Identification of novel neutrophil very long chain plasmalogen molecular species and their myeloperoxidase mediated oxidation products in human sepsis

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ABSTRACT

Plasmalogens are a class of phospholipids containing vinyl ether linked aliphatic groups at the sn-1 position. Plasmalogens are known to contain 16- and 18-carbon aliphatic groups at the sn-1 position. Here, we reveal that the human neutrophil plasmenylethanolamine pool uniquely includes molecular species with very long carbon chain (VLC) aliphatic groups, including 20-, 22- and 24-carbon vinyl ether linked aliphatic groups at the sn-1 position. We identified these novel VLC plasmalogen species by electrospray ionization mass spectrometry methods. VLC plasmalogens were only found in the neutrophil plasmenylethanolamine pool. During neutrophil activation, VLC plasmalogen species undergo myeloperoxidase-dependent oxidation to produce VLC 2-chlorofatty aldehydes and their oxidation products, 2-chlorofatty acid (2-CIFA). Furthermore, plasma concentrations of VLC 2-CIFA are elevated in human sepsis. These studies demonstrate for the first time VLC plasmenylethanolamine molecular species, their myeloperoxidase-mediated chlorolipid products and the presence of these chlorolipids in human sepsis.

1. Introduction

Plasmalogens are a subclass of glycerophospholipids characterized by a vinyl ether bond linking the aliphatic group at the sn-1 position. These lipids are present in cell membranes and lipid rafts of many cell types including neutrophils, monocytes, cardiac cells, endothelial cells, and smooth muscle cells [1–5]. The plasmalogens, plasmenylethanolamine and plasmenylcholine, are predominantly present in ethanolamine (PE) and choline (PC) glycerophospholipid pools, respectively.

The sn-1 position of plasmalogens have been characterized to contain C16:0 (C indicates carbon and XX:Y indicates the # of carbons: # of double bonds), C18:0, and C18:1 aliphatic groups. Additionally, the LIPIDMAPS consortium include C20:0 plasmalogens in their mass spectral library and they have been shown in human red blood cells [6].

During neutrophil activation, myeloperoxidase (MPO)-derived HOCl targets the sn-1 vinyl ether bond of plasmalogens resulting in 2-chlorofatty aldehyde (2-CIFALD) release [7]. 2-CIFALDs are readily oxidized to 2-chlorofatty acids (2-CIFA), which are stable metabolites. The most

Abbreviations: 2-CIFA, 2-chlorofatty acid; 2-CIFALD, 2-chlorofatty aldehyde; ESI MS/MS, electrospray ionization tandem mass spectrometry; FA, fatty acid; FALD, fatty aldehyde; GC/MS, gas chromatography mass spectrometry; HCl, hydrochloric acid; HOCI, hypochlorous acid; LPE, lysophosphatidylethanolamine; MPO, myeloperoxidase; PC, choline glycerophospholipid; PE, ethanolamine glycerophospholipid; PMA, phorbol 12-myristate 13-acetate; TLC, thin layer chromatography; VLC, very long chain.

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common 2-CIFALDs and 2-CIFAs identified are C16:0 and C18:0 species and these chlorinated lipids have been associated with inflammatory conditions such as endotoxemia, atherosclerosis, and myocardial infarction [8–10]. Moreover, increased plasma 2-CIFA levels are associated with acute respiratory distress syndrome and 30-day mortality in sepsis patients [11]. Chlorinated lipids elicit diverse cellular effects including the induction of neutrophil chemotaxis and neutrophil extracellular trap formation [12,13], endothelial dysfunction [14,15], and activation of monocyte apoptosis [16]. Additionally, these lipids have antibacterial properties against E.coli [17]. Since chlorolipids and their precursor plasmalogens are important in the milieu of oxidative stress and sepsis, the present study was designed to determine the possibility of novel molecular species of chlorolipids and their precursor plasmalogens.

2. Materials and Methods

2.1. Neutrophil and monocyte preparations

Human neutrophils were isolated from healthy donors as previously described [17] and approved by St. Louis University IRB protocol 9952. Human monocytes, purchased through Gulf Coast Regional Blood Center, were isolated as previously described [18].

2.2. Human plasma specimens and analysis

Sepsis plasma samples were obtained from subjects admitted to the intensive care unit (ICU) with suspected infection and acute organ dysfunction (sepsis) on the day of ICU admission (day 0) and days 2 and 7 if subjects remained in the ICU. The cohort has been previously described [19]. The cohort study is approved by the University of Pennsylvania institutional review board (IRB protocol #808542) and all subjects or their proxies provided informed consent to participate. Control healthy plasma samples were obtained at Saint Louis University under IRB protocol 26646. Plasma concentrations were compared between healthy control subjects and sepsis subjects by Wilcoxon rank sum test. For visualization, we display each VLC species by quartile of 16:0 2-CIFA.

2.3. Neutrophil lipids were extracted by modified Bligh Dyer extraction using lipid class internal standards as previously described [20–22]. Lipid-specific mass spectrometry scan parameters are included in Table S1. 2-Chloro-[d4]-hexadecan-2-yl hexadecanoic acid were used as internal standards for 2-CIFALD and 2-CIFA measurements, respectively [13,23]. 2-CIFALD, dried lipid extracts were incubated with Amplifex Keto reagent (SCIEX; catalog no. 4465962) as described with the reagent and quantified by LC-MS. 2-CIFA species in the lipid extracts and plasma were measured as previously described [11,24].

LC-MS were performed using parallel reaction monitoring with a Q Exactive mass spectrometer equipped with a Vanquish UHPLC System (Thermo Scientific). Lipids were separated on an Acquity ultrabead C18 column 2.1 × 150 mm (Thermo Scientific) with mobile phase A comprised of 60% acetonitrile, 40% water, 10 mM ammonium formate, 0.1% formic acid and mobile phase B comprised of 90% isopropanol, 10% acetonitrile with 2 mM ammonium formate, and 0.02% formic acid. Initial conditions were 30% B with a discontinuous gradient to 100% B at a flow rate of 0.260 ml/min. Shotgun lipidomics were performed on both the total lipid extract to quantitate sphingomyelin, ceramide, and PC and fluoronylmethoxy carbonyl-Cl derivatized PE species, as described previously [25]. Samples were analyzed using ESI/MS/MS (TSQ Quantum Ultra, Thermo Scientific). FA from human neutrophil lipid extracts were determined as 2,3,4,5,6-pentafluorobenzoyl ester derivatives as previously described [24,26]. Fatty alcohol concentrations from neutrophil lipid extracts were determined via derivatization with 2,3,4,5,6-pentafluorobenzoyl chloride, as previously described [27].

3. Results and discussion

3.1. Identification of VLC plasmalogens

We investigated the presence of VLC plasmalogens in neutrophil lipid extracts using high resolution MS and shotgun lipidomics. Novel VLC molecular species of plasmenylethanolamine were detected using both lipid mass spectrometry methods (Fig. 1). High-resolution MS chromatograms for PE P-24:1/18:1 (P indicates plasmalogen and XX/Y indicate sn-1/sn-2 constituents), PE P-24:1/18:2, PE P-22:1/18:1, and PE P-22:1/18:2 species (Fig. 1A) and their representative fragments (Fig. 1C) confirm the presence of these VLC plasmenylethanolamine molecular species. The fragment ion for glycerol phosphoethanolamine (m/z 196) and the ethanolamine phosphate ion (m/z 140) confirm they are PE species, while loss of the sn-2 acyl chain ketene from [M – H] (m/z 518 for 22:1 species and m/z 546 for 24:1 species), neutral loss of the sn-2 fatty acid group from [M – H] (m/z 500 for 22:1 species and m/z 528 for 24:1 species), and the fatty acid ion confirm the respective sn-1 and sn-2 composition. Plasmenalogens are acid-labile [28,29]. Chromatographic peaks from the novel molecular species of plasmenylethanolamines were not observed following HCl vapor exposure (Fig. 1B). Diacyl PE (18:0/20:4) was not altered by acid treatment. Additionally, thin layer chromatography (TLC)-purified neutrophil PE lipids and whole lipid extracts of neutrophils were acid treated and plasmalogen-derived FALD species were detected by mass spectrometry following Amplifex derivatization (Fig. 1D). TLC-purified plasmenylethanolamines consist of 6.8% and 6.6% 22:1 and 24:1 FALD, respectively. Interestingly, the percentages of FALD species liberated from TLC-purified plasmenylethanolamine and neutrophil whole lipid extracts were nearly identical, suggesting that the majority of VLC plasmalogens exist in the plasmenylethanolamine pool. Shotgun lipidomics was also employed as an alternative strategy to identify these novel plasmenylethanolamine molecular species. The spectra with respective m/z for fluoronylmethoxy carbonyl-derivatized plasmenylethanolamines before and after acid treatment are shown in Fig. 1E. Acid treatment led to the selective disappearance of plasmenylethanolamine molecular species. Plasmenylethanolamine molecular species in neutrophil lipid extracts were quantified using liquid chromatography with Q-Exactive MS/MS detection (Table 1). Similar quantitative results for these plasmenylethanolamine molecular species were determined using shotgun lipidomics (Table S2).

Previous studies have shown that 66% of human neutrophil phospholipids consist of plasmenylethanolamines while only 9% consist of plasmanyle cholines [30]. We did not detect VLC plasmenylcholine molecular species in human neutrophils. Our limit of detection for plasmenylethanolamine is ~1 ng for each molecular species containing a 24:1 VLC as the vinyl ether group (Fig. S1). Under these conditions we did not detect VLC plasmenylethanolamine molecular species in any other tissues and cells tested including monocytes, endothelial cells, epithelial cells, and heart tissue. This finding may indicate distinct functions of VLC plasmenylethanolamines in neutrophils. It has long been known that plasmanogens have structural roles in membrane integrity [31–33]; therefore, it is possible that VLC plasmenylethanolamines may provide additional membrane stabilizing or destabilizing properties during phagocytosis that require further investigation. Lodhi et al. demonstrated that the inhibition of either lipid synthesis resulted in neutrophil apoptosis and endoplasmic reticulum stress [34]. Moreover, blocking ether lipid synthesis in pexiromes led to neutropenia in mice [34]. Thus, the presence of these VLC plasmenylethanolamines may also be associated with the viability of the neutrophils.

We also quantified ceramides, sphingomyelin, fatty acid, and fatty alcohols to determine the abundance of VLC aliphatic groups in the
neutrophils and monocyte lipidomes (Table S3). 22:1 and 24:1 containing FA were found in low abundance compared to 16:0 and 18:0 FA. Neutrophil PE and PC diacyl molecular species containing 22:1 and 24:1 aliphatic groups were found at lower concentrations compared to levels of VLC plasmenylethanolamines (Table S3 and Table 1). 24:1 containing sphingomyelin and ceramide molecular species were detected in both monocytes and neutrophils (Table S3).

3.2. VLC chlorolipid production in activated neutrophils

We have previously shown that the vinyl ether bonds of plasmalogens are targeted by MPO-derived HOCl to produce chlorolipids [7]. Neutrophils stimulated with PMA produce 16:0 and 18:0 2-ClFALD and 2-ClFA [8, 13]. We hypothesized that the novel VLC plasmenylethanolamine molecular species may also be oxidized to produce VLC 2-ClFALD and 2-ClFA during neutrophil activation. Indeed, PMA-activated neutrophils showed significant increases in 22:1 2-ClFALD, 24:1 2-ClFALD, 22:1 2-ClFA and 24:1 2-ClFA (Fig. 2A and B). Additionally, both 2-ClFALD and 2-ClFA production were reduced by MPO inhibition by 3-amino-1,2,4-triazole. This result demonstrates that novel VLC chlorolipid production is MPO dependent. We examined plasmenylethanolamine molecular species levels following PMA activation of neutrophils. Concomitant with the appearance of VLC chlorolipids during neutrophil activation were increases in LPE molecular species containing predominantly C18:1, C18:2 and C20:4 fatty acids suggesting they are derived from VLC plasmenylethanolamine molecular species. Lysoplasmenylethanolamine molecular species were not detectable.

| Molecular Species                         | ng/10⁶ neutrophils |
|-----------------------------------------|-------------------|
| PE(P-16:0/18:1)                          | 291.59 ± 10.24    |
| PE(P-16:0/18:2)                          | 97.84 ± 4.34      |
| PE(P-16:0/20:4)                          | 240.29 ± 9.64     |
| PE(P-18:0/18:1)                          | 269.27 ± 9.59     |
| PE(P-18:0/18:2)                          | 316.24 ± 13.53    |
| PE(P-18:0/20:4)                          | 596.81 ± 24.37    |
| PE(P-18:1/18:1)                          | 315.06 ± 11.41    |
| PE(P-18:1/18:2)                          | 84.45 ± 6.54      |
| PE(P-18:1/20:4)                          | 128.58 ± 6.78     |
| PE(P-20:0/18:1)                          | 37.39 ± 3.74      |
| PE(P-20:0/18:2)                          | 42.47 ± 3.49      |
| PE(P-20:0/20:4)                          | 78.14 ± 3.51      |
| PE(P-22:0/18:1)                          | 8.63 ± 1.83       |
| PE(P-22:0/18:2)                          | ND                |
| PE(P-22:1/18:1)                          | 38.02 ± 1.43      |
| PE(P-22:1/18:2)                          | 57.41 ± 1.43      |
| PE(P-22:1/20:4)                          | 64.88 ± 3.35      |
| PE(P-24:1/18:1)                          | 42.78 ± 4.92      |
| PE(P-24:1/18:2)                          | 64.73 ± 3.54      |
| PE(P-24:1/20:4)                          | 72.24 ± 4.61      |
3.3. Elevation of plasma VLC 2-ClFA in human sepsis

Human sepsis patients have previously been shown to have elevated plasma 16:0 and 18:0 2-ClFA concentrations compared to healthy, non-septic controls. Moreover, 2-ClFA levels were associated with acute respiratory distress syndrome of sepsis patients [11]. Accordingly, we examined the novel VLC 2-ClFA species in human sepsis plasma. Plasma concentrations of 24:1 2-ClFA, 20:1 2-ClFA, 20:0 2-ClFA and 22:1 2-ClFA were significantly elevated in sepsis patient plasma compared to healthy controls (Fig. 3). For visualization, we display the sepsis subjects by quartile of measured plasma 16:0 2-ClFA to emphasize the heterogeneity among the sepsis population. Between 21 and 26% of the sepsis population had detectable VLC species. Statistical comparisons are between the healthy controls and all four quartiles as an aggregate by...
Wilcoxon rank sum test. 2-CIFALD and 2-CIFA have been shown to induce multiple effects on cells such as endothelial dysfunction, monocyte apoptosis, neutrophil chemotaxis, and NETosis [8,14,16]. Most of these effects are deleterious to host cells. However, they may also serve as antibacterial compounds, which is beneficial during infection [17]. We speculate that VLC 2-CIFALD and 2-CIFA species may also cause cellular effects that need further investigation. Furthermore, plasma VLC 2-CIFA levels in sepsis may associate with specific outcomes during sepsis compared to long chain 2-CIFA molecular species, which may be attributed to their precursors being specifically localized to the neutrophils.

4. Conclusions

We have discovered VLC plasmenylethanolamine molecular species with C22:1 and C24:1 at the sn-1 position. They are uniquely found in neutrophils. These VLC plasmenylethanolamine molecular species under MPO-dependent oxidation to produce VLC chlorolipids, which are significantly elevated in human sepsis patients. These observations warrant further study of VLC plasmenylethanolamine biosynthesis, their specific roles in neutrophils and the clinical importance of VLC chlorolipids in inflammation and sepsis.

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Appendix A. Supplementary data

Supplementary data related to this article can be found at https://doi.org/10.1016/j.redox.2021.102208.

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