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Research paper

Lipid peroxidation biomarkers in adolescents with or at high-risk for bipolar disorder

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ABSTRACT

Background: Prior work suggests that adult bipolar disorder (BD) is associated with increased oxidative stress and inflammation. This exploratory study examined markers of lipid and protein oxidation and inflammation in adolescents with and at varying risk for BD type I (BD-I).

Methods: Blood was obtained from four groups of adolescents (9–20 years of age): (1) healthy comparison subjects with no personal or family history of psychiatric disorders ($n=13$), (2) subjects with no psychiatric diagnosis and at least one parent with BD-I ('high-risk', $n=15$), (3) subjects with at least one parent with BD-I and a diagnosis of depressive disorder not-otherwise-specified ('ultra-high-risk', $n=20$), and (4) first-episode patients exhibiting mixed or manic symptoms that received a diagnosis of BD-I ($n=16$). Plasma levels of lipid peroxidation (LPH, 4-HNE, 8-ISO), protein carbonyl, and inflammation (IL-1α-β, IL-6, IL-10, IFNγ, TNFα) were assessed using analysis of variance and covariance models.

Results: LPH was lower in adolescents with fully syndromal BD than controls, while LPH levels in the at-risk groups were between healthy controls and fully syndromal BD. Post-hoc analysis showed a non-significant increase in the (4-HNE+8-ISO)/LPH ratio suggesting a potential conversion of LPH into late-stage markers of lipid peroxidation. There were no significant differences among protein carbonyl content and inflammatory markers.

Conclusions: In adolescents, fully syndromal BD is associated with significant reductions in LPH levels, and LPH levels decrease along the spectrum of risk for BD-I. Quantifying lipid peroxidation in longitudinal studies may help clarify the role of LPH in BD risk progression.

1. Introduction

The initial onset of mania, and by definition bipolar I disorder (BD), typically occurs during adolescence and is associated with significant psychosocial morbidity and increased risk for suicide (Perlis et al., 2004, 2009). Misdiagnosis, heterogeneity of clinical symptoms, profound functional impairment, and identification of effective pharmacologic treatments are common challenges for youth and clinicians in treatment planning. The pathophysiology of BD is multifaceted and incompletely understood. Heritability estimates for BD range from 60% to 87% (Smoller and Finn, 2003), and family studies suggest that having a first-degree relative with BD substantially increases risk for developing bipolar disorder (DelBello and Geller, 2001; Mortensen et al., 2003). Retrospective and prospective longitudinal studies additionally suggest that mood symptoms, including episodic subsyndromal depressive

Abbreviations: 4-HNE, 4-hydroxy-2-nonenal; 8-ISO, 8-isoprostane; ADHD, attention deficit hyperactivity disorder; Akt, protein kinase B; AP-1, activator protein 1; BD, bipolar disorder; BMI, body mass index; Cys, cysteine; DD-NOS, depressive disorder not otherwise specified; ELISA, enzyme-linked immunosorbent assay; Erk1/2, extracellular signal-regulated protein kinases 1 and 2; HDRS, Hamilton Depression Rating Scale; His, histidine; IL, interleukins; JNK, c-Jun N-terminal kinases; LPH, lipid hydroperoxides; Lys, lysine; MDD, major depressive disorder; NF-κB, nuclear factor kappa-light-chain-enhancer of activated B cells; Nrf2, nuclear factor (erythroid-derived 2)-like 2; p38 MAP kinases, p38 mitogen-activated protein kinases; PI3, phosphoinositide-3-kinase; PPAR, peroxisome proliferator-activated receptors; TNFα, tumor necrosis factor α; VEGF, vascular endothelial growth factor; YMRS, Young Mania Rating Scale

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symptoms and major depressive disorder (MDD), frequently precede the initial onset of mania by several years (Correll et al., 2014; Egeland et al., 2000; Howes et al., 2011; Skjelstad et al., 2010), and increase risk for developing mania in adolescent bipolar offspring (Axelson et al., 2015). While emerging retrospective and prospective evidence suggests that adolescent age, positive family history, and prodromal mood symptoms can serve as criteria to identify individuals at increased risk for developing bipolar disorder (Bechdolf et al., 2010, 2014), the addition of prodromal biomarkers may augment prognostic accuracy as well as inform early intervention strategies (Conus et al., 2008; McGorry et al., 2014; McNamara et al., 2010).

Several candidate biomarkers are emerging and hold the promise of elucidating relevant biologic underpinnings of BD, identifying subsets, facilitating early identification, characterizing staging approaches, personalizing treatment, and improving outcomes. One compelling approach focuses on peripheral measures of oxidative stress and inflammation. Prior work suggests that mitochondrial dysfunction and oxidative stress play a central role in the etiopathology of BD (Scola et al., 2013; Versace et al., 2014). Recent meta-analytic studies and systematic reviews indicate that lipid peroxidation is increased in adult BD (Brown et al., 2014). Lipid peroxidation is initiated by reactive oxygen species, thereby forming lipid hydroperoxides (LPH), 4-hyroxy-2-nonenal (4-HNE), and 8-isoprostane (8-ISO) (Halliwell, 2007). Independent studies have consistently demonstrated elevations in these indices of oxidative stress in adult BD (Andreazza et al., 2013; Versace et al., 2014; Wang et al., 2009). Inflammation also orchestrates important molecular changes that may be involved in the pathophysiology of BD (Scola and Andreazza, 2014). For example, pro-inflammatory cytokines, such as IL-1α-β, IL-6 and TNFα, are increased in adult BD (aged 35 and older) with BD (Goldstein et al., 2009; Hope et al., 2011; Rao et al., 2010; Scola and Andreazza, 2014) as well as children and adolescents with BD (Mitchell and Goldstein, 2014).

There is currently a dearth of research focused on measures of oxidative stress in adolescents and at-risk populations. Magalhães and colleagues reported that young adults (aged 18–24) with BD had higher protein carbonyl content compared to healthy controls (Magalhães et al., 2012). Decreased glutathione levels (a marker of oxidative stress) were also observed in the anterior cingulate cortex of patients with evolving BD by proton magnetic resonance spectroscopy (Chitty et al., 2015). More recently, Hatch and colleagues found that adolescents with BD had lower LPH as compared to prior samples of adult patients with BD (Hatch et al., 2015). While prior research suggests that lipid peroxidation and inflammation are increased in adult patients with BD, it is not known whether these abnormalities exist during acute illness or if it represents a risk biomarker for BD. In the present, exploratory, cross-sectional study, we determined levels of lipid peroxidation and inflammatory biomarkers in adolescents with and at varying risk for BD. Based on extant evidence, our primary hypothesis was that lipid peroxidation and inflammatory biomarkers would be elevated in first-episode BD patients compared with healthy adolescents, and these increases would be graded in asymptomatic and symptomatic high risk adolescents.

2. Methods

2.1. Study participants

Study participants were recruited from inpatient units and outpatient clinics at Cincinnati Children's Hospital Medical Center and University of Cincinnati Medical Center. Demographically matched healthy comparison participants were recruited from the communities in which the other participants resided. The cohort consisted of 4 groups of adolescents (9–20 years of age): (1) healthy comparison (HC) participants with no personal or family history of psychiatric disorders, (2) participants with no *DSM-IV-TR* Axis I diagnosis and at least one parent with bipolar I disorder (high-risk; HR), (3) adolescents with at least one parent with bipolar I disorder and a *DSM-IV-TR* Axis I diagnosis of MDD or depressive disorder not-otherwise-specified ('ultra-high-risk' UHR), and (4) first-episode patients exhibiting mixed or manic symptoms that received a *DSM-IV-TR* Axis I of bipolar I disorder. *DSM-IV-TR* diagnoses were determined using the Washington University in St. Louis Kiddie-Schedule for Affective Disorders and Schizophrenia (WASH-U-KSADS) (Geller et al., 2001). Parental diagnoses were determined using the Structured Clinical Interview for DSM-IV-TR (SCID) (First et al., 1995) and confirmed using the Family Interview for Genetic Studies (FIGS) (Maxwell, 1999). All participants were assessed by board-certified psychiatrists with established inter-rater reliabilities (kappa $>$ 0.9). IQ was estimated using the Wechsler Abbreviated Scale of Intelligence (WASI) (Wechsler, 1997). Symptom severity ratings were obtained with the Young Mania Rating Scale (YMRS) (Young et al., 1978) for mania and the 28-item Hamilton Depression Rating Scale (HDRS/HAM-D) (Hamilton, 1960) for depression. Pubertal development was measured using a self-rated Tanner Scale. Exclusion criteria included a diagnosis of substance dependence within the previous 3 months (McLellan et al., 1992), any major medical or neurological illnesses (e.g., head trauma resulting in loss of consciousness for more than 5 min), an IQ $<$ 70, and a positive urine pregnancy test in females. Cigarette use in the past 30 days was documented but not exclusionary. All patients were free of psychotropic medications or medications with central nervous system effects, at the time of the blood draw, with the exception of psychostimulants in participants with ADHD. Ultra-high risk patients were required to be antidepressant-free for at least 5 days prior to the blood draw (or at least one month for fluoxetine because of its long half-life). In first-episode patients, the blood draw occurred prior to initial treatment with antipsychotics or mood stabilizer medication. Written informed consent and assent were provided by a legal guardian and the patient, respectively. This study was approved by the Institutional Review Boards of University of Cincinnati College of Medicine.

2.2. Lipid peroxidation assessments

Serum levels of lipid peroxidation (lipid hydroperoxides, LPH; 8-isoprostane, 8-Iso; 4-hydroxy-2-nonenal, 4-HNE) were measured. LPH was assessed using the colorimetric LPH assay kit (Cayman Chemical item number 705002) according to manufacturer's instructions. The sensitivity of this kit is in between 0.25 and 5 nmol hydroperoxides. The intra-assay presented a coefficient of variation (CV) in between 2% and 15% and the inter-assay presented a CV of 9%. Data are presented as the amount of LPH (μM). Levels of 4-HNE proteins via Michael addition to lysine (Lys), histidine (His) or cysteine (Cys) were measured using ELISA as previously described with slight modifications. For each sample, 30 μg/mL of total proteins were used. The sensitivity of this assay is in between 0.5 and 10 μg/mL of 4-HNE. The intra-assay presented a coefficient of variation in between 1% and 16% and the inter-assay presented a CV of 7.5%. Results were expressed as fmol/μg of protein. 8-ISO levels were quantified using a standard competitive sandwich ELISA (Cayman Chemical item number 516351) according to manufacturer's instructions. The sensitivity of this assay is in between 0.8 and 500 pg/mL of 8-ISO. The intra-assay presented a coefficient of variation in between 1% and 19% and the inter-assay presented a CV of 9.1%. Results were expressed as fmol/μg of protein or pg/mL.