Psychiatry Research 198 (2012) 347–352

Contents lists available at SciVerse ScienceDirect

Psychiatry Research

journal homepage: www.elsevier.com/locate/psychres

Impaired plasmalogens in patients with schizophrenia

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ARTICLE INFO

Article history:
Received 22 March 2011
Received in revised form 16 February 2012
Accepted 20 February 2012

Keywords:
Schizophrenia
Drug-naïve
Metabolomics
Lipidomics
Plasmalogen
Phospholipids

ABSTRACT

Plasmalogens are a subclass of glycerophospholipids and ubiquitous constituents of cellular membranes and serum lipoproteins. Several neurological disorders show decreased level of plasmogens. An earlier study found differences in plasma phospholipids between unmedicated patients with schizophrenia and matched healthy control subjects. We here report a comparison of plasma plasmalogen levels across 20 drug-naïve patients experiencing first psychotic episodes, 20 recently unmedicated patients experiencing psychotic relapses after failing to comply with prescribed medications, and 17 matched healthy control subjects.
Multiple plasma phosphatidylcholine and phosphatidylethanolamine plasmalogen levels were significantly lower in first episode patients and patients with recurrent disease compared to healthy controls. Reduced plasmalogen levels appear to be a trait evident at the onset of psychotic illness and after multiple psychotic relapses. It is implied that reductions in plasmalogen levels are not related to antipsychotic treatment but due to the illness itself. Reduced plasmalogen levels suggest impairments in membrane structure and function in patients with schizophrenia that might happen early in development. This may serve as a clue to the neurobiology of schizophrenia and should be studied as a potential biomarker for individuals at risk for schizophrenia.

1. Introduction

Schizophrenia is arguably one of the most serious psychiatric brain disorders with a worldwide prevalence of about 1%. It has a large socioeconomic impact due to its early age of onset, the enduring disability it produces, and the costs of the long-term care required for afflicted patients. Although there has been substantial progress in understanding the neurobiology and neuropharmacology of the psychotic symptoms of schizophrenia over the past half century, many critically important questions on the pathogenesis of schizophrenia remain wholly or partially unanswered (Ho et al., 2003). Theories of the pathophysiology underlying schizophrenia have centered on neurotransmitters and their receptors but recent evidence has emerged proposing that schizophrenia may be viewed as a neurodevelopmental disorder, suggesting that a focus on early detection and intervention could yield substantial improvements in outcomes (Insel, 2010). Over the last two decades evidence has accrued that phospholipids, which play a critical role in the structure and function of membranes, seem to be altered (Pettegrew et al., 1993; Horrobin, 1998; Mahadik and Yao, 2006; Kaddurah-Daouk et al., 2007). Neuronal cell membranes form the vesicles in which neurotransmitters are stored and released as well as the matrix in which receptors, channels and other proteins must be properly configured; hence membrane lipid changes could have a direct effect on proper neurotransmission.

Plasmalogens are a phospholipid subclass which contains vinyl-ether double bonds in the sn-1 position of the phospholipid. These lipids are ubiquitous throughout tissue membranes and serum lipoproteins, but are found in relatively high levels in the brain, lung, heart, muscle, and red blood cells. Plasmalogens contribute to the structure of lipid bilayer membranes (with phosphatidylcholine (PC), phosphatidylethanolamine (PE) and other phospholipids), maintain fluidity and oxidation in lipid

Abbreviations: PC, phosphatidylcholine; PE, phosphatidylethanolamine.

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doi:10.1016/j.psychres.2012.02.019

rafts (Pike et al., 2002), function as reservoirs for lipid second messengers (Mazza et al., 2007), facilitate membrane fusion (Lohner, 1996) and ion transport (Gross, 1985; Chen and Gross, 1994; Zeng et al., 1998) and are involved in the regulation of cholesterol efflux (Munn et al., 2003). Plasmalogens are required for intracellular cholesterol transport and may decrease cholesterol oxidation in low density lipoprotein (LDL). Decreases in these essential structural phospholipids and the related increases in membrane cholesterol may result in loss of membrane fluidity (Hashimoto et al., 1999) and alterations in ion channel and/or receptor function (Hashimoto et al., 2005).

Levels of plasmalogens have been shown to be decreased in Alzheimer's disease (Farooqui and Horrocks, 2001; Pettegrew et al., 2001; Han, 2005; Goodenowe et al., 2007; Hartmann et al., 2007; Mazza et al., 2007; Lessig and Fuchs, 2009), but not altered in other neurodegenerative diseases such as Huntington's or Parkinson's disease (Ginsberg et al., 1995; Cheng et al., 2003). A recent study also showed that plasmalogens are regulated by the amyloid precursor protein under physiological conditions (Grimm et al., 2011). Plasmalogens have also been found to be decreased in patients suffering from ischemic cerebrovascular disease (Hoerrmann et al., 1991) and in patients with atherosclerosis (McHowat and Creer, 2001; Stenvinkel et al., 2004; Brosche et al., 2007; Lessig and Fuchs, 2009). Finally, as we reported in our previous pilot study, plasmalogen levels were significantly reduced in unmedicated patients with schizophrenia compared to healthy controls (Kaddurah-Daouk et al., 2007).

Metabolomics is the study of metabolism at the global level. It involves systematic study of the metabolome, the complete repertoire of small molecules present in cells, tissues or organisms. Sophisticated metabolomic analytical platforms and informatics tools enable mapping of pathways implicated in disease and in response to therapy (Kristal et al., 2007; Kaddurah-Daouk et al., 2008; Kaddurah-Daouk and Krishnan, 2009; Ji et al., 2011; Kaddurah-Daouk et al., 2011).

The aim of the present study was to use a targeted metabolomic platform to replicate and extend our previous findings by comparing early biochemical changes in drug free first episode schizophrenia patients to chronic patients with recurrent psychotic relapses and to healthy controls. Based on the results of our 2007 study, we hypothesized that the decreased concentrations of plasmalogens in patients with schizophrenia was a marker of the disease and not significantly affected by recurrence of psychotic episodes; therefore both groups of patients with schizophrenia (first episode and recurrent) may show equally reduced levels of plasmalogens compared to healthy controls.

2. Experimental design/materials and methods

2.1. Patient population

Medication-naïve patients presenting for treatment at Duke University Medical Center for a first psychotic episode ($n=20$), and patients presenting for treatment of a psychotic relapse of schizophrenia or schizoaffective disorder related to their non-adherence with prescribed outpatient treatment ($n=20$) consented to participate in the study; no patient's medication was discontinued as a condition for participation in the study. All available information from patients, family members, and treating clinicians indicated that each recurrent patient had not received antipsychotic medication for at least the preceding 2 weeks and in most cases much longer. All patients had at least two positive symptom items from the Brief Psychiatric Rating Scale (BPRS) rated ≥4 at baseline (Rhoades and Overall, 1988).

Control subjects: Controls with similar age and Body mass index (BMI) to the patients, who had no personal history of psychotic illness or first or second degree relatives with psychotic illnesses, were recruited from hospital staff and their family members and friends ($n=17$). Individuals receiving treatment for diabetes mellitus or hyper-lipidemia were excluded.

2.2. Assessments

The Structured Clinical Interview for Diagnostic and Statistical Manual of Mental Disorders IV (DSM-IV) (SCID) was administered to all subjects and controls; age, gender, and race were all recorded. The number of prior hospitalizations and years since first hospitalization were recorded for all subjects. All medications the subjects or controls had been taking over the preceding 14 days were recorded. Weight, BMI and waist circumference was measured. Psychopathology was assessed at baseline by means of the Brief Psychiatric Rating Scale (BPRS), administered by a rater trained for inter-rater reliability through the Clinical Antipsychotic Trials in Intervention Effectiveness (CATIE) trials and numerous industry FDA trials (Lieberman et al., 2005). Adverse events/side effects (AE/SE) were recorded on the AE/SE form utilized in the CATIE trial that includes 18 of the most common complaints associated with antipsychotic treatment, at baseline and after 4 weeks of treatment.

2.3. Plasma sampling

All blood samples were drawn in the morning after at least 10 h of fasting and immediately placed on ice. Blood was drawn into vacutainer tubes containing sodium heparin, immediately placed on ice, and centrifuged within 30 min; the plasma was transferred into polypropylene tubes and stored in a −30 °C freezer for less than 60 days and then transferred to a −80 °C freezer until analysis. Samples were stored at −80 °C for less than 6 months before analysis to minimize any storage-related changes in the lipids (Fox et al., 2003; Zivkovic et al., 2009). There were no differences between groups in the average length of time that samples were frozen (data not shown).

Lipid analysis: An assessment of plasma lipid profiles was performed, as described previously (Watkins et al., 2002). Quantitative measurements of fatty acids in lipid classes were determined as nmol fatty acid/gram of plasma. Briefly, lipids were extracted in the presence of internal standards by the method of Folch et al. (1957) using chloroform–methanol (2:1, v/v) with 0.05% butylated hydroxytoluene. Individual lipid classes were separated by preparative high-performance liquid chromatography for the phospholipid classes and by thin-layer chromatography for the neutral lipid classes. Each isolated lipid class fraction was trans-esterified in 3 N methanolic-HCl in a sealed vial under N_2 at 100 °C for 60 min. The resulting fatty acid methyl esters were extracted from the mixture with hexane containing 0.05% butylated hydroxytoluene and prepared for gas chromatography by sealing the hexane extracts under N_2. FA methyl esters were separated and quantified by capillary GC using a gas chromatograph (Agilent Technologies model 6890, Wilmington, DE) equipped with a 30-m DB-225MS capillary column (Agilent Technologies, Folsom, CA) and a flame-ionization detector. All fatty acids in both neutral lipids: cholesterol esters, diglycerides, triglycerides, free fatty acids, and phospholipids: phosphatidylcholine, lysophosphatidylcholine, and phosphatidylethanolamine and free cholesterol were measured as part of this study. However, the analysis presented in this paper focuses on the plasmalogen subclass of the phospholipids. Analyses of the remaining data, including that of the n-3 and n-6 phospholipid metabolites, will be described in other publications. Quantitative measurements of fatty acids in lipid classes were determined as nmol fatty acid/gram of plasma. Metabolites were identified by the lipid class and the fatty acid moiety, e.g. arachidonic acid in phosphatidylcholine: PC20:4n6.

2.4. Statistical analyses

Statistically significant differences in plasma lipid concentrations between patients and controls were assessed by Wilcoxon rank sum test. First episode patients and recurrent patients were also compared to controls separately using the same method. Associations between baseline measures of plasma lipids and BPRS score, age, weight, BMI, or waist circumference were obtained using Spearman's correlation coefficient. Q values were obtained to correct for multiple tests, which measures the minimum false discovery rate that is incurred when calling this specific test significant (Storey and Tibshirani, 2003). The statistical analysis was done using the statistical software R (R Development Core Team, 2006). The following programs were used for the analysis: Wilcoxon rank sum test: wilcox.test, Spearman's correlation coefficient: cor.test, q value: qvalue (in the qvalue library).

Since there is some indication in the literature that overall phosphatidylcholine and phosphatidylethanolamine contents are higher in women than in men (Geppert et al., 2009), we conducted an interaction analysis to test if there were any gender by group (patients vs. controls) effects on the baseline metabolite concentrations that were found to be significantly different between patients and controls.

3. Results

3.1. Characteristics of patients and healthy controls

There were no significant differences between the first episode psychosis patients vs. patients with psychotic relapse of schizophrenia/schizoaffective disorder in weight, or waist circumference (see Table 1). The first episode patients were significantly younger ($p=0.01$) and had lower levels of BPRS score ($p=0.03$) at enrollment than the recurrent patients. As a group, the patients did not differ in body composition measurements or age compared to the controls (see Table 1).

3.2. Baseline differences between all patients vs. controls

Tables 2 and 3 incorporate all plasmalogens measured with the mean levels ± standard deviation (S.D.) presented for the three groups. We present p and q values (q value provides the estimated