Published in final edited form as:

Anal Biochem. 2013 December 15; 443(2): . doi:10.1016/j.ab.2013.09.016.

Approaches for the Analysis of Chlorinated Lipids

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Abstract

Leukocytes are key cellular mediators of human diseases through their role in inflammation. Identifying unique molecules produced by leukocytes may provide new biomarkers and mechanistic insights into the role of leukocytes in disease. Chlorinated lipids are generated as a result of myeloperoxidase-containing leukocyte-derived hypochlorous acid targeting the vinyl ether bond of plasmalogens. The initial product of this reaction is α -chlorofatty aldehyde. α -Chlorofatty aldehyde is both oxidized to α -chlorofatty acid and reduced to α -chlorofatty alcohol by cellular metabolism. This review focuses on the separation techniques and quantitative analysis for these chlorinated lipids. For α -chlorofatty acid the negative charge of carboxylic acids is exploited to detect the chlorinated lipid species of these acids by electrospray ionization mass spectrometry in the negative ion mode. In contrast, α -chlorofatty aldehyde and α -chlorofatty alcohol are converted to pentafluorobenzyl oxime and pentafluorobenzoyl ester derivatives, which are detected by negative ion-chemical ionization mass spectrometry. These two detection methods coupled with the use of stable isotope internal standards and either liquid chromatography or gas chromatography provide highly sensitive analytical approaches to measure these novel lipids.

Keywords

fatty aldehyde; fatty acid; mass spectrometry; monocytes; chlorinated lipids; chromatography

Introduction

Myeloperoxidase (MPO) is an abundant and important protein found in neutrophils, monocytes and macrophages. MPO amplifies the oxidant response in these leukocytes during activation resulting in the conversion of hydrogen peroxide to hypochlorous acid (HOCl) [1; 2]. While HOCl contributes to the antimicrobial and cytotoxic properties of these leukocytes, this powerful oxidant also targets host tissues and may be involved in the pathogenesis of several diseases [3; 4]. Inflammatory reactions mediated by MPO-enriched phagocytes contribute to the pathogenesis of atherosclerosis, ischemic/reperfusion injury to numerous tissues, and other disorders. HOCl is a two-electron oxidant that can target reactive sites of proteins and lipids. The reactive sites of lipids that can potentially be chlorinated by HOCl include amine, alkene, and vinyl ether functional groups.

The rate constant for HOCl reacting with vinyl ether bonds, which is the defining structural component of plasmalogens, is much greater than that of alkenes [5]. Plasmalogens are a

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major lipid subclass found in many mammalian cell types, including endothelial cells, macrophages, neutrophils, smooth muscle cells, cardiac myocytes, neurons, and glia [6; 7; 8]. Plasmalogens have antioxidative properties based on two electron free oxidants reacting with the vinyl ether bond leading to the production of stable products [9; 10]. However, reaction products from HOCl targeting plasmalogens have been associated with cardiovascular disease [3]. Figure 1 shows the precursor, plasmalogen, reacting with HOCl resulting in the formation of the products, lysophospholipid and α -chlorofatty aldehyde (α -CIFALD). The major plasmalogens, plasmenylethanolamine and plasmenylcholine, are both targets of HOCl resulting in the production of α -CIFALD and the lysophospholipids, lysophosphatidylethanolamine and lysophosphatidylcholine, respectively. α -CIFALD can be either oxidized to α -chlorofatty acid (α -CIFA) or reduced to α -chlorofatty alcohol (α -CIFOH). Oxidation of the aldehyde to the α -CIFA metabolite is catalyzed by a fatty aldehyde dehydrogenase [11]. α -Oxidation of α -CIFA is initiated by an α -hydroxylation step, followed by conversion of the intermediate to an α -chlorodicarboxylic acid. Sequential α -oxidation from the α -end of the dicarboxylic acids leads to the production of 2-chloroadipic acid (2-ClAdA). The *in vivo* metabolism of α -CIFA to 2-ClAdA has been demonstrated with the final product, 2-ClAdA, being excreted in the urine [12].

α -CIFALD accumulates in activated human neutrophils, activated human monocytes, human atherosclerotic lesions, infarcted rodent myocardium, and brain of LPS-challenged mice [13; 14; 15; 16; 17]. α -CIFA is found in activated neutrophils and plasma of rats treated with LPS, and α -CIFOH is also found in activated neutrophil [11; 12]. Concomitant with elevations in α -CIFA in the plasma of LPS-treated rats is an increased excretion of 2-ClAdA in the urine [12]. The biological activities of these chlorinated lipids thus far include α -CIFALD: 1) having chemoattractant properties towards neutrophils [14]; 2) being an inhibitor of eNOS activity and expression in endothelial cells [18]; 3) eliciting myocardial contractile dysfunction and endothelial dysfunction [15; 19]; and 4) inducing COX-2 expression in human coronary artery endothelial cells [20]. Additionally α -CIFA induces COX-2 expression in endothelial cells suggesting that the activity of α -CIFALD may be due to its metabolism to α -CIFA [20]. Collectively these findings suggest the importance of chlorinated lipids in disease mediated by MPO-containing leukocytes, and, accordingly accurate analytical techniques for the measurement of these lipids is critical as we gain new insights into the biological role of these novel lipids.

Figure 2 shows the structures of the chlorinated lipids and their derivatives as well as an overview of the chromatography and mass spectrometry approaches that have been developed to detect and quantify these chlorinated lipids. The functional groups of the analytes dictate the derivatizations employed, chromatographic characteristics and mass spectrometry ionization choices. In this review details will be outlined for the analytical approaches used to quantify: 1) α -CIFALD as pentafluorobenzyl oximes (PFBO) using gas chromatography (GC)-mass spectrometry (MS) with negative ion chemical ionization (NICI); 2) α -CIFOH as pentafluorobenzoyl (PFB) esters; and 3) α -CIFA by reversed phase liquid chromatography with electrospray ionization (ESI)-MS and selected reaction monitoring (SRM) for detection.

Preparation of Synthetic Standards

For each of the chlorinated lipid classes, stable isotope-labeled internal standards are the best approach for quantitative analysis. For α -CIFALD analysis, the internal standard used is 2-chloro- $[d_4, 7, 7, 8, 8]$ -hexadecanal (2-Cl- $[d_4]$ HDA). For α -CIFA analysis, the internal standard used is 2-chloro- $[d_4, 7, 7, 8, 8]$ -hexadecanoic acid (2-Cl- $[d_4]$ HA). For 2-CIFOH analysis, the internal standard used is 2-chloro- $[d_4, 7, 7, 8, 8]$ -hexadecanol (2-Cl- $[d_4]$ HOH).

2-Cl-[d_4]HDA has been previously synthesized [15] by the following steps: 1) synthesis of [7,7,8,8- d_4]-hexadecanol from [7,7,8,8- d_4]-hexadecanoic acid (Medical Isotopes, Inc.) using sodium bis(2-methoxyethoxy)aluminum hydride; 2) synthesis of [7,7,8,8- d_4]-hexadecanal by partial oxidation at 70 °C utilizing oxalyl chloride-activated DMSO as catalyst (30); 3) synthesis of the dimethyl acetal of [7,7,8,8- d_4]-hexadecanal by acid methanolysis; 4) synthesis of the dimethyl acetal of 2-Cl-[d_4]HDA by acetal chlorination employing MnO_2 -trimethylchlorosilane (31); and 5) synthesis of 2-Cl-[d_4]HDA by reflux in 1:1 trifluoroacetic acid/dichloromethane. The product is purified by semi-preparative TLC and quantitated [15].

For the synthesis of 2-Cl-[d_4]HA, [d_4]-hexadecanoic acid ([d_4]-16:0 FA) is subjected to α -chlorination with Cl_2 using the Hell-Vollard-Zelinsky reaction and phosphorous as catalyst [21]. [d_4]-16:0 FA is melted at 80°C before an equimolar amount of phosphorous trichloride in dichloromethane is added to the reaction vial. Cl_2 is then slowly bubbled into the reaction mixture for 1 h. The crude product is sequentially extracted and purified first by TLC and then reversed phase HPLC as previously described [22]. For the synthesis of 2-Cl-[d_4]HOH, 2-Cl-[d_4]HDA is reduced with Vitride™ reagent (sodium bis(2-methoxyethoxy)aluminum hydride), and the resultant alcohol is purified by TLC (petroleum ether/ethyl ether/acetic acid (70/30/1, v/v/v)) (R_f = 0.41).

Lipid extraction

Figure 3 shows a flow chart for the extraction procedures used for chlorinated lipids from either tissues, cells, cell culture media or plasma. For lipid extractions, derivatizations, and chromatography, HPLC grade solvents should be used. For Bligh-Dyer extractions of cells or tissues the use of chloroform and methanol purchased from Fisher is recommended.

Cellular or tissue lipids (e.g., from 1×10^6 neutrophils) are extracted by a modification of the methods of Bligh and Dyer [13; 23] in the presence of 20 pmol each of 2-Cl-[d_4]HDA, 2-Cl-[d_4]HA, and 2-Cl-[d_4]HOH (e.g., in the case of 1×10^6 neutrophils extracted) that are added as internal standards for α -CIFALD, α -CIFA, and α -CIFOH quantitation, respectively. These cellular or tissue lipid extracts are then used in subsequent analytical steps to quantify α -CIFALD, free α -CIFA, and α -CIFOH as described below.

For cell culture media and plasma analysis of free α -CIFA, a modified Dole extraction is routinely performed [11; 12]. After drying the organic phase extracts containing α -CIFA, these samples are suspended in 150 μl of methanol/water (85/15, v/v) containing 0.1% formic acid and transferred to an autosampler vial with an insert, and these samples will subsequently be analyzed using LC-MS. Similarly Bligh-Dyer lipid extracts are suspended in 150 μl of methanol/water (85/15, v/v) containing 0.1% formic acid for the analysis of cellular or tissue free α -CIFA levels.

For total α -CIFA (including free α -CIFA and α -CIFA esterified to complex lipids) in either cells, tissue, cell culture media or plasma, samples are subjected to base hydrolysis. For tissue and cell samples, base hydrolysis is performed on Bligh-Dyer lipid extracts [11; 12]. In contrast, cell culture media and plasma are first subjected to base hydrolysis. Following base hydrolysis a modified Dole extraction is used to extract the hydrolysis products that are subsequently resuspended in methanol/water (85/15, v/v) containing 0.1% formic acid prior to LC-MS analyses.

Thin layer chromatography of α -CIFALD

Previous studies have shown that a purification step is essential for the analysis of α -CIFALD in some tissues [15]. For example in myocardial tissue, α -CIFALD is purified by

thin layer chromatography. 2-CIHDA from crude lipid extract suspended can be purified on TLC using silica gel 60-A plates and a mobile phase comprised of petroleum ether/ethyl ether/acetic acid (90/10/1, v/v/v)(relative migration 0.46). Other long-chain α -CIFALD, including 2-CIODA, copurify with 2-CIHDA using this TLC procedure. The silica corresponding to the purified α -CIFALD is scrapped from the plate and extracted using two sequential single phase extractions with methanol/chloroform (1/1), and then saline/methanol/chloroform (0.8/2/1). Additional chloroform and saline are added to the combined TLC silica extracts to bring the saline/methanol/chloroform to (0.8/1/1), and then the lower phase chloroform is collected for subsequent α -CIFALD by GC-MS.

α -CIFALD analysis

To quantify α -CIFALD, the aldehyde is first converted to its PFBO and then this derivative is subjected to GC-MS with NICI. This method has been used by the Ford laboratory group, and the Malle and Sattler laboratory group [13; 14; 15; 16; 17; 19]. One minor difference between the method described below (Ford group method) and that of the Malle and Sattler group is the use of different stable isotope labeled internal standards (e.g., the Malle and Sattler group uses 2-chloro-[2,4,6,8,10,12,14,16- $^{13}\text{C}_8$]-hexadecanal as internal standard) [17; 19]. In each case, either lipid extracts or TLC-purified α -CIFALD from tissues are derivatized to their PFBO before quantitation by GC-MS. Bligh-Dyer extracted lipids from either tissue, cells, plasma or media that are in chloroform are sequentially dried under nitrogen, suspended in 300 μl of ethanol, and combined with 300 μl of 6 mg/ml pentafluorobenzyl hydroxylamine (Sigma Aldrich) in water. After vortexing, the reaction is kept at room temperature for 25 min and then terminated by adding 1.2 ml of Milli-Q water followed by 2 ml of cyclohexane/ethyl ether (4/1, v/v) and subsequent vortex mixing. After centrifugation, the upper phase is collected and the remaining lower phase is re-extracted. The extracted reaction products are sequentially dried under nitrogen and suspended in 100 μl of petroleum ether before analysis by GC-MS using a DB-1 column and Agilent 6890 gas chromatograph, as described before [15]. The injector and the transfer lines are maintained at 250 $^{\circ}\text{C}$ and 280 $^{\circ}\text{C}$, respectively. The GC oven is initially at 150 $^{\circ}\text{C}$ for 3.5 min and increased at a rate of 25 $^{\circ}\text{C}/\text{min}$ to 310 $^{\circ}\text{C}$. The oven temperature is held at 310 $^{\circ}\text{C}$ for an additional 5 min. All spectra are acquired on an Agilent 5973 mass spectrometer that is operated in the NICI mode with methane as the reagent gas and helium as the carrier gas. The source temperature is set at 150 $^{\circ}\text{C}$. The electron energy is 170 eV, and the emission current was 49 mA. These PFBO of α -CIFALD were quantified by SIM of the major ion fragment of the derivative and this fragment contains the structurally-relevant component derived from the α -CIFALD (see Figure 2). The integrated area of the *anti* and the *syn* isomers of the PFBO derivatives are summed together in this analyses. The PFBO of 2-CIHDA is detected by selected ion monitoring (SIM) m/z 288, while the PFBO of the internal standard, 2-chloro-[7,7,8,8- d_4] hexadecanal is detected by SIM m/z 292 (see Figure 2 for the structure of this derivative, and its fragmentation). The PFBO of 2-chlorooctadecanal (2-CIODA) is also detected by SIM m/z 316. Under these conditions described there is a linear response of natural 2-CIHDA to 2-Cl-[d_4]HDA that has been documented between 5-60 pmol of 2-CIHDA using 20 pmol of 2-Cl-[d_4]HDA [14]. This responsiveness has not been determined for 2-CIODA, but it is assumed to be similar for 2-CIHDA using 2-Cl-[d_4]HDA as internal standard. It is assumed that the sensitivity of this assay by others is similar. However, their studies assessing 2-CIHDA metabolism in brain microvascular endothelial cells employed over an order of magnitude greater amount of internal standard [19].

α -CIFA analysis

To date, two methods have been described to quantify α -CIFA. The Malle and Sattler group has converted α -CIFA to its PFB ester using PFB bromide derivatizing reagent and resolved this derivative using GC with NICI-MS of the α -CIFA PFB ester [19]. Also in these studies both pentadecanoic and heptadecanoic acids (at 100 ng, ~300 pmol) were used as internal standards [19]. In contrast, the method used by the Ford group employs LC-MS using 2-Cl-[d_4]HA as an internal standard. For this method α -CIFA molecular species are separated on Discovery HS C18, 150 $\times$ 2.1 mm, 5 μ m (Supelco). We use a Thermo Fisher Surveyor LC system with a Thermo Fisher Quantum Ultra electrospray ionization mass spectrometer used as a detector. For LC, the mobile phases used are as follows: (A) 70/30 (methanol/water) with 5 mM ammonium acetate; (B) methanol with 5 mM ammonium acetate; and (C) methanol. Initial conditions are 100 % A at a flow rate of 200 μ l/min. Two minutes following injection a linear gradient from 100% A to 100% B is applied over 4 min, and the 100% B is held for 6 min. The mobile phase is then switched to 100% C and the flow rate is increased to 400 μ l/min and the column is washed with this step for 3 min followed by a re-equilibration to the initial conditions. The 2-chlorohexadecanoic acid (2-CIHA) is detected using SRM transition, by observing the loss of H³⁵Cl from the target chlorinated fatty acid. SRM in the negative ion mode of m/z 289 $\rightarrow$ 253 for natural 2-CIHA is used to measure 2-CIHA. The internal standard, 2-Cl-[7,7,8,8- d_4]-HA is detected at m/z 293 $\rightarrow$ 257. For electrospray ionization MS, the ionization energy and temperature were 3200 V and 310 $^{\circ}$ C, respectively. For fragmentation, the collision energy is 13 V, and 1.0 millitorr argon is used as the collision gas. Under these conditions the retention time for 2-CIHA is ~7.6 min.

This method is sensitive and can reliably detect 2-CIHA as low as 2 fmol and reliably quantify 10 fmol in plasma [12]. In general much higher levels are found in activated leukocytes and tissues impacted by activated leukocytes. The detection of 2-CIHA in plasma is challenging, and care must be directed toward avoiding contamination during the workup of samples as well as between samples during sequential analyses. Using blanks throughout a sequence of samples allows monitoring for contamination across samples. Additionally the use of 100% methanol as an elution step, and washing the injection needle in 100% methanol reduces contamination in our hands to a point that contamination is negligible. In the future the use of monolithic C18 columns may also reduce contamination issues and allow rapid changes in mobile phases to allow faster analyses of multiple samples. When testing other commercial brands of C18 columns it is recommended that additional method development be considered to optimize resolution and sensitivity. For example in our preliminary tests with other C18 columns adding 0.05% acetic acid to mobile phase A, and altering the proportion of water and methanol in mobile A had significant effects on resolution and sensitivity.

As previously stated alternative approaches can be used for the analyses of α -CIFA that include GC. α -CIFA can be converted to its PFB ester, which is then analyzed by GC with NICI-MS detection. Alternatively, although not as sensitive, α -CIFA can be converted to its fatty acid methyl ester that can be detected following GC by either flame ionization detection, electron ionization mass spectrometry or positive ion-CI mass spectrometry. Another option for α -CIFA measurements in the future would be the use of LC-MS with derivatives that provide a charge reversal of the negative ion carboxylic acid. Recently, charge reversal strategies have been shown to significantly enhance the detection of fatty acids [24] and it is possible that using a derivative such as N-(4-aminomethylphenyl)pyridinium may provide extraordinary sensitivity that may be very useful for the analysis of plasma levels of α -CIFA.

α -CIFOH analysis

Similar to our group, the Malle and Sattler group has quantified α -CIFOH by first derivatizing it to its PFB ester that is then quantitated using GC-MS [19]. There are differences however in the internal standards used with the Malle and Sattler group using pentadecanol while our group uses 2-Cl-[d_4]HOH. Typically Bligh Dyer lipid extracts in chloroform are dried under nitrogen and 50 μ l of PFB chloride is subsequently added [11; 22]. Samples are capped under nitrogen and incubated at 60 °C for 45 min. At the end of incubations, the reaction is cooled down to room temperature for 10 min, and then dried under nitrogen. The derivatized products in the dried residue are suspended in 2ml of hexane and dried under nitrogen with two repetitions of this process. The final residue resuspended in hexane is transferred to an autosampler vial insert, followed by drying and resuspending in 100 μ l of ethyl acetate which is then subjected to GC-MS. GC-MS analysis of PFB esters is performed using a Hewlett Packard (Palo Alto, CA) 6890 gas chromatograph and 5973 mass spectrometer using the negative ion chemical ionization (NICI) mode with methane as the reagent gas. The source temperature is set at 150 °C. The electron energy is 240 eV, and the emission current is 300 A. The PFB derivatives are separated on a J&W Scientific (Folsom, CA) DB-1 column (12.5 m, 0.2 mm inner diameter, 0.33- μ m film thickness). The injector and the transfer line temperatures are maintained at 250 °C. The GC oven is maintained at 150 °C for 3.5 min, increased at a rate of 30 °C/min to 270 °C, and held at 270 °C for an additional 2 min. While other reports detect these molecules (based on the amount of internal standard added) at levels around 300 pmol [19], our previous studies have detected α -CIFOH in the range of 10-50 pmol [11].

Conclusions

The key step for the production of chlorinated lipids is the release of α -CIFALD from the masked aldehyde vinyl ether linkage of plasmalogens [14; 25]. α -CIFALD is the initial chlorinated lipid produced and is the precursor of the other chlorinated lipids. Since α -CIFALD is less polar, analyses by ESI is not very robust, and our experience indicates that α -CIFALD is unstable under ESI conditions. Accordingly, derivatizing α -CIFALD to its PFBO enhances the ionization of this molecule, and this compound is easily detected by negative ionization chemical ionization (NICI) mass spectrometry in conjunction with gas chromatography. This methodology can be used to detect α -CIFALD into the fmol range [14; 25]. Alternatively, one can detect α -CIFALD by converting it to its dimethyl acetal and separating this derivative by GC with flame ionization detection [25]. This is a good alternative when a mass spectrometer is not available, but it is not as sensitive as the PFBO derivative that is detected by NICI-GC-MS.

In the methods shown in this review we show that α -CIFA can be directly analyzed (without derivatization) by LC-MS employing selected reaction monitoring on a tandem mass spectrometry instrument. In general this method is very sensitive and is capable of detecting 1 nM levels of α -CIFA in 25 μ l of plasma [12]. Due to the extremely low levels of α -CIFA in some biological tissues considerable care must be given to avoid contamination, and thus rigorous washing of the column and injection port with methanol are critical, as well as repetitive measures of each biological sample. For plasma samples we have only detected α -CIFA. It is likely that α -CIFALD is readily metabolized to α -CIFA in cells capable of oxidation of this aldehyde and this is the preferred metabolite compared α -CIFOH [11; 22]. It would be interesting to determine if tissue or plasma α -CIFOH levels might rise under pathophysiological conditions during which α -CIFALD oxidation to α -CIFA is impaired (e.g., ischemic conditions).

It should also be noted that similar strategies can be applied for the quantification of other fatty acids, fatty aldehydes and fatty alcohols with derivatizations that enhance NICI detection and the development of specific SRMs that enable sensitive detection by LC-MS. One similarly modified lipid family are the brominated lipids that can be formed by MPO-mediated production of HOBr that target plasmalogens to produce γ -bromofatty aldehyde (γ -BrFALD) by a similar mechanism as that for γ -ClFALD production [26]. Indeed, γ -BrFALD is produced in activated human neutrophils [26]. Furthermore, eosinophil peroxidase preferentially produces HOBr, and activation of eosinophils leads to γ -BrFALD production [27]. γ -BrFALD is quantified by similar strategies with its PFBO derivative quantified following GC utilizing NICI-MS detection [26].

Chlorinated lipids including γ -ClFALD, γ -ClFA, and γ -ClFOH are novel lipids produced as a result of phagocyte activation due to the targeting of the vinyl ether bond of plasmalogens by HOCl [11; 12; 13; 14; 22; 25]. Since they are produced by these activated cells that are involved in inflammation and multiple diseases it is possible that they can be used as tools to show the signature of cell activation resulting in MPO activity. These lipids also are relatively unexplored as mediators of cellular injury and signalling in disease processes involving these phagocytes. This analytical review highlights the analytical tools that are currently used to measure the levels of these lipids in biological samples. These tools can also be used to follow the metabolism of these compounds under conditions of exogenous addition to tissues or cells to examine the biological activities of these compounds.

Acknowledgments

This work was supported in part by National Institutes of Health Grants HL-074214, HL-111906 and RR-019232 to D.A.F.

References

1. Weiss SJ, Klein R, Slivka A, Wei M. Chlorination of taurine by human neutrophils. Evidence for hypochlorous acid generation. *J Clin Invest.* 1982; 70:598–607. [PubMed: 6286728]
2. Klebanoff SJ. Oxygen metabolism and the toxic properties of phagocytes. *Ann Intern Med.* 1980; 93:480–9. [PubMed: 6254418]
3. Ford DA. Lipid oxidation by hypochlorous acid: chlorinated lipids in atherosclerosis and myocardial ischemia. *Clin Lipidol.* 2010; 5:835–852. [PubMed: 21339854]
4. Spickett C. Chlorinated lipids and fatty acids: An emerging role in pathology. *Pharmacol Therapeut.* 2007; 115:400–409.
5. Skaff O, Pattison DI, Davies MJ. The vinyl ether linkages of plasmalogens are favored targets for myeloperoxidase-derived oxidants: A kinetic study. *Biochemistry.* 2008; 47:8237–8245. [PubMed: 18605737]
6. Farooqui AA, Horrocks LA. Plasmalogen-selective phospholipase A2 and its involvement in Alzheimer's disease. *Biochem Soc Trans.* 1998; 26:243–6. [PubMed: 9649755]
7. Gross RW. High plasmalogen and arachidonic acid content of canine myocardial sarcolemma: a fast atom bombardment mass spectroscopic and gas chromatography-mass spectroscopic characterization. *Biochemistry.* 1984; 23:158–65. [PubMed: 6419772]
8. Murphy EJ, Joseph L, Stephens R, Horrocks LA. Phospholipid composition of cultured human endothelial cells. *Lipids.* 1992; 27:150–3. [PubMed: 1315902]
9. Vance JE. Lipoproteins secreted by cultured rat hepatocytes contain the antioxidant 1-alk-1-enyl-2-acylglycerophosphoethanolamine. *Biochim Biophys Acta.* 1990; 1045:128–34. [PubMed: 2116174]
10. Zoeller RA, Grazia TJ, LaCamera P, Park J, Gaposchkin DP, Farber HW. Increasing plasmalogen levels protects human endothelial cells during hypoxia. *Am J Physiol-Heart Circ Physiol.* 2002; 283:H671–9. [PubMed: 12124215]

11. Anbukumar DS, Shornick LP, Albert CJ, Steward MM, Zoeller RA, Neumann WL, Ford DA. Chlorinated lipid species in activated human neutrophils: Lipid metabolites of 2-chlorohexadecanal. *J Lipid Res.* 2010; 51:1085–1092. [PubMed: 20019386]
12. Brahmabhatt VV, Albert CJ, Anbukumar DS, Cunningham BA, Neumann WL, Ford DA. {Omega}-oxidation of {alpha}-chlorinated fatty acids: identification of {alpha}-chlorinated dicarboxylic acids. *J Biol Chem.* 2010; 285:41255–69. [PubMed: 20956542]
13. Thukkani AK, Albert CJ, Wildsmith KR, Messner MC, Martinson BD, Hsu FF, Ford DA. Myeloperoxidase-derived reactive chlorinating species from human monocytes target plasmalogens in low density lipoprotein. *J Biol Chem.* 2003; 278:36365–72. [PubMed: 12869568]
14. Thukkani AK, Hsu FF, Crowley JR, Wysolmerski RB, Albert CJ, Ford DA. Reactive chlorinating species produced during neutrophil activation target tissue plasmalogens: production of the chemoattractant, 2-chlorohexadecanal. *J Biol Chem.* 2002; 277:3842–9. [PubMed: 11724792]
15. Thukkani AK, Martinson BD, Albert CJ, Vogler GA, Ford DA. Neutrophil-mediated accumulation of 2-CIHDA during myocardial infarction: 2- CIHDA-mediated myocardial injury. *Am J Physiol-Heart Circ Physiol.* 2005; 288:H2955–64. [PubMed: 15681699]
16. Thukkani AK, McHowat J, Hsu FF, Brennan ML, Hazen SL, Ford DA. Identification of alpha-chloro fatty aldehydes and unsaturated lysophosphatidylcholine molecular species in human atherosclerotic lesions. *Circulation.* 2003; 108:3128–33. [PubMed: 14638540]
17. Ullen A, Fauler G, Kofeler H, Walzl S, Nussold C, Bernhart E, Reicher H, Leis HJ, Wintersperger A, Malle E, Sattler W. Mouse brain plasmalogens are targets for hypochlorous acid-mediated modification in vitro and in vivo. *Free Radic Biol Med.* 2010; 49:1655–65. [PubMed: 20807565]
18. Marsche G, Heller R, Fauler G, Kovacevic A, Nuskowski A, Graier W, Sattler W, Malle E. 2-chlorohexadecanal derived from hypochlorite- modified high-density lipoprotein-associated plasmalogen is a natural inhibitor of endothelial nitric oxide biosynthesis. *Arterioscler Thromb Vasc Biol.* 2004; 24:2302–2306. [PubMed: 15514213]
19. Ullen A, Fauler G, Bernhart E, Nussold C, Reicher H, Leis HJ, Malle E, Sattler W. Phloretin ameliorates 2-chlorohexadecanal-mediated brain microvascular endothelial cell dysfunction in vitro. *Free Radic Biol Med.* 2012; 53:1770–81. [PubMed: 22982051]
20. Messner MC, Albert CJ, Ford DA. 2-Chlorohexadecanal and 2-chlorohexadecanoic acid induce COX-2 expression in human coronary artery endothelial cells. *Lipids.* 2008; 43:581–8. [PubMed: 18493808]
21. Van Denbergen H, Daloze D, Braekman JC. Synthesis of analogues of 2-iodohexadecanal, a regulator of iodine metabolism in the thyroid gland. *J Braz Chem Soc.* 1999; 10:1–12.
22. Wildsmith KR, Albert CJ, Anbukumar DS, Ford DA. Metabolism of myeloperoxidase-derived 2-chlorohexadecanal. *J Biol Chem.* 2006; 281:16849–16860. [PubMed: 16611638]
23. Bligh EG, Dyer WJ. A rapid method of total lipid extraction and purification. *Can J Biochem Physiol.* 1959; 37:911–7. [PubMed: 13671378]
24. Bollinger JG, Rohan G, Sadilek M, Gelb MH. Liquid chromatography/electrospray mass spectrometric detection of fatty acid by charge reversal derivatization with more than 4-orders of magnitude improvement in sensitivity. *J Lipid Res.* Aug 14, 2013 Epub ahead of print.
25. Albert CJ, Crowley JR, Hsu FF, Thukkani AK, Ford DA. Reactive chlorinating species produced by myeloperoxidase target the vinyl ether bond of plasmalogens: identification of 2-chlorohexadecanal. *J Biol Chem.* 2001; 276:23733–41. [PubMed: 11301330]
26. Albert CJ, Crowley JR, Hsu FF, Thukkani AK, Ford DA. Reactive brominating species produced by myeloperoxidase target the vinyl ether bond of plasmalogens: disparate utilization of sodium halides in the production of alpha-halo fatty aldehydes. *J Biol Chem.* 2002; 277:4694–703. [PubMed: 11836259]
27. Albert CJ, Thukkani AK, Heuertz RM, Slungaard A, Hazen SL, Ford DA. Eosinophil peroxidase-derived reactive brominating species target the vinyl ether bond of plasmalogens generating a novel chemoattractant, alpha-bromo fatty aldehyde. *J Biol Chem.* 2003; 278:8942–50. [PubMed: 12643282]

Figure 1.

Leukocyte activation and the chlorinated lipidome. Abbreviations include α -chlorofatty aldehyde (α -CIFALD), α -chlorofatty acid (α -CIFA), α -chlorofatty alcohol (α -CIFOH), myeloperoxidase (MPO), and polar head groups including choline and ethanolamine (X).

Figure 2.

Analytical strategies for measuring, and structures of, chlorinated lipids produced from HOCl oxidation of plasmalogens. Abbreviations include aliphatic chain (R), -chlorofatty aldehyde (-CIFALD), -chlorofatty acid (-CIFA), -chlorofatty alcohol (-CIFOH), gas chromatography-mass spectrometry (GC-MS), hydroxylamine (HA), liquid chromatography-mass spectrometry (LC-MS), lysophosphatidylcholine (LPC), myeloperoxidase (MPO), negative ion chemical ionization (NICI), pentafluorobenzyl hydroxylamine (PFB-HA), pentafluorobenzoyl chloride (PFB-Cl), pentafluorobenzoyl (PFB), selected ion monitoring (SIM), selected reaction monitoring (SRM) and 2-chlorohexadecanal (2-ClHDA).

Figure 3.

Flowchart for lipid extraction for the members of the chlorinated lipidome. Abbreviations include -chlorofatty aldehyde (-CIFALD), -chlorofatty acid (-CIFA), -chlorofatty alcohol (-CIFOH), and thin layer chromatography (TLC).