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Strategies for the analysis of chlorinated lipids in biological systems

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ABSTRACT

Myeloperoxidase-derived HOCl reacts with the vinyl ether bond of plasmalogens yielding α-chlorofatty aldehydes. These chlorinated aldehydes can be purified using thin-layer chromatography, which is essential for subsequent analysis of extracts from some tissues such as myocardium. The α-chlorofatty aldehyde 2-chlorohexadecanal (2-ClHDA) is quantified after conversion to its pentafluorobenzyl oxime derivative using gas chromatography–mass spectrometry and negative-ion chemical ionization detection. 2-ClHDA accumulates in activated human neutrophils and monocytes, as well as in atherosclerotic lesions and infarcted myocardium. Metabolites of 2-ClHDA have also been identified, including the oxidation product, 2-chlorohexadecanoic acid (2-ClHA), and the reduction product, 2-chlorohexadecanol. 2-ClHA can be quantified using LC–MS with selected reaction monitoring (SRM) detection. 2-ClHA can be ω-oxidized by hepatocytes and subsequently β-oxidized from the ω-end, leading to the production of the dicarboxylic acid, 2-chloroadipic acid. This dicarboxylic acid is excreted in the urine and can also be quantified using LC–MS methods with SRM detection. Quantitative analyses of these novel chlorinated lipids are essential to identify the role of these lipids in leukocyte-mediated injury and disease.

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Introduction

Inflammatory reactions mediated by myeloperoxidase (MPO)-enriched phagocytes including neutrophils, monocytes, and some macrophages contribute to the pathogenesis of atherosclerosis, ischemic/reperfusion injury to numerous tissues, and other disorders. MPO released from activated phagocytes leads to the formation of the reactive chlorinating species HOCl, via the peroxidation of chloride ions [1,2]. The chemical reactivity of HOCl gives it potent antimicrobial and cytotoxic properties, which are important for the immune response, but also has the potential to mediate pathogenesis in many diseases and disorders [3,4]. HOCl is a two-electron oxidant of proteins and lipids. Lipids can potentially be chlorinated by HOCl at amine, alkene, and vinyl ether functional groups.

Plasmalogens are preferentially targeted by HOCl because of the relatively high rate constant for reactions with the vinyl ether bond compared to other reactive targets including alkenes [5]. Plasmalogens are abundant phospholipid components in many cell types, including endothelial cells, macrophages, neutrophils, smooth muscle cells, cardiac myocytes, neurons, and glia [6–8]. Although plasmalogens have been described as having antioxidant capabilities [9,10], their reactivity with HOCl leads to the production of reaction products that have been associated with cardiovascular disease. Plasmalogens react with HOCl leading to the production of α-chlorofatty aldehydes (α-ClFALD) and lysophospholipids (Fig. 1). The α-ClFALD can be further metabolized to α-chlorofatty acid (α-ClFA) and α-chlorofatty alcohol (α-ClFOH). Oxidation of the aldehyde to the α-ClFA metabolite is catalyzed by a fatty aldehyde dehydrogenase [11]. α-ClFA can be further catabolized by ω-oxidation, which is initiated by an ω-hydroxylation step, followed by conversion of the intermediate to an α-chlorodicarboxylic acid. Sequential β-oxidation from the ω-end of the dicarboxylic acids (through multiple dicarboxylic acid intermediates) leads to the eventual production of 2-chloroadipic acid (2-ClAdA). The in vivo metabolism of α-ClFA to 2-ClAdA is efficient, with the final product 2-ClAdA being excreted in the urine [12].

Chlorinated lipids have been identified under several pathophysiological and physiological conditions involving MPO-laden phagocytes. Activated neutrophils and monocytes transiently accumulate α-ClFALD [13,14]. Human aortic atherosclerotic plaques show a nearly 1400-fold increase in levels of the α-ClFALD, 2-chlorohexadecanal (2-ClHDA), compared to normal aortic tissue [15]. Additionally, 2-ClHDA acts as a chemoattractant to neutrophils, suggesting that it may play a role in the recruitment of neutrophils to sites of inflammation [14]. Both unsaturated molecular species of lysophosphatidylcholine (the accompanying product from the degradation of plasmalogens by HOCl) and lysophosphatidylcholine-chlorohydrin, a product of HOCl attack of alkenes present in the *sn*-2 aliphatic chain of unsaturated lysophosphatidylcholine molecular species, induce P-selectin surface expression on human coronary artery endothelial cells [16]. Endothelial cells show inhibition of endothelial nitric oxide synthase (eNOS) expression caused

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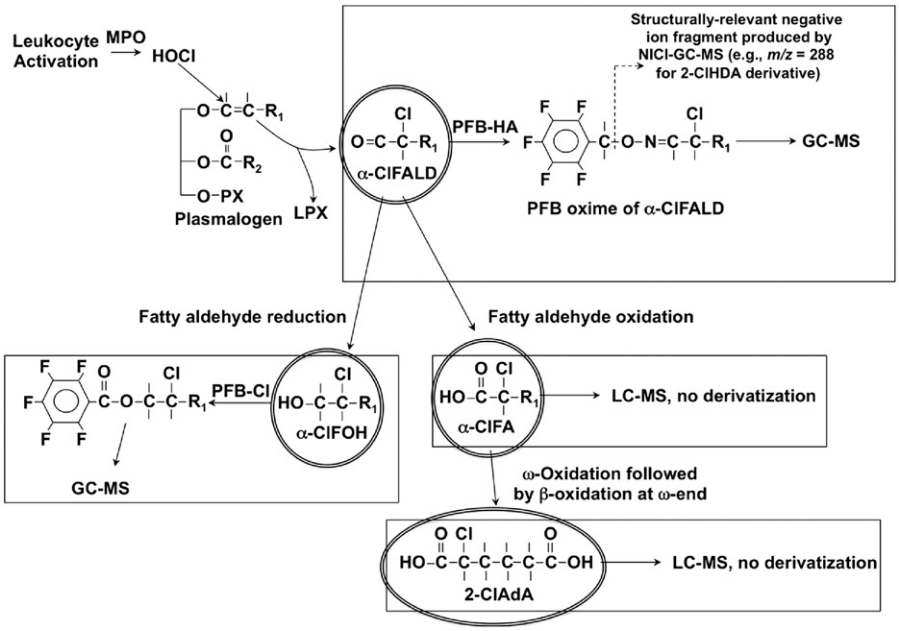

Fig. 1. Structures of the members of the chlorinated lipidome derived from HOCl oxidation of plasmalogens and abbreviated strategies for their quantification. PX, LPX, PFB-HA, and PFB-Cl are the abbreviations for phospho-head group (e.g., phosphorylcholine), lysophospholipids, pentafluorobenzyl hydroxylamine, and pentafluorobenzoyl chloride.

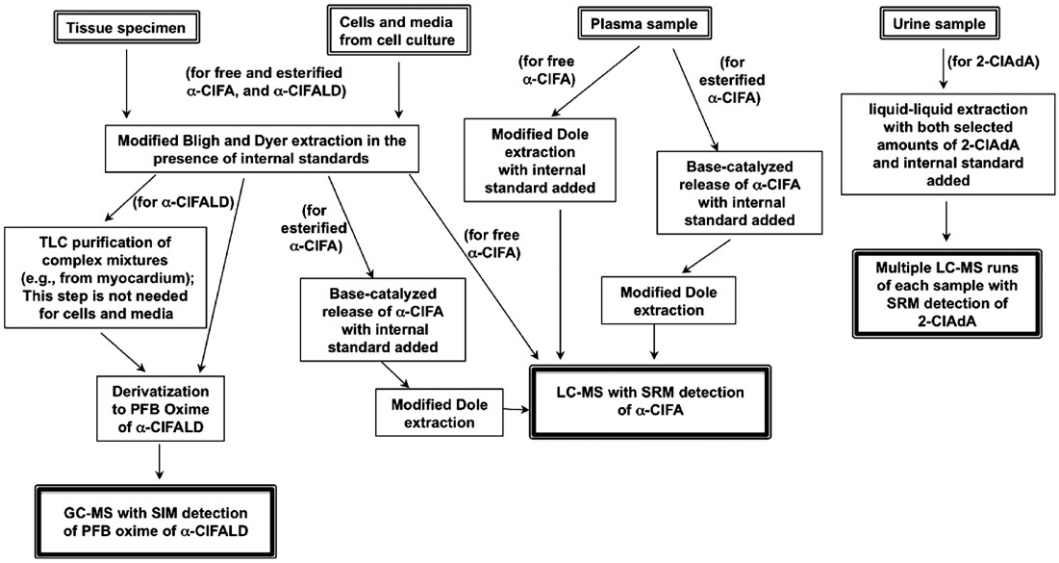

Fig. 2. Flowchart of strategies to quantify members of the chlorinated lipidome.

by 2-ClHDA, as well as reduced eNOS expression localized to the plasma membrane [17]. 2-ClHDA has been shown to increase in infarcted myocardial tissue and can depress contractile function [18]. Furthermore, both 2-ClHDA and 2-chlorohexadecanoic acid (2-ClHA) induce COX-2 expression in human coronary artery endothelial cells [19]. Thus, it is clear that chlorinated lipids accumulate in an array of cardiovascular pathophysiological conditions, and the accurate measurement of these novel lipids is essential and may lead to further insights into the role of these compounds either as putative biomarkers or mediators of proinflammatory mechanisms.

Several mass spectrometry-based methods have been developed to detect and accurately quantify these chlorinated lipids (see Fig. 2 for flowchart of these quantification strategies and Fig. 1 for the structures that are quantified). The choices of (1) whether to derivatize the chlorinated lipids, (2) the type of chromatography, and (3) the mass spectrometry (MS) scan mode are dependent on the functional groups of the analytes that dictate their chromatographic characteristics and ionization by mass spectrometry. Accordingly, in this review of the methodological strategies employed to measure chlorinated lipids, the quantification of (1) α-chlorofatty aldehydes as pentafluorobenzyl (PFB) oximes using gas chromatography (GC)–MS with negative ion chemical ionization (NICI), (2) α-ClFA by reversed-phase liquid chromatography (LC) with electrospray ionization (ESI)–MS and selected reaction monitoring (SRM) for detection, and (3) 2-ClAdA by reversed-phase LC with ESI–MS and SRM for detection will be detailed. For this review, we focus on the accurate measurement of three chlorinated lipids: the α-ClFALD 2-ClHDA, the α-ClFA 2-ClHA, and the α-Cl-dicarboxylic acid 2-ClAdA. These are the predominant chlorinated lipids that have been detected in vivo. Methods to quantify the less predominant

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