP, 2-hydrazinopyridine; HPLC, High-pressure liquid chromatography; IsoPs, Isoprostanes; LOD, limit of detection; LOQ, limit of quantification; *m/z*, Mass-to-charge ratio; MRM, Multiple reaction monitoring; MS, Mass spectrometry; NeuroPs, Neuroprostanes; OS, Oxidative stress; PhytoPs, Phytoprostanes; PA, 2-picolylamine; PUFAs, Polyunsaturated fatty acids; ROS, Reactive oxygen species; SPE, Solid-phase extraction; SRM, Selected-reaction monitoring; S/N, signal to noise ratio; TPP, Triphenylphosphine.

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spectrometry (LC–MS/MS). The lipid portion from various biological samples was extracted prior to LC–MS/MS evaluation. For all the isoprostanoids LOD and LOQ, and the method was validated on plasma samples for matrix effect, yield of extraction and reproducibility were determined. The methodology was further tested for the isoprostanoids profiles in brain and liver of LDLR$^{-/-}$ mice with and without docosahexaenoic acid (DHA) supplementation. Our analysis showed similar levels of total F_2-isoprostanes and F_4-neuroprostanes in the liver and brain of non-supplemented LDLR$^{-/-}$ mice. The distribution of different F_2-isoprostane isomers varied between tissues but not for F_4-neuroprostanes which were predominated by the 4(*RS*)-4-F_{4t}-neuroprostane isomer. DHA supplementation to LDLR$^{-/-}$ mice concomitantly increased total F_4-neuroprostanes levels compared to F_2-isoprostanes but this effect was more pronounced in the liver than brain.

1. Introduction

Excessive free radicals *in vivo* have been implicated in a number of human diseases such as neurodegenerative, cardiovascular, pulmonary disorder and cancer [1,2]. The most common free radicals are reactive oxygen species (ROS), which can modify lipids, proteins and nucleic acids. Of the lipids in particular, the polyunsaturated fatty acids (PUFA) form a wide variety of oxygenated metabolites [3,4]. Among them, the isoprostanes (IsoPs) appears to be a promising group of biomarkers to be assessed for oxidative stress (OS) assessment *in vivo* for over two decades due to its specificity and sensitivity [5,6]. These compounds are formed *in situ* on membrane phospholipids and then released into their free form via phospholipase A_2 and platelet activating factor hydrolase for circulation. Elevation of IsoPs, in particular those originated from arachidonic acid (AA, 20:4 n-6) also known as F_2-IsoPs in biological fluids (e.g. plasma and urines) are recognized as the reference biomarker for lipid peroxidation and OS in most biological systems. Beyond their capacity of OS as biomarker, IsoPs from n-3 PUFA also demonstrated to be biologically active [7–9]. Therefore it is crucial to be able to quantify the different isoforms in a large panel of biological samples to integrate this chemical and biological complexity.

Unlike PUFAs, the isoprostanoids are quite complex to assess since the concentration range is very low (from picogram to nanogram) in most biological samples. Moreover, depending on the parent PUFAs, a large diversity of molecule has been discovered as shown in Fig. 1. Analysis of these metabolites in biological samples is a challenge and depends on the robustness of the analytical instrumentation. Further, it requires one or several preparation steps, including hydrolysis and extraction from their biological matrix before analysis by radio immunological methods (RIA) or gas chromatography–mass spectrometry (GC–MS) or liquid chromatography–mass spectrometry (LC–MS), which are often coupled to another mass spectrometer (MS/MS) to increase the sensitivity [10]. It is well known to analysts, that RIA is not specific enough to provide efficient quantification of different IsoPs [11]. To date, LC–MS/MS is the most common technique to quantify these biomarkers [12], even if the mass spectrometry is not the perfect method to perform absolute quantification compared to GC–MS because of the various ionization efficiency between different molecules. These changes can be very important when comparing compounds with very close structures especially for lipids including the IsoPs. In order to optimize ionization efficacy for each compound, it is essential to have the pure standard to develop a rigorous quantitative method. Although some standards are available commercially, many of the novel ones are unavailable. Through total synthesis, Durand's group was able to synthesize [13–21] these novel standards, for example dihomo-IsoPs from adrenic acid (C22:4 n-6, AdA) for mass spectrometry analysis.

In this study, we developed a complete quantitative profiling of IsoPs by LC–MS/MS. As IsoPs are present in a very low concentration it was imperative to improve largely the sensitivity of the method therefore we also tried two different derivatization procedures of the carboxylic acid function to improve the ionization of molecules. For both profiles, with and without derivatization, chromatographic separation has been optimized and sensitivity compared. The two methods have been tested on human plasma and the best one was applied in this study. The final methodology was then validated on plasma sample and applied to other biological samples, namely cerebrospinal fluid (CSF), urine, and brain, liver and muscle tissues. Our methodology was finally checked on a mouse model, in which the goal was to determine the isoprostanoids profiling in the brain and liver of LDLR$^{-/-}$ mice and to investigate the effect of docosahexaenoic acid (C22:6 n-3, DHA) supplementation on these profiles.

2. Material and methods

2.1. Chemicals

Commercially available IsoP standards (d_4-15-F_{2t}-IsoP and 2,3-dinor-15-F_{2t}-IsoP) were purchased from Cayman Chemicals (Ann Arbor, MI, USA). Others standards *Ent*-16-*epi*-16-F_{1t}-PhytoP, *Ent*-16-F_{1t}-PhytoP, 9-F_{1t}-PhytoP, 9-*epi*-9-F_{1t}-PhytoP, *Ent*-15(*RS*)-2,3-dinor-5,6 dihydro-15-F_{2t}-IsoP, 8-F_{3t}-IsoP, 8-*epi*-8-F_{3t}-IsoP, 5-F_{3t}-IsoP, 5-*epi*-5-F_{3t}-IsoP, 15-F_{2t}-IsoP, 15-*epi*-15-F_{2t}-IsoP, 5-F_{2t}-IsoP, 5-*epi*-5-F_{2t}-IsoP, 10-F_{4t}-NeuroP, 10-*epi*-10-F_{4t}-NeuroP, 14(*RS*)-14-F_{4t}-NeuroP, 4(*RS*)-4-F_{4t}-NeuroP, *Ent*-7(*RS*)-7-F_{2t}-dihomo-IsoP, 17(*RS*)-17-F_{2t}-dihomo-IsoP, C21-15-F_{2t}-IsoP, d_4-10-*epi*-10-F_{4t}-NeuroP, d_4-10-F_{4t}-NeuroP, d_4-4(*RS*)-4-F_{4t}-NeuroP were synthesized according to our published procedures. Hexane, ethanol absolute, acetic acid potassium hydroxide (KOH), methanol (MeOH; HPLC gradient Grade), butylated hydroxytoluene (BHT) and formic acid were purchased from Sigma Aldrich (Saint Quentin Fallavier, France). Acetonitrile (ACN; HPLC grade) was obtained from Acros Organics (Illkirch, France). Ammonia solution 30% (NH_4OH) was purchased from Carlo Erba Reagenti (Cornaredo, Italy). Water used in this study was purified on a milliQ system (Millipore). The 96 well-plate for solid extraction (SPE) (Oasis Max, 60 mg) was purchased from Waters (Saint-Quentin en Yvelines, France).

2.2. Standards preparation for linearity and reproducibility assessment

Standard solutions with or without derivatization were prepared in MeOH at the following concentrations, 0.06, 0.12, 0.24, 0.49, 0.98, 1.95, 3.91, 7.81, 15.63, 31.25, 62.5, 125, 250, and 500 ng. mL^{-1} for all primary standards. The concentration of the deuterated internal standards (IS) used 5 ng taken from