Oxidatively-induced DNA damage and base excision repair in euthymic patients with bipolar disorder

Deniz Ceylana,b,⁎, Gamze Tuna, Güldal Kirkald, Zeliha Tuncae, Güneş Canf, Hidayet Ece Aratg, Melis Kanth, Miral Dizdaroglu,⁎⁎, Ayşegül Özerdemb,e

a Vocational School of Health Services, Izmir University of Economics, Izmir, Turkey
b Department of Neuroscience, Health Sciences Institute, Dokuz Eylül University, Izmir, Turkey
c Department of Molecular Medicine, Health Sciences Institute, Dokuz Eylül University, Izmir, Turkey
d Thoracic and Gastrointestinal Oncology Branch, Center for Cancer Research, National Cancer Institute, National Institutes of Health, Building 10, Bethesda, MD, 20892, USA
e Department of Psychiatry, School of Medicine, Dokuz Eylül University, Izmir, Turkey
f Department of Psychology, Istanbul Gelisim University, Istanbul, Turkey
h Department of Medical Biochemistry, Health Sciences Institute, Dokuz Eylül University, Izmir, Turkey
i Biomolecular Measurement Division, National Institute of Standards and Technology, Gaithersburg, MD, 20899, USA

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ABSTRACT
Oxidatively-induced DNA damage has previously been associated with bipolar disorder. More recently, impairments in DNA repair mechanisms have also been reported. We aimed to investigate oxidatively-induced DNA lesions and expression of DNA glycosylases involved in base excision repair in euthymic patients with bipolar disorder compared to healthy individuals. DNA base lesions including both base and nucleoside modifications were measured using gas chromatography-tandem mass spectrometry and liquid chromatography-tandem mass spectrometry with isotope-dilution in DNA samples isolated from leukocytes of euthymic patients with bipolar disorder (n = 32) and healthy individuals (n = 51). The expression of DNA repair enzymes OGG1 and NEL1 were measured using quantitative real-time polymerase chain reaction. The levels of malondialdehyde were measured using high performance liquid chromatography. Seven DNA base lesions in DNA of leukocytes of patients and healthy individuals were identified and quantified. Three of them had significantly elevated levels in bipolar patients when compared to healthy individuals. No elevation of lipid peroxidation marker malondialdehyde was observed. The level of OGG1 expression was significantly reduced in bipolar patients compared to healthy individuals, whereas the two groups exhibited similar levels of NEL1 expression. Our results suggest that oxidatively-induced DNA damage occurs and base excision repair capacity may be decreased in bipolar patients when compared to healthy individuals. Measurement of oxidatively-induced DNA base lesions and the expression of DNA repair enzymes may be of great importance for large scale basic research and clinical studies of bipolar disorder.

1. Introduction

Bipolar disorder (BD) is a chronic, severe and highly disabling psychiatric disorder, which is considered as one of the leading causes of disability amongst all medical and psychiatric conditions [1–3]. BD has previously been associated with increased mortality and morbidity due to general medical conditions such as cardiovascular, metabolic or inflammatory diseases [4–12]. Despite vast uncertainties about the underlying molecular mechanisms, recent evidence has shown that increased oxidatively-induced DNA damage may have a central role in the pathophysiology of BD and increased cellular aging and comorbidity in BD [13–15]. Oxygen-derived free radicals are constantly generated as...
by-products of aerobic metabolism. Oxidative stress occurs when enzymatic and non-enzymatic antioxidant defense systems are overwhelmed by elevated levels of oxygen-derived free radicals [16]. Oxidative stress damages biological molecules such as DNA, proteins and lipids, causing multiple forms of DNA damage including base and sugar modifications, strand breaks and DNA-protein cross-links [17]. Oxidatively-induced damage to DNA can initiate mutagenic processes and early aging [18]. This type of DNA damage has been shown to play a role in the pathophysiology of cardiovascular diseases, diabetes mellitus, various cancers and psychiatric disorders including BD [18–20].

Previous studies focusing on antioxidant enzymes and oxidatively-induced damage to proteins and lipids in BD reported consistent and significant alterations in antioxidant enzymes, lipid peroxidation and nitric oxide levels [21–23]. Increased levels of DNA single- or double-strand breaks have been shown in both postmortem brain tissues [24–26] and lymphocytes of patients with BD [27]. Moreover, levels of 8-hydroxy-2′-deoxyguanosine (8-OH-dG) have been reported to be increased in blood [28,29] and urine samples of patients with BD [30,31]. Despite a plethora of known oxidatively-induced DNA base lesions, previous research in psychiatric disorders focused on 8-OH-dG only [20]. Therefore, there are no data on the alterations of the levels of DNA base lesions other than that of 8-OH-dG in BD.

Various DNA repair mechanisms exist to repair oxidatively-induced DNA base damage. The base excision repair (BER) is the major mechanism for the repair of this type of DNA damage. It recognizes and removes modified DNA bases by DNA glycosylases, followed by the activity of other enzymes to complete DNA repair [32–34]. In BER, OGG1 is a specific enzyme for the excision of 8-OH-Gua and 2,6-diamino-4-hydroxy-5-formamidopyrimidine (FapyGua), whereas 4,6-diamino-5-formamidopyrimidine (FapyAde) and FapyGua are removed by NEIL1 and NEIL3, but not 8-OH-Gua [34]. Two studies showed that increases in expression of OGG1 were associated with depressive symptoms in cancer patients [35,36]. A decrease in BER capacity in recurrent depressive disorder [37], and down regulated OGG1 levels in rapid-cycling BD [38] have also been reported.

The objective of the present study was to investigate a more extensive set of markers of oxidatively-induced DNA damage and DNA repair enzymes in DNA samples isolated from leukocytes of euthymic patients with BD in comparison to healthy individuals.

2. Materials and methods

2.1. Participants

Patients with BD (n = 32) and healthy individuals (n = 51) were included in this study. The patients who had been euthymic for at least 6 months were recruited from the Bipolar Disorders Outpatient Unit, Department of Psychiatry, Dokuz Eylul University, Izmir, Turkey. Diagnoses were confirmed using the Structured Clinical Interview for the Diagnostic Manual of Mental Disorders [39] and clinical variables were recorded by experienced clinicians of the research team. Patients with neurological disorders, history of head trauma, chronic medical condition (e.g., hypertension, diabetes mellitus) and substance use were excluded. Other exclusion criteria included comorbid Axis I psychiatric diagnosis, neurodegenerative diseases, epilepsy or previous brain surgery, auditory or visual impairment, and being pregnant or breast-feeding. Symptomatic severity was assessed using Young Mania Rating Scale (YMRS) [40], Hamilton Depression Scale-17 (HAM-D) [41], Clinical Global Impression Scale (CGI) [42] and Global Assessment of Functionality (GAF) [43]. Healthy individuals with no known medical problems, no family history of major psychiatric or no neurological disorders, including dementia, mental retardation, cancer, cardiovascular disease or diabetes mellitus in the first-degree relatives or psychiatric history were enrolled in this study. Psychiatric conditions of the healthy individuals were confirmed by the Structural Clinical Interview for DSM-IV interview [38]. The study was approved by Dokuz Eylul University Hospital Ethics Committee (Approval date: 12.07.2012; protocol no: 2012/16-13). All participants provided written informed consent.

2.2. Collection of the blood samples

Each participant provided 10 mL blood sample collected in EDTA-coagulated tubes (for leucocyte, RNA and plasma isolation) by venipuncture. At the day of the venipuncture, leukocytes were isolated from blood samples by density gradient separation using Histopaque-1119 and total RNA was extracted from 500 μL blood samples using GeneJet RNA Purification Kit (Fermentas, MA, USA). Leukocytes were frozen at −80 °C until DNA isolation. The RNA samples were frozen at −80 °C until they were converted to first-strand cDNA with an oligo-2′-deoxythymidine (dT) 18 primer. The RNA samples were converted to first-strand cDNA using the First Strand cDNA Synthesis Kit (Fermentas, MA, USA) and were frozen at −80 °C until quantitative real-time polymerase chain reaction (QRT-PCR) was performed.

2.3. DNA isolation and analysis

DNA was isolated from leukocytes by using salting-out/NaCl method [44]. DNA concentration was measured by recording the UV spectrum of each sample using an absorption spectrophotometer between the wavelengths of 200 nm and 350 nm. The absorbance at 260 nm was used to measure the DNA concentration. Subsequently, 50 μg aliquots of DNA samples were dried in a SpeedVac under vacuum. According to a Material Transfer Agreement between Dokuz Eylul University, Izmir, Turkey and National Institute of Standards and Technology (NIST), Gaithersburg, MD, USA, DNA samples were sent to NIST for analysis by gas chromatography-tandem mass spectrometry (GC–MS/MS) and liquid chromatography-tandem mass spectrometry (LC–MS/MS).

2.4. Gas chromatography-tandem mass spectrometry

GC–MS/MS with isotope dilution was used to identify and quantify FapyAde, FapyGua, 8-OH-Gua, thymine glycol (ThyGly), 5-hydroxycytosine (5-OH-Cyt) and 5-hydroxy-5-methylhydantoin (5-OH-5-MeHyd). Aliquots (50 μg) of DNA samples were supplemented with aliquots of internal standards FapyAde-13C,15N5, FapyGua-13C,15N5, 8-OH-Gua-15N5, ThyGly-15N4, 5-OH-Cyt-13C,15N2 and 5-OH-5-MeHyd-13C,15N2. DNA samples were dissolved in 50 μL of an incubation buffer consisting of 50 mM phosphate buffer (pH 7.4), 100 mM KCl, 1 mM EDTA, and 0.1 mM diithiothreitol, and then incubated with 2 μg of E. coli Fpg and 2 μg of E. coli Nth for 1 h at 37 °C to release DNA base lesions from DNA. Subsequently, 100 μL ethanol were added to precipitate DNA. After centrifugation, supernatant fractions were separated, lyophilized and trimethylsilylated. Derivatized samples were analyzed by GC–MS/MS as described previously [45].

2.5. Liquid chromatography-tandem mass spectrometry

LC–MS/MS with isotope dilution was used to measure the levels of (5′S)′-5′-cyclo-2′-deoxyadenosine (5′-cdA) and 8-OH-dG, which is the 2′-deoxynucleoside form of 8-OH-Gua. Aliquots of S-cdA-13C,15N3 and 8-OH-dG-15N5 as internal standards were added to an aliquot of 50 μg of DNA samples, which were then dried in a SpeedVac. Subsequently, DNA samples were hydrolyzed with a mixture of nuclease P1, phosphodiesterase I and alkaline phosphatase according to a published procedure [45]. All samples were filtered using Millipore Microcon Ultracel YM-3 ultrafiltration membranes (Millipore, Bedford, MA) with molecular mass cutoff of 3 kDa by centrifugation at 12000×g for 30 min. LC–MS/MS analyses were performed using a Thermo-Scientific Finnigan TSQ Quantum Ultra AM triple quadrupole MS/MS system with an installed heated electrospray-ionization source, as described previously.
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