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Peripheral blood mRNA expressions of stress biomarkers in manic episode and subsequent remission

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ABSTRACT

Theoretical models of the neuroprogressive nature of bipolar disorder (BD) are based on the hypothesis that it is an accelerated aging disease, with the allostatic load playing a major role. Glucocorticoids, oxidative stress markers, inflammatory cytokines and neurotrophins play important roles in BD. The messenger ribonucleic acid (mRNA) expressions of brain-derived neurotrophic factor (BDNF), tissue plasminogen activator (tPA), glucocorticoid receptor (GR), heat shock protein 70 (HSP70), tumour necrosis factor-alpha (TNF-α) were examined in the peripheral blood of 20 adult male, drug-free BD patients during manic and remission periods and in 20 adult male, healthy controls. mRNA expression was measured using the quantitative real-time polymerase chain reaction (qRT-PCR). Compared to the controls, the expressions of BDNF and tPA mRNA were down-regulated in mania. In remission, BNDF and tPA mRNA levels increased, but they were still lower than those of the controls. Between mania and remission periods, only the change in mRNA levels of BDNF reached statistical significance. The results suggest that BDNF and tPA may be biomarkers of BD and that proteolytic conversion of BDNF may be important in the pathophysiology of BD. The change in BDNF levels between mania and remission could be adaptive and used to follow the progression of BD.

1. Introduction

Bipolar disorder (BD) is a cyclic and progressive disorder (Belmaker, 2004). In patients with BD, there are symptomatologic differences between manic and depressive periods. The identification of biomarkers of these periods would be useful in the management of the disease.

Neuroprogressive decay within years, together with increased morbidity and mortality may put the disorder in the category of accelerated aging diseases (acceleration of cellular/biological aging). The regeneration ability of the brain and body diminishes with age and with chronic diseases (Rizzo et al., 2014). Progressive exposure to cellular stress also leads to allostatic load (homeostatic derangements). Multiple mediators of adaptation interact with each other and by time over/under production of primary biomarkers (stress hormones and inflammatory cytokines) affect systemic parameters (Juster et al., 2010). In BD, allostasis was proposed to explain the link between disease progression and medical comorbidity (Kapczinski et al., 2008).

In response to acute and chronic stress, the chemistry of the brain changes; its morphology may also change (Jeanneteau and Chao, 2013; McEwen, 2008). These changes are largely reversible if the stress is short term (i.e. weeks) but may be irreversible if the stress is long term (months or years) (Juster et al., 2010; McEwen, 2008). Glucocorticoids, oxidative stress markers, inflammatory cytokines and neurotrophins are thought to play significant roles in the homeostatic regulation (Maletic and Raison, 2014). One of the first responses of the brain against stress is activation of the hypothalamic-pituitary-adrenal (HPA) axis to secrete glucocorticoids. The effects of glucocorticoids are mediated by binding to the intracellular glucocorticoid receptor (GR). In the absence of glucocorticoids, GRs and heat shock proteins (HSPs) remain as a complex in the cytoplasm. In the presence of high levels of glucocorticoids, they bind to GRs, which then dissociate from the HSPs (Bei et al., 2013).

HSPs perform a chaperone function by facilitating protein refolding and maintaining normal cellular homeostasis in response to stress. They are also referred to as stress proteins and up-regulated as part of the stress response (Santoro, 2000). Increased Hsp70 expression is a universal response to cell damage caused by increased oxidative stress (Martinez de Toda and De la Fuente, 2015). Increased expression of the HSP70-GR complex was shown in BD patients as compared to controls. The authors interpreted

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the increase as due to changes in the affinity of HSP70-GR interactions or alterations in the relative amounts of total cellular GR and HSP70 (Bei et al., 2013). HSP70 is also thought to protect the brain, and up-regulation of HSP70 was shown to exert anti-inflammatory effects on the brain by inhibiting the release of pro-inflammatory cytokines (Yu et al., 2015). In the rat hippocampus, acute immobilisation stress led to a decrease in mRNA levels of the brain-derived neurotrophic factor (BDNF) and an increase in the levels of HSP70 (Kim et al., 2014).

A recent meta-analysis of the HPA axis in BD concluded that the disorder was associated with state and trait hyperactivity of the HPA axis, resulting from abnormalities in glucocorticoid signalling. In that meta-analysis, the cortisol levels of the BD patients, especially those with mania, were higher than those of the controls (Belvederi Murri et al., 2015). Cortisol-bound GR functions as a transcription factor to modulate the expression of a variety of genes, including the transcription of many genes involved in inflammatory signalling. Interestingly, high levels of cytokines have been shown to trigger increased secretion of cortisol, which in turn cyclically repressed immune gene expression. A disruption in any part of this pathway led to increased inflammation in the periphery system or central nervous system and consequent cellular damage. According to an earlier study, dysregulation of cytokine mRNA expression in psychiatric disorders may be associated with GR mRNA abnormalities (Fillman et al., 2014). Dysregulation of GR was observed in the brains of both schizophrenia and BD patients (Sinclair et al., 2011).

Stress-induced activation of GR stimulates pro-BDNF and tissue plasminogen activator (tPA) proteins, which induce an increase in mature BDNF, because tPA mediates the conversion of pro-BDNF (apoptotic) to mature BDNF (neuroprotective) (Pang et al., 2004; Revest et al., 2014). tPA is a serine protease produced by the endothelial cells of blood vessels. It converts plasminogen to plasmin, which is responsible for the removal of fibrin deposits, and is used as a thrombolytic agent for myocardial infarction and stroke. tPA is also found in neurons and functions independently of plasminogen-plasmin conversion (Yepes, 2015). Both BDNF and tPA contribute to synaptic plasticity and have neuroprotective effects (Rothman and Mattson, 2013; Yepes, 2015). In animal models, tPA was associated with both acute and chronic stress responses (Melchor et al., 2003; Pawlak et al., 2005). The ratio of mature and pro-BDNF and the levels of mature BDNF were higher in patients with BD than in controls. However, in the same study, serum pro-BDNF levels were lower in the BD patients compared to the controls, which suggests that the alteration in the conversion of the pro to the mature form of BDNF may be associated with the pathophysiology of BD (Sodersten et al., 2014).

A recent meta-analysis showed that compared to healthy controls, peripheral blood BDNF levels were decreased in bipolar episodes and increased after treatment of acute manic but not depressive episodes. BDNF levels were negatively correlated with the severity of either episode, but they were not associated with the duration of the illness. The authors suggested that peripheral BDNF may be used in the future as part of a blood protein composite measure to assess disease activity in BD (Fernandes et al., 2015). In pre-clinical models, BDNF modulated the coupling of neurogenesis and vasculogenesis, and vascular levels of BDNF mRNA expression were comparable to those in the brain (Li et al., 2006). According to a previous study, BDNF and endothelial function may share a bidirectional association, as vascular endothelial cells produce BDNF (Nakahashi et al., 2000). Recent findings from the Collaborative Depression Study cohort indicated that after controlling for age, gender and smoking, manic symptoms were associated with poorer endothelial function, whereas this association was not observed for depressive symptoms (Fiedorowicz et al., 2012).

Kapczinski et al. (2011) proposed a 'systemic toxicity index' composed of the following dimensions: neurotrophins, oxidative stress markers and inflammatory markers. They concluded that peripheral markers of allostatic adaptations in BD may aid understanding of the pathophysiology and progression of this disorder. Further, they suggested that state markers could help in differentiating between different episodes of BD or that they may only be useful when measured during a specific mood episode.

Most previous studies consisted of mixed groups of BD patients (mania, hypomania, depression or euthymia) and controls (Fernandes et al., 2015; Frey et al., 2013; Goldstein and Young, 2013; Kapczinski et al., 2011). According to a literature search, no previous human studies have examined the relationship between BD and tPA. As mania is a clinical hallmark of BD (Frey et al., 2013), the present study included only BD patients with mania. The hypothesis of the present study was that BDNF and tPA gene expression would be altered in manic patients. To shed light on the neurogenesis process, we selected the studied possible biomarkers, focusing on evidence based on findings in peripheral blood (Fernandes et al., 2015; Frey et al., 2013; Goldstein and Young, 2013; Kapczinski et al., 2011; Leboyer et al., 2012; Maletic and Raison, 2014; Rizzo et al., 2014). In the present study, the mRNA expressions of BDNF, tPA, the GR, HSP70 and tumour necrosis factor-alpha (TNF-α) were examined in the peripheral blood of adult male, drug-free BD patients during manic and remission periods and a healthy male control group.

2. Methods

2.1. Study design and participants

This was a case-control study with a prospective design. The study consisted of 20 male, drug-free manic patients and 20 age- and gender-matched healthy controls. Males were selected as the study group to eliminate effects of the menstrual cycle on the studied parameters and to avoid confounding of the results by gender bias. To exclude potential age- and weight-related differences, inclusion criteria included being 19–45 years of age and having normal weight. The body mass index (BMI) was defined as the body mass divided by the square of the body height, with normal weight considered 18.5–25 kg/m^2. The diagnosis of BD I and lack of psychiatric diagnoses in the controls were confirmed through use of the Structured Clinical Interview for Diagnostic and Statistical Manual of Mental Disorders, fourth edition (SCID-I). The patients were assessed additionally by Young Mania Rating Scale (YMRS) and Hamilton Depression Rating Scale (HDRS). BD patients with a total score of $\geq$25 on the YMRS (defining markedly ill) and a score of $\leq$7 on the HDRS were recruited from our inpatient clinic.

Exclusion criteria for the patient and the control groups were major medical diseases, including cardiovascular and endocrine diseases, especially diabetes, obesity and metabolic syndrome. Patients with neurological diseases, including epilepsy and a history of stroke, were also excluded. For both medical and neurological diseases, exclusion was based on the results of general medical and neurological examinations. Patients and controls with mental retardation, according to a psychiatric examination, current or past substance abuse and drug dependence, except nicotine were excluded, as well as those with active infection (verified by total cell counts and C-reactive protein levels) and a history of head injury, with cognitive sequelae.

Psychotrophic drug-using patients were also excluded. Purification periods for psychotrophic drug were 2 weeks for oral drugs, 1 month for parenteral drugs and blood levels of 0 for mood stabilisers. Control subjects having first-degree relatives with BD, schizophrenia or other psychotic disorders were excluded. All the patients and controls stated that they were not on a diet and that they did not take part in strenuous exercise activity. They stated