CrossMark

Neurotrophic and inflammatory markers in bipolar disorder: A prospective study

Seline van den Ameele[a,b,*], Violette Coppens[a,b], Jeroen Schuermans[a,b], Peter De Boer[c], Maarten Timmers[d,e], Erik Fransen[f], Bernard Sabbe[a,b], Manuel Morrens[a,b]

[a] Collaborative Antwerp Psychiatric Research Institute (CAPRI), Department of Biomedical Sciences, University of Antwerp, Belgium
[b] University Psychiatric Hospital Duffel, VZW Emmaüs, Duffel, Belgium
[c] Experimental Medicine Neuroscience, Janssen Pharmaceutica N.V., Beerse, Belgium
[d] Janssen Research and Development, a division of Janssen Pharmaceutica N.V., Beerse, Belgium
[e] Reference Center for Biological Markers of Dementia (BIODEM), Institute Born-Bunge, University of Antwerp, Belgium
[f] StatUa − Center for Statistics, University of Antwerp, Belgium

ARTICLE INFO

ABSTRACT

Altered neurotrophic signaling is thought to impair neuroplasticity in bipolar disorder (BD). Brain-derived neurotrophic factor (BDNF) is proposed as a neurotrophic marker in BD. However, the current evidence for its use in monitoring disease activity and illness progression is conflicting and an exploration of additional neurotrophic markers is needed. This prospective case-control study investigated mood-specific changes in potential neurotrophic markers and their association to inflammatory activity. Patients with BD were included during an acute mood episode, either depressive (n = 35) or (hypo)manic (n = 32). Fifty-nine patients (88%) and 29 healthy controls (97%) completed the study. Peripheral blood levels of BDNF, vascular endothelial growth factor A (VEGF), soluble fms-like tyrosine kinase-1 (sFlt-1) and tumor necrosis factor alpha (TNF-α) were measured at baseline and after 2 months. Biomarker levels in patients were compared to controls and correlated to HDRS-17 and YMRS total scores and the PANSS positive subscale scores. Linear mixed model analysis revealed no significant differences in neurotrophic markers between patients and controls. We found significantly increased TNF-α levels in patients and a subsequent normalization during euthymia. None of the biomarkers strongly correlated to mood symptom severity. Despite standardized methodological practices, BDNF and VEGF levels had a wide distribution range. We need a better understanding of methodological aspects influencing the analysis of neurotrophic factors to improve future research on markers for mood state monitoring and illness progression in BD.

1. Introduction

Changes in neurotrophic signaling leading to impaired neuroplasticity are thought to play a role in the pathophysiology of bipolar disorder (BD) (Berk et al., 2011; Grande et al., 2012). Several neurotrophins and trophic factors are thought to be involved, with most evidence for changes in brain-derived neurotrophic factor (BDNF) expression in patients with BD (Scola and Andreazza, 2015). Decreased BDNF levels were shown during acute mood episodes and correlated negatively to the severity of mood symptoms (Cunha et al., 2006; Machado-Vieira et al., 2007). In line with the latter, drug responders had increasing BDNF levels while non-responders had stable lower

levels (de Sousa et al., 2011; Tramontina et al., 2009). On the long term, a negative correlation between BDNF levels and duration of illness was found (Kauer-Sant'Anna et al., 2009). These findings put BDNF forward as a promising biomarker for both acute mood state monitoring and documenting illness progression in BD (Grande et al., 2010). However, the evidence on BDNF as a biomarker in BD is increasingly conflicting. While decreased BDNF levels in patients with BD compared to healthy controls (HCs) are confirmed in recent meta-analyses, state-related BDNF alterations or a link to illness progression were not consistent (Fernandes et al., 2015; Munkholm et al., 2016; Polyakova et al., 2015). Furthermore, the meta-analyses point out methodological problems such as between-study heterogeneity, insufficient control for

confounding factors, publication bias and a lack of longitudinal designs (Munkholm et al., 2016). Consequently, the initial enthusiasm on BDNF has tempered and efforts shifted towards an exploration of other potential biomarkers in BD (Scola and Andreazza, 2015). Vascular growth factor A (VEGF) is an angiogenic protein with neurotrophic capacities induced by hypoxia and pro-inflammatory cytokines (Ruiz de Almodovar et al., 2009). VEGF signals through the VEGF receptor (VEGFR)-1 and VEGFR-2, which are both involved in neuroprotection and neuroregeneration. The soluble form of VEGFR-1 (soluble fms-like tyrosine kinase-1, sFlt-1) is induced by hypoxia and acts as an anti-angiogenic factor by scavenging free VEGF and attenuating its trophic effects (Ruiz de Almodovar et al., 2009). Soluble Flt-1 also sensitizes endothelial cells for pro-inflammatory cytokines making them more susceptible to damage (Lizano et al., 2016). Disturbances in VEGF signaling were reported in schizophrenia (Lee et al., 2015; Lopes et al., 2015), but studies in BD are scarce (Scola and Andreazza, 2015). A single study showed a mood state-related increase in VEGF levels in major depressive disorder (MDD) and BD (Lee and Kim, 2012). Lithium treatment downregulated *VEGF* transcription in leukocytes of patients with BD (Kikuchi et al., 2011). These findings may represent mood state-related VEGF changes, but without prospective studies, the evidence remains weak. Increased sFlt-1 levels were found in a young population at high risk for psychosis, but again there are no studies in BD (Lizano et al., 2016).

Immune system alterations in patients with BD have been demonstrated extensively (Haarman et al., 2014; Munkholm et al., 2013; Rosenblat et al., 2014). An increased pro-inflammatory state during mood episodes as well as a role of inflammation in illness progression have been described (Berk et al., 2010; van den Ameele et al., 2016). Although both neurotrophic factors and inflammatory cytokines are proposed as biomarkers in BD, study designs exploring the interaction between alterations in neurotrophic signaling and immune system activity are scarce (Jacoby et al., 2016; Kauer-Sant'Anna et al., 2009). A better exploration of possible interactions between neurotrophic and inflammatory pathways could contribute to better insight in the underlying pathophysiology of acute mood episodes and illness progression.

We aimed to prospectively investigate mood state-specific changes in neurotrophic markers (BDNF, VEGF and sFlt-1) in patients with BD compared to HCs. Since there is great evidence on increased tumor necrosis factor-alfa (TNF-α) levels in BD (Modabbernia et al., 2013; Munkholm et al., 2013) with even indications of a mood state-related increase (Fiedorowicz et al., 2015; Modabbernia et al., 2013; van den Ameele et al., 2016), TNF-α was included as a marker of inflammation to study the interaction between inflammatory changes and neurotrophic markers in BD. We hypothesized TNF-α, VEGF and sFlt-1 levels to be increased in patients with BD in all affective states compared to HCs and that these levels would positively correlate to the severity of mood symptoms. The inverse was hypothesized for BDNF.

2. Methods

2.1. Participants

Inpatients were recruited in 3 psychiatric centers in the region of Antwerp, Belgium. Outpatients were recruited *via* the Flemish patient association. The inclusion criteria were age 18–65 years, DSM-IV diagnosis of BD type I, type II or schizoaffective disorder and suffering from a depressive or (hypo)manic episode. Healthy controls were recruited mainly among staff members. Patients and HCs were age and gender matched because of the impact of these factors on inflammatory activity and neurotrophic factors (Bus et al., 2011; Michaud et al., 2013). We aimed for an equal distribution of inclusions of HCs and patients throughout the year to account for seasonality in immune system activity and BDNF levels (Molendijk et al., 2012; Nelson, 2004). Exclusion criteria for both patient and control group were: substance

abuse, use of anti-inflammatory drugs within 2 weeks before screening or test days, acute infection, autoimmune diseases, chronic inflammatory or neurological diseases, pregnancy or breastfeeding, electroconvulsive therapy (ECT) within 6 months before screening or during follow-up, mental retardation, significant disturbances on a screening blood test evaluating a complete blood count, blood chemistry, fasting glucose, lipid profile, liver, kidney and thyroid function, and serology (human immunodeficiency virus, hepatitis B and C). Urine drug testing was routinely done at screening and repeated on subsequent test days when drug abuse was suspected (e.g. history of substance abuse, unreliable anamnesis). In the control group, additional exclusion criteria were applied: current or past diagnosis of MDD, BD or psychotic syndrome as defined by DSM-IV criteria and BD or psychotic syndrome in a first-degree family member, use of psychopharmacological drugs, benzodiazepines or benzodiazepine-like products. There were no other restrictions regarding medication use.

Participants were recruited between March 2015 and May 2016. The study was approved by the Committee for Medical Ethics of the University Hospital Antwerp and the Antwerp University with protocol number B300201421645. The local ethical committees of the participating centers approved the protocol. All participants agreed to participate in the study and signed informed consent. The study complied with the Declaration of Helsinki.

2.2. Study design

Patients were recruited during an acute mood episode, either depressed (BD-D) or (hypo)manic (BD-M). Patients with mixed features were allocated to the mood state of which the symptoms were most pronounced, according to clinical assessments at screening. All participants were tested for the first time within 1–5 days after screening and a second time at 2 months follow-up. Clinical assessments and blood draws were always done on the same day. During the study period, patients received treatment as usual without intervention of the investigators.

2.3. Clinical assessments

The M.I.N.I.-plus, International Neuropsychiatric Interview, version 5.0.0 was chosen as diagnostic instrument in patients and HCs because of its accurate structured DSM-IV diagnosis and convenience to administer (Sheehan et al., 1998). In patients, mood symptom severity was assessed by the 17-item Hamilton Depression Rating Scaling (HDRS-17) (Hamilton, 1960) and the Young Mania Rating Scale (YMRS) (Young et al., 1978) at screening and on both test days. At screening, threshold scores for inclusion were set at ≥17 for the HDRS-17 or ≥13 for the YMRS, corresponding to moderate depression and hypomania respectively (Vieta, 2009; Zimmerman et al., 2013). Positive psychotic symptoms were evaluated on test days with the positive subscale of the Positive and Negative Syndrome Scale (PANSS) (Kay et al., 1987). In the control group, the occurrence of mood episodes during follow-up was evaluated based on a short screening questionnaire on the 2nd test day. For all participants, we assessed medication use and the occurrence of any exclusion criteria on both test days. All clinical assessments were done by a psychiatrist in training (SvdA) and supervised by a specialist in psychiatry (MM).

2.4. Laboratory assessments

Blood samples were obtained between 8.00 AM. and 10.30 AM. Blood was drawn by venipuncture into an EDTA-coated vacuum tube (5 ml). Tubes were immediately stored at 4 °C and centrifuged at 2g and 4 °C for 10 min within 2 h after the blood draw. Plasma was aliquoted and stored at −70 °C until assayed.

TNF-α, BDNF, VEGF and sFlt-1 were measured in duplicate by an electrochemiluminescence immunoassay technique (Mesoscale