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Dysfunctional plasmalogen dynamics in the plasma and platelets of patients with schizophrenia

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ABSTRACT

Background: Based upon the concept of a prodromal risk syndrome for first psychosis, intense efforts are being applied to define potential biomarkers of disease onset in schizophrenia. One such tentative biomarker may be plasmalogens, complex structural glycerophospholipids that serve multiple functions in all cells.

Methods: To further investigate this possibility, we undertook a lipidomics analysis of choline and ethanolamine plasmalogens in the plasma and platelets of 23 patients with schizophrenia and 27 age-matched controls.

Results: Plasma levels of both choline and ethanolamine plasmalogens were decreased by 23 to 45% in patients with schizophrenia. In platelets, from patients with schizophrenia, ethanolamine plasmalogens also were decreased while choline plasmalogen levels were increased. Levels of docosahexaenoic acid (DHA) were decreased by approximately 30% both in plasma and platelets.

Conclusions: Our results suggest that alterations in lipid transport or lipid remodeling/metabolism of plasmalogens are present in schizophrenia and that changes in the steady-state levels of these complex lipid pools may be involved in altered neuronal function.

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1. Introduction

Schizophrenia is a chronic debilitating mental illness that affects approximately 1% of the world's population (an der Heiden and Häfner, 2000; Saha et al., 2007). Integration of current clinical research data has led to the concept that schizophrenia may be a neurodevelopmental disorder that results in abnormal neuronal circuits supporting sensory processing, cognition, and emotion (Lewis and Sweet, 2009; Insel, 2009). Discovery of the underlying biochemical processes responsible for this dysfunction in the development of brain neuronal circuits has remained elusive despite intensive research efforts (Oertel-Knöchel et al., 2011). Furthermore, since early intervention has already been demonstrated to lead to increased clinical success in the treatment of young patients with schizophrenia (McFarlane et al., 2014), it is important to define biomarkers of the prodromal phase of the disease (Woods et al., 2009).

In this regard, decreased circulating plasmalogens have been demonstrated in first onset and recurrent schizophrenia patients (Kaddurah-Daouk et al., 2012). Plasmalogens are complex structural glycerophospholipids that possess a vinyl-ether linked fatty alcohol at the sn-1 position of the glycerol backbone and either phosphoethanolamine or phosphocholine at sn-3 (Wood, 2012). Plasmalogens also serve as

reservoirs for fatty acid mediators such as arachidonic acid and docosahexaenoic acid (DHA) which are released from the sn-2 position of the glycerol backbone via phospholipases in a dynamic deacylation–reacylation cycle termed “lipid remodeling” (Wood, 2012). As major components of membranes, plasmalogens are essential for membrane fluidity, lipid raft formation, membrane fusion for neurotransmitter release, ion transport, and regulation of cholesterol efflux (Wood, 2012). Plasmalogens are also essential in brain development, both in white and gray matter (Niemoller and Bazan, 2010; Aubourg and Wanders, 2013).

In this study, we undertook a validation of the findings of reduced circulating plasmalogens by Kaddurah-Daouk et al. (2012). Since circulating plasmalogens are derived mainly from the liver and gastrointestinal epithelium (Wood, 2012), we also evaluated cellular plasmalogens in platelets, which possess the cellular machinery required for plasmalogen synthesis.

2. Materials and methods

2.1. Study participants

Caucasian schizophrenia patients were recruited from the clinical practice at Southwestern Behavioral Healthcare Inc. All patients were maintained on second generation antipsychotics and were capable of understanding and providing informed consent for the study. Healthy volunteers were recruited from the staff of SWBH. The clinical study was approved by the Lincoln Memorial University Institutional Review

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Table 1
Demographics of study participants.

Parameter	Controls	Schizophrenia patients
Average age (range)	47 (25–65)	47 (25–66)
Age of onset	–	24 (16–30)
Females (N)	18	5
Males (N)	9	18
Statin use (N)	5	7
BMI (mean \pm SD)	31.3 \pm 7.4	31.3 \pm 8.2

Board. The demographics of the study participants are presented in Table 1.

2.2. Sample collection

After an overnight fast, blood was collected in EDTA Vacutainers and placed on ice. Plasma was isolated by centrifugation at 3000 \times g for 15 min. Platelets were obtained by first centrifuging 8 mL of EDTA blood at 400 \times g for 15 min. The platelet-rich plasma was next centrifuged at 18,000 \times g for 15 min to obtain the platelet pellet which was washed once with 8 mL of cold PBS. Samples were stored at -80°C prior to analyses.

2.3. Lipidomics analyses

Platelet pellets or 100 μL plasma samples were extracted utilizing tert-butylmethyl ether and methanol for extraction of lipids (Schuhmann et al., 2012; Wood and Shirley, 2013). The extraction solution contained [$^2\text{H}_8$] arachidonic acid, [$^{13}\text{C}_{16}$] palmitic acid, [$^2\text{H}_7$] cholesterol sulfate, [$^2\text{H}_{31}$] PtdEtn 34:1, [$^2\text{H}_{54}$] PtdEtn 28:0, [$^2\text{H}_{31}$] PtdCh 34:1, [$^2\text{H}_{54}$] PtdCh 28:0, [$^2\text{H}_{62}$] PtdCh 32:0, [$^2\text{H}_{31}$] SM 16:0, [$^2\text{H}_{31}$] PtdSer 36:1, bromocriptine, and glyburide as internal standards. Stable isotope internal standards were obtained from Avanti polar lipids, Cambridge Isotope Laboratories, and Cayman Chemical. Extracts were dried by centrifugal vacuum evaporation prior to dissolution in isopropanol:methanol:chloroform (4:2:1) containing 7.5 mM ammonium acetate. Shotgun lipidomics were performed utilizing high-resolution (140,000 at 200 amu) data acquisition, with sub-milligram accuracy on an Orbitrap mass spectrometer (Thermo QExactive, Thermo Scientific), with successive switching between polarity modes (Schuhmann et al., 2012; Wood and Shirley, 2013). Samples were infused for 2 min at 5 $\mu\text{L}/\text{min}$ followed by successive 500 μL washes of the infusion line with methanol and hexane/ethyl acetate (3:2) to minimize ghost effects. In negative ion ESI (3.2 kV, capillary temp. of 320 $^{\circ}\text{C}$, sheath gas of 10), the anions of ethanolamine plasmalogens (alkenyl-acyl), ethanolamine lysoplasmalogens, phosphatidylethanolamines (diacyl), and docosahexaenoic acid (DHA; 22:6) were monitored and lipid identities validated by MS². In positive ion ESI (4.3 kV, capillary temp. of 320 $^{\circ}\text{C}$, sheath gas of 10), the cations of choline plasmalogens (alkenyl-acyl), choline lysoplasmalogens, and phosphatidylcholines (diacyl) were monitored and lipid identities validated by MS/MS. The anion of bromocriptine and cations of both bromocriptine and glyburide were used to monitor for potential mass axis drift. Platelet proteins were determined utilizing the Pierce BCA Protein Assay kit (Thermo Fisher).

2.4. Data analyses

Data are presented as R values (ratio of the endogenous lipid to the peak area of an appropriate internal standard), in bar graphs \pm SEM. In the case of platelets, these values were corrected for protein content. Data comparisons of control and schizophrenia groups utilized the Student's t-test. Gender differences were evaluated by a two-way ANOVA and age differences analyzed via regression analysis.

3. Results

3.1. Assay validation

The MS² analyses of plasmalogens extracted from plasma are presented in Fig. 1. The major fragment for choline plasmalogens was phosphocholine (184.0732) as presented in the upper plot of Fig. 1. In the case of ethanolamine plasmalogens, the main fragment comes from the fatty acid substitution at sn-2 of the glycerol backbone. In the case of PlsE 40:6 this was DHA (22:6) thereby supporting 16:0 as the substituent at sn-1 (lower plot of Fig. 1). Since these analyses are direct infusion, they cannot rule out isobars that are 1-alkyl substituents with an unsaturated side-chain rather than an alkenyl substituent. However, 1-alkyl-2-acyl glycerophospholipids are small lipid pools (Ford and Gross, 1989) while plasmalogens are at high concentrations in all membranes and are micromolar in plasma lipoproteins (Wiesner et al., 2009).

3.2. Choline plasmalogens

Choline plasmalogens were decreased by 10 to 30% in the plasma of patients with schizophrenia (Fig. 2). This included plasmalogens containing fatty acids with low levels of unsaturation as well as polyunsaturated fatty acids. In contrast cellular levels of choline plasmalogens in platelets were increased in patients with schizophrenia (Fig. 2). No gender or age differences were observed within each group in the levels of choline plasmalogens. Similarly, no differences in phosphatidylcholines (diacyl) between controls and patients with schizophrenia were observed.

3.3. Ethanolamine plasmalogens

Ethanolamine plasmalogens were decreased by 20 to 40% in the plasma and platelets of patients with schizophrenia (Fig. 3). No gender or age differences were observed within each group in the levels of ethanolamine plasmalogens. No differences in phosphatidylethanolamines (diacyl) between controls and patients with schizophrenia were observed.

3.4. Lysoplasmalogens and DHA

In plasma, the predominant choline lysoplasmalogen was LPCp 16:0 while in platelets LPCp 18:0 was the predominant lipid species. Neither of these choline lysoplasmalogens were altered in plasma (data not shown) or platelets (Fig. 4). The predominant ethanolamine lysoplasmalogens in platelets were LPEp 16:0 and LPEp 18:1, neither of which were altered in patients with schizophrenia (Fig. 4). DHA was decreased in plasma (68% of control) and platelets (72% of control) in patients with schizophrenia (Fig. 4).

4. Discussion

Biomarker research in schizophrenia is complicated by the complex polygenetics and heterogeneous phenotype of this patient population, with regard to disease onset and progression (Gottesman and Erlenmeyer-Kimling, 2001). In addition, lipidomics analyses are complicated by the high incidence of metabolic syndrome in schizophrenia and its induction/worsening by treatment with antipsychotics (Malhotra et al., 2013). However, in the case of plasmalogen analyses, approximately 20% decreases in circulating plasmalogens in 20 first episode and 20 recurrent schizophrenic patients have been reported (Kaddurah-Daouk et al., 2012) suggesting that this may represent an intrinsic biochemical deficit involved in this developmental disorder. This initial report involved the traditional analyses of 16:0 and 18:0 dimethylacetals, a procedure that does not allow for the quantitation of individual plasmalogens. In our analyses we analyzed over 36 individual choline plasmalogens and

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