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Research report

Peripheral markers of oxidative stress and antioxidative defense in euthymia of bipolar disorder—Gender and obesity effects

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ABSTRACT

Introduction: Oxidative and nitrosative stress are implicated in the pathogenesis of uni- and bipolar disorder. Herein we primarily sought to characterize markers of oxidative/nitrosative stress during euthymia in adults with bipolar disorder (BD). Oxidative markers were further evaluated in this BD sample in synopsis with excess overweight or obesity and/or comorbid metabolic syndrome (MetS).

Methods: Peripheral markers of oxidative stress [i.e. thiobarbituric acid reactive substance, (TBARS), malondialdehyde (MDA), and carbonyl proteins] and antioxidant markers [e.g. total antioxidative capacity (TAC), superoxide dismutase (SOD), glutathione *S*-transferase (GST)] were obtained in a cohort of euthymic adults with BD ($N=113$) and compared to healthy controls (CG) ($N=78$). Additionally, anthropometric measures included the body mass index (BMI) [kg/m^2], waist and hip circumference [cm], waist-to-hip-ratio (WHR), waist to height ratio (WtHR) as well as the IDF-defined MetS.

Results: The major finding was a significantly decreased TAC in BD compared to the CG ($p<0.01$; BD: M 1.18, SD 0.47; CG: M 1.39, SD 0.49). MDA was significantly and TBARS by trend higher in the CG compared to the euthymic bipolar test persons (MDA: $p<0.01$, BD: M 0.70, SD 0.18; CG: M 0.81, SD 0.25; TBARS: $p<0.1$, BD: M 0.78, SD 0.28; CG: M 0.76, SD 0.30). The antioxidative enzyme GST was significantly elevated in both patients and controls (BD: M 298.24, SD 133.02; CG: M 307.27 SD 118.18). Subgroup analysis revealed that the CG with concurrent MetS and obesity had significantly elevated TAC when compared to CG without concurrent MetS ($p<0.05$, no MetS: M 1.33, SD 0.50; MetS: M 1.67, SD 0.32), as well as persons with BD with or without current MetS (no MetS: M 1.18, SD 0.44; MetS: M 1.15, SD 0.49). Significant correlations between GST and anthropometric variables were found in male study participants. Multivariate analysis indicated a significant gender effect concerning TBARS values in all patients and CG ($p<0.01$, females: M 0.73, SD 0.29; males: M 0.83, SD 0.28).

Conclusion: Euthymic bipolar adults exhibit peripheral evidence of a disturbed biosignature of oxidative stress and antioxidative defense. Male test persons showed significantly higher peripheral markers of oxidative stress than women- female sex may exert protective effects. Furthermore, the biosignature of oxidative stress obtained herein was more pronounced in males with concurrent metabolic disorders. Our results further extend knowledge by introducing the moderating influence of gender and obesity on oxidative stress and BD.

1. Introduction

Bipolar disorder (BD) is a severe brain disorder heterogeneous in pathogenesis. Several lines of evidence implicate disturbances in oxidative and nitrosative stress (O&NS) as causally related to the onset and progression of several brain disorders including but not limited to BD and major depressive disorder (Andreazza et al., 2008; Berk et al.,

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2011, 2013; Bortolasci et al., 2014; Kapczinski et al., 2011b; Soczynska et al., 2011; Stefanescu and Ciobica, 2012). Moreover, preliminary evidence indicates that O&NS may also be more pronounced in more severe presentations e.g. suicidality (Vargas et al., 2013).

Activated O&NS pathways are in interplay with several systems also implicated in the pathogenesis of several brain disorders and comorbidity, e.g. immuno-inflammation (Berk et al., 2011; Maes et al., 2013a, 2013b). Collectively, O&NS and related systems are thought to be pertinent to the phenotypic and neurobiological progression of brain disorders (e.g. apoptosis, altered neuroplasticity). In addition to exerting the direct hazard on organ systems implicated in the pathogenesis of mood disorders, O&NS can additionally lead to a cascade of secondary autoimmune responses with consequent modulatory effects on disparate neurochemicals and immune targets (Berk et al., 2011; Maes et al., 2011, 2013a, 2013b; Moylan et al., 2013).

Reactive oxygen species (ROS) are generated along the electron transport chain as a product of mitochondrial respiration. Under physiological conditions, ROS are detoxified by endogenous antioxidant enzymes (e.g. superoxide dismutase). Under pathogenic conditions, an imbalance occurs between ROS accumulation and elimination with hazardous effects on lipids, proteins, DNA as well as immune systems e.g. neoepitopes (Dalvi et al., 2013; Frey et al., 2007; Lenaz et al., 2000; Maes et al., 2013a, 2013b; Mangge et al., 2004, 2010; Reininghaus et al., 2014).

O&NS are also implicated as both causative and consequential to obesity/metabolic syndrome; a common comorbidity in adults with BD (Furukawa et al., 2004; Mangge et al., 2010). Moreover, more replicated evidence implicates that the occurrence of obesity/metabolic syndrome is associated with a more complex BD illness presentation and course, suggesting a contributory role of O&NS in the underline progressive process (Leboyer et al., 2012; Fagiolini et al., 2005 2008; McIntyre et al., 2010a, 2010b, 2010c). Nevertheless, a recent study found none of the key metabolic characteristics of MetS to be fully specific for mood disorders (Vargas et al., 2014). An additional observation has been the differential risk of obesity/metabolic syndrome in females with BD, inviting the need to parse the moderational influence of gender. The forgoing collection of observations provides the impetus for exploring O&NS biosignature in adults with asymptomatic BD with a particular emphasis on concurrent obesity/metabolic syndrome and gender influences (Andreazza et al., 2008; Dietrich et al., 2013; Gergerlioglu et al., 2007; Kapczinski et al., 2011c; Kiray et al., 2007; Kuloglu et al., 2002; Machado-Vieira et al., 2007; Moorthy et al., 2005; Ozcan et al., 2004; Ranjekar et al., 2003a; Savas et al., 2006; Selek et al., 2008; Spence et al., 2013). Moreover, the moderational influence of psychotropic agents on peripheral markers of O&NS needs to be also considered (Albayrak et al., 2013; Bakare et al., 2009; Diniz et al., 2013; Jornada et al., 2011; Khairova et al., 2012; Nciri et al., 2013; Riadh et al., 2011).

Herein we sought to characterize the “oxidative signature” in adults with BD in a well characterized sample of euthymic adults with BD subgrouped on the basis of the presence vs. absence of concurrent obesity/metabolic syndrome. The bipolar sample was compared to a healthy control sample with an additional emphasis given to the moderational influence of gender.

2. Methods

2.1. Study participants

All subjects in the analysis herein were part of the BIPFAT study which had been described elsewhere (Reininghaus et al., 2013). Briefly, the BIPFAT study broadly aimed to characterize biological abnormalities in adults with BD. Subjects in the analysis herein included Caucasian individuals with BD ($N=113$) compared to a healthy control group ($N=78$).

The recruited bipolar study participants had been former in- or outpatients of the Medical University of Graz and had been diagnosed with BD according to the DSM-IV criteria (verified by SCID-I) (Wittchen et al., 1997). Bipolar subjects had been euthymic at the time of inclusion (HAMD < 11, YMRS < 9) and had given written informed consent prior to participating in the study. Exclusion criteria were the presence of chronic obstructive pulmonary disease, rheumatoid arthritis, systemic lupus erythematosus, inflammatory bowel disease, neurodegenerative and neuroinflammatory disorders (i.e. Alzheimer's, Huntington's and Parkinson's disorder, multiple sclerosis), hemodialysis and interferon-α-based immunotherapy. Further exclusion criteria for controls were the presence of lifetime psychiatric diagnoses (verified by SCID I) and first and second grade relationship to relatives with psychiatric disorders.

The BIPFAT study had been approved by the local ethics committee (Medical University of Graz, Austria) in compliance with the current revision of the Declaration of Helsinki, ICH guideline for Good Clinical Practice and current regulations (EK-number: 24-123 ex 11/12).

Peripheral serum markers for oxidative stress and antioxidative defense, metabolic and anthropometric measures were measured for this present study. Fasting blood samples were taken between 8:30 and 10:00 a.m. The oxidative stress markers thiobarbituric acid (TBARS), malondialdehyde (MDA) and carbonyl proteins and the antioxidative measures (Cu/Zn superoxide dismutase: SOD, glutathione *S*-transferase: GST, total antioxidative capacity: TAC) were analyzed in serum. One blood sample (concerning MDA analysis) was excluded due to the presence of severe hemolysis.

Malondialdehyde is a marker for lipid oxidation and belongs to the class of TBA-reactive substances (TBARS). Since a range of substances react with TBA (i.e. urobilinogen, hemoglobin), the TBA-reaction is not exclusively measuring malondialdehyde and can produce only a rather unprecise measure of malondialdehyde. Nevertheless, the major part of former studies has used the TBA-reaction. Therefore, we used two techniques (TBA reaction and gas chromatography–mass spectrometry, GCMS) to make our results comparable to the previous literature. To our knowledge, few papers have measured malondialdehyde levels by more exact GCMS. The latter was based on derivation of MDA with 2,4-dinitrophenylhydrazine. In this present study, ions were detected at m/z 204 for MDA and at m/z 206 for the deuterated analog (MDA-d 2) as internal standard by capillary column gas chromatography-negative-ion chemical ionization mass spectrometry (GC–MS–NIC) with methane as collision gas (flow 2.0 ml/min) on a Thermo Trace GC Ultra coupled to a Thermo DSQ II mass spectrometer (from Thermo Fisher Scientific, CA, USA) (Zelzer et al., 2013). The TBA-reaction was followed by high-performance liquid chromatography (HPLC, Merck-Hitachi, Stuttgart, Germany) analysis and fluorometric detection. Carbonyl proteins were analyzed by the Carbonyl Protein ELISA Kit and the BCA Test distributed by Immundiagnostik AG, Bensheim, Germany.

The peripheral antioxidative serum markers comprised of TAC, SOD and GST. TAC was determined by the TAC (Total Antioxidative Capacity) ELISA Kit manufactured by the Omnignostica Forschungs GmbH, Vienna, Austria. The TAC (in the literature also described as total reactive antioxidant potential TRAP or total antioxidant status TAS) refers to the general ability of the organism to scavenge ROS. TAC is a summary marker for all antioxidative enzymes and scavengers, which participate in detoxification and neutralization of O&NS.

SOD was analyzed by the Serazym® Cu/Zn SOD ELISA Kit by Seramun Diagnostica GmbH, Heidesee, Germany. GST was detected by the GST-π ELISA Kit distributed by Imundiagnostik AG, Bensheim, Germany.

The inter-assay coefficients of variability for all analytes were less than 10%. Assays with a coefficient of variation > 10% were reanalyzed.