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Review Article

Oxidative Phospholipidomics in health and disease: Achievements, challenges and hopes☆

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ABSTRACT

Phospholipid peroxidation products are recognized as important bioactive lipid mediators playing an active role as modulators in signalling events in inflammation, immunity and infection. The biochemical responses are determined by the oxidation structural features present in oxPL modulating biophysical and biological properties in model membranes and lipoproteins. In spite of the extensive work conducted with model systems over the last 20 years, the study of oxPL in biological systems has virtually stagnated. In fact, very little is known concerning the predominant oxPL in fluids and tissues, their basal levels, and any variations introduced with age, gender and ethnicity in health and disease. In consequence, knowledge on oxPL has not yet translated into clinical diagnostic, in the early and timely diagnosis of "silent" diseases such as atherosclerosis and cardiovascular diseases, or as prognosis tools in disease stratification and particularly useful in the context of multimorbidities. Their use as therapeutic solutions or the development of innovative functional biomaterials remains to be explored.

This review summarizes the achievements made in the identification of oxPL revealing an enormous structural diversity. A brief overview of the challenges associated with the analysis of such diverse array of products is given and a critical evaluation on key aspects in the analysis pipeline that need to be addressed. Once these issues are addressed, Oxidative Phospholipidomics will hopefully lead to major breakthrough discoveries in biochemistry, pharmaceutical, and clinical areas for the upcoming 20 years. This article is part of Special Issue entitled 4-Hydroxynonenal and Related Lipid Oxidation Products.

1. Introductory perspective and focus

Oxidized phospholipids (oxPL) are widely accepted as key biomolecules participating in inflammatory signalling events [1–5]. The establishment of oxPL as mediators in biochemical events has come a long way since their first discovery. The term "oxidized phospholipids" was initially used by Frederik Bernheim [6] to describe the compounds formed after the addition of vanadium salts onto brain, liver and heart tissues. The presence of "oxidized phospholipid" compounds was proposed based on oxygen uptake by tissues [6]. Following this, and in line with the free radical theory [7] modification of (phospho)lipids under aerobic oxidative conditions gained popularity and is currently accepted as key to maintain cellular homeostasis.

For many years, analysis of polar molecules with high molecular weight and low thermal stability, including oxidized (phospho)lipids, was restricted by the available techniques such as gas chromatography (GC) involving laborious derivatisation protocols designed to increase volatility of fatty acids released after saponification. However, derivatisation protocols were often source of experimental artifacts confounding the final outcome, and alternative Mass Spectrometry (MS)

Abbreviations and Definitions: DHB, 2,5-dihydroxybenzoic acid; HILIC, hydrophilic interaction liquid chromatography; HOL•, hydroxy-alkyl lipid radical; HETE-PL, hydroxy-eicosatetraenoic phospholipid derivative; LDL, low density lipoprotein; LLE, liquid-liquid extraction; LUV, large unilamellar vesicles; L•, alkyl lipid radical; LOO•, peroxyl lipid radical; LO•, alkoxyl lipid radical; KETE-PL, keto-eicosatetraenoic phospholipid derivative; MALDI, Matrix-assisted Laser Desorption Ionization; MLV, multi-lamellar vesicles; MS/MS, tandem mass spectrometry; OL•, epoxy-alkyl lipid radical; OLOO•, epoxy-peroxyl lipid radical; oxPC, oxidized phosphatidylcholine; oxPE, oxidized phosphatidylethanolamine; OxPL, oxidized phospholipid; oxPS, oxidized phosphatidylserine; PAPC, 1-palmitoyl-2-arachidonoyl-phosphatidylcholine; PAzPC, 1-palmitoyl-2-azeloyl-phosphatidylcholine; PC, phosphatidylcholines lipids; *d*PC, diacyl-phosphatidylcholines lipids; *p*PC, plasmenyl-phosphatidylcholines lipids; PE, phosphatidylethanolamine lipids; PEIPC, 1-palmitoyl-2-epoxy-isoprostane-phosphatidylcholine; PGPC, 1-palmitoyl-2-glutaroyl-phosphatidylcholine; PIS, precursor ion scanning; PhGPx, phospholipid hydroperoxide glutathione peroxidase; PL, phospholipid; PLA2, phospholipase A2; PLOOH, phospholipid hydroperoxides; POVPC, 1-palmitoyl-2-oxovaleroyl-phosphatidylcholine; PS, phosphatidylserine lipids; PONPC, 1-palmitoyl-2-(oxo-nonanoyl)-phosphatidylcholine; POVPC, 1-palmitoyl-2-(oxo-valeroyl)-phosphatidylcholine; RA, Relative Abundance; ROS, reactive oxygen species; RP, reverse phase; SazPC, 1-stearoyl-2-azeloyl-phosphatidylcholine; SGPC, 1-steraoyl-2-glutaroyl-phosphatidylcholine; SM, sphingomyelins; SMase, sphingomyelinase; SOVPC, 1-stearoyl-2-oxovaleroyl-phosphatidylcholine; SONPC, 1-stearoyl-2-(oxo-nonanoyl)-phosphatidylcholine; SUV, small unilamellar vesicles; TAG, Triacylglycerides

☆ This review is dedicated to Hermann Esterbauer who with his work pioneered the world of oxidative lipidomics.

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approaches such as Fast Atom Bombardment (FAB-MS), chemical ionization (CI-MS) and electron impact (EI-MS) involved the deposition of high energy amounts on the analyte resulting in sample degradation, high background noise and complex mass spectra [8–10].

The 1980's was prolific with ground-breaking work in the fields of Physics and Biochemistry. Advances made in these 2 unrelated fields of Science set the basis and boosted the scientific community interest in the area of lipid peroxidation and the study of oxidatively modified lipids.

Earlier studies carried out by John B. Fenn on supersonic free jet expansion shaped his interests to work and replicate the initial experiments by Dole on the dispersion of analyte solution using high electric fields beamed into a bath gas [11]. The ions observed as clusters in the mass spectra without thermal degradation changed the panorama for the analysis of large biomolecules in solution [12]. Across the Pacific Ocean Dr Koichi Tanaka, working for Shimadzu, investigated the use of laser irradiated inorganic salts (cobalt embedded in glycerol) to promote the ionization of biomolecules into the gas plume [13] initiating the use of a matrix compound for the ionization of biomolecules without loss of structure. Meantime, in Europe, Michael Karas working at Hillenkamp's group, attempted a similar approach by using organic molecules as support (matrix) compounds to assist the laser ionization process [14]. Over time, organic UV-absorbing molecules became the gold-standard method in MALDI sample preparation in detriment to the use of inorganic UV-absorbing salts. Analysis of high molecular weight polar biomolecules in complex matrices was greatly simplified by the advances made on the "*development of soft desorption ionization methods for mass spectrometric analyses of biological macromolecules*" and the development of electrospray ionization (ESI) and matrix-assisted laser desorption ionization (MALDI) leading to the attribution of the Nobel Prize in Chemistry to John B. Fenn and Koichi Tanaka in 2002 (http://www.nobelprize.org/nobel_prizes/chemistry/laureates/2002/).

In another field, Prof Hermann Esterbauer, who had initially participated in the discovery of 4-hydroxy-nonenal (HNE) [15], described the modification of lysine and tyrosine amino acids by HNE in human LDL [16] and the impact on the uptake of HNE-modified LDL by tissue macrophages [17]. His work, on the targets and cytotoxic effects of hydroxyalkenals and the pathophysiological implications of HNE-modified molecules [18] with emphasis on LDL [17–20], was crucial to the growing interest of lipid peroxidation and our current understanding of lipid peroxidation reactions and consequent implications of these reactions to the homeostasis in biological systems. Between 1988 and 1993, Prof Esterbauer published and co-authored > 50 papers covering the many aspects of synthesis, separation, characterization, and biological role of HNE and other aldehydes in biological systems influencing and inspiring a whole new generation of scientists. Together with the pivotal work developed by Prof Esterbauer, work on the signalling properties of 1-palmitoyl-2-(5-oxo-pentanoyl) – 3-glycero-PC (POVPC) for the platelet-activating acetylhydrolase enzyme found in human plasma [21], and the discovery of other phosphatidylcholine derivatives, who were chemically similar to platelet-aggregating factor (PAF) [10,22] boosted researcher's interest into what was, at the time, the unexciting field of oxidatively modified lipids.

Since then, oxPL have been identified in fluids [23–27], cells [28–33] and tissues [34–37], in health [23,24,38] and disease [27,37,39] reinforcing the notion that oxidized phospholipids are no longer mere by-products and spectators formed during lipid peroxidation reactions but exciting molecules and key players in inflammation [1,3,5], infection [40] and immune response [41,42].

The discovery of these natural compounds and their improved detection in complex matrices, prompted by the development of "soft" ionization methods, became a hallmark for the rise of the Oxidative (Phospho)lipidomics field in the past 25 years. The growing number of publications focusing on the identification, detection and role of oxPL in model systems and biological samples reflects the increasing curiosity and interest around oxPL over the years. Despite the many studies,

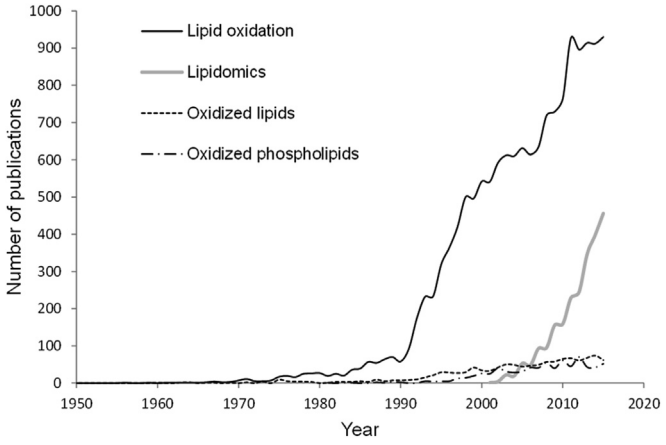

Fig. 1. Number of publications in lipid-related topics spanning from 1950 to 2015 (Scopus search, October 2016).

routine analysis of oxPL in fluids and tissues is still limited and knowledge on the basal levels of oxPL with age, gender, ethnicity in health and disease remain elusive. In consequence, the clinical significance of oxPL *in vivo* has not yet translated into the discovery of disease markers, novel drug targets or even resulted in the advance of innovative therapeutic solutions. Strangely, while the topic of lipid peroxidation remains popular and lipidomic field has continuously grown for the past 10 years, the field of **Oxidative (Phospho)lipidomics** has virtually remained stagnated (Fig. 1).

One of the main reasons for the stagnation may be related to the enormous complexity of structural features typically present in oxPL, requiring multiple analytical strategies, able to detect oxPL present in a wide mass range, over a wide concentration range in complex biological matrices.

The following sections summarize the current knowledge on oxPL identified in models and biological samples, with focus on the analytical strategies adopted to extract, detect, identify and accurately quantify residual oxPL using mass spectrometry. Emphasis on current limitations associated with the routine analysis of oxPL that require additional efforts by researchers and manufacturers is also described. Once these issues are addressed, the exploratory analysis of oxPL in *ex vivo* samples in large cohorts will permit to pinpoint the oxPL signature in fluids and cells, in health and disease and through the integration with additional omic platforms redirect the field of Oxidative Phospholipidomics to exciting new possibilities in the biomedical field.

2. The structural diversity behind oxidized phospholipids

Oxidized phospholipids comprise hundreds of structures from different phospholipid classes, several chemical groups with varied carbon chain lengths. Phospholipids (glycerol-based or sphingosine-based) containing unsaturated acyl chains in its structure are susceptible to oxidative modification initiated by the attack of oxygen centered free radicals formed under aerobic conditions ($HO^{\bullet}$, $OONO^{\bullet}$, $O_2^{\bullet}$). Due to its very reactive nature and short half-life, free radicals easily abstract hydrogen atoms located between two double bonds (bis-allylic H atoms) [43]. The abstraction of H atoms has poor selectivity and oxidative modification is mostly governed by the accessibility of initiating free radical specie generating lipid radicals at various positions along the carbon chain. Free radical reactions occur randomly along the carbon chain and are typically characterized by initiation, propagation and termination steps, with modification of unsaturated fatty acid chains by oxygen centered radicals leading to the formation of primary peroxidation products (hydroperoxide, hydroxyl and keto derivatives), by decomposition of hydroperoxide groups to the formation of secondary peroxidation products (carbonyl-based derivatives), and finally by cross-linking reactions of carbonyl groups (electrophiles) with amino