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Elevated neurotrophin-3 and neurotrophin 4/5 levels in unmedicated bipolar depression and the effects of lithium

Alexandre A. Loch [a], Marcus V. Zanetti [a,b,c], Rafael T. de Sousa [a], Tiffany M. Chaim [c], Mauricio H. Serpa [c], Wagner F. Gattaz [a,b], Antonio L. Teixeira [e], Rodrigo Machado-Vieira [a,b,d,*]

[a] Laboratory of Neuroscience, LIM-27, Institute and Department of Psychiatry, University of Sao Paulo, Brazil
[b] Center for Interdisciplinary Research on Applied Neurosciences (NAPNA), University of Sao Paulo, Brazil
[c] Laboratory of Psychiatric Neuroimaging, LIM-21, Department and Institute of Psychiatry, University of Sao Paulo, Brazil
[d] Experimental Therapeutics and Pathophysiology Branch (ETPB), National Institute of Mental Health, NIH, Bethesda, MD, USA
[e] Interdisciplinary Laboratory of Medical Investigation, Faculty of Medicine of Minas Gerais, Belo Horizonte, Brazil

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ABSTRACT

Background: Bipolar disorder (BD) has been associated with diverse abnormalities in neural plasticity and cellular resilience. Neurotrophin-3 (NT-3) and neurotrophin-4/5 (NT-4/5) support synaptic neuronal survival and differentiation. NT-3 and NT-4/5 levels were found to be altered in BD, potentially representing a physiological response against cellular stress. However, the use of psychopharmacological agents and heterogeneous mood states may constitute important biases in such studies. Thus, we aimed to assess NT-3 and NT-4/5 levels in medication-free BD type I or II individuals in a current depressive episode, before and after 6weeks of lithium monotherapy and matched with healthy controls.

Methods: Twenty-three patients with BD type I or II during a depressive episode and 28 healthy controls were studied. Patients were required to have a 21-item Hamilton Depression Rating Scale score ≥18 and had not undergone any psychopharmacological treatment for at least 6 weeks prior to study entry. Patients were treated with lithium for 6 weeks and plasma NT-3 and NT-4/5 levels were determined at baseline and endpoint using ELISA method.

Results: Baseline plasma levels of both NT-3 and NT-4/5 were significantly increased in acutely depressed BD subjects in comparison to healthy controls ($p = 0.040$ and 0.039, respectively). The NT-3 and NT-4/5 levels did not significantly change after lithium treatment. NT-3 and NT-4/5 levels were positively correlated to illness duration in BD ($p = 0.032$ and 0.034, respectively).

Conclusion: Our findings suggest that NT-3 and NT-4/5 levels are increased in the depressive phase of BD, which seems directly associated with illness duration. The increased levels of NT-3 and NT-4/5 may underlie a biological response to cellular stress associated with the course of BD.

1. Introduction

Bipolar disorder (BD) is a chronic, severe and disabling disorder affecting neural plasticity and cellular resilience at multiple levels (Machado-Vieira et al., 2013). Neurotrophins (NT) are thought to play a major role in such neuronal processes, since they are implicated in neuronal growth, development and plasticity (Lu and Figurov, 1997). NTs exert their actions through the tyrosine kinases (Trk) family of receptors, which activate gene expression and generate products that are essential for neurite outgrowth and neuronal survival (Huang and Reichardt, 2001).

Abnormalities in the production/release of NTs during acute mood episodes have been described (Barbosa et al., 2014; Braga et al., 2009; Hashimoto et al., 2004; Kapczinski et al., 2008; Machado-Vieira et al., 2007). In this regard, brain-derived neurotrophic factor (BDNF) has been the most studied NT in BD. A recent review has shown that BDNF is usually decreased in the plasma of BD individuals during mood episodes, when compared to euthymic patients and healthy controls (Scola and Andreazza, 2015). Mood stabilizers are thought to normalize BDNF levels. Recently NT-3 and NT-4/5 have also become a focus of investigation in mood disorders because of their role in neuronal processes and association with antidepressant actions (Altar,

Abbreviations: BD, Bipolar Disorder; NT, Neurotrophins; BDNF, brain-derived neurotrophic factor; Trk, tyrosine kinases; BD-1, Bipolar Disorder I; YMRS, Young Mania Rating Scale; CGI, Clinical Global Impression; HAM-D, Hamilton Depression Rating Scale; DSM-IV, Diagnostic Structured Manual IV Edition; ANOVA, Analysis of variance.

* Corresponding author at: Laboratory of Neuroscience (LIM27), Department and Institute of Psychiatry, University of Sao Paulo, Rua Dr. Ovídio Pires de Campos, 785, Sao Paulo, Brazil.
E-mail address: machadovieirar@gmail.com (R. Machado-Vieira).

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1999; Duman, 2004). Moreover, as they cross the brain–blood barrier and show strong correlation between brain and peripheral levels, these may be good candidates to be assessed in clinical samples (Pan et al., 1998). Previous studies investigating NT-3 levels in BD (Walz et al., 2007) found elevated NT-3 levels in both manic and depressed medicated BD patients. Fernandes et al. (2010) also observed increased NT-3 levels in BD subjects during the acute phases of BD. Meanwhile, NT-4/5 has been shown to specifically protect dopaminergic neurons (Hyman et al., 1994; Sauer et al., 1995). Serum NT-4/5 levels were increased in 154 BD patients relative to healthy controls, regardless of their mood phase (euthymia, depression or mania) (Walz et al., 2009). It was proposed that the increased NT-3 and NT-4/5 might represent a compensating cellular mechanism for ongoing increased oxidative stress observed during mood phases (Walz et al., 2009). Nevertheless, these studies mostly evaluated BD patients taking psychotropic medications and at different phases of the illness. Also, previous studies have assessed NT-3 and NT-4/5 levels only in patients with BD type I (BD-I).

The aim of our study was to assess plasma NT-3 and NT-4/5 levels in medication-free BD individuals during an acute depressive episode and after 6 weeks of lithium treatment. We hypothesized that NT-3 and NT-4/5 would be increased in these acutely depressed BD patients relative to the controls, possibly due to a physiological response to neuronal insult associated with mood phase and illness course.

2. Methods

2.1. Subjects

Twenty-three drug-free patients were recruited at the Mood Disorders Program, LIM-27, Institute of Psychiatry, University of Sao Paulo, Brazil, between August 2010 and June 2012. Male and female outpatients, aged 18–45, were eligible for study entry. Inclusion criteria comprised a DSM-IV diagnosis of BD type I or II at an acute depressive episode according to the SCID interview (First et al., 1997), and a 21-item Hamilton Depression Rating Scale (HAM-D) score ≥ 18. Also, patients had less than three lifetime mood episodes and no more than 5 years of illness duration at study entry. Subjects had not undergone any psychopharmacological treatment for at least 6 weeks prior to the study entry. Exclusion criteria specific for the patients included: (a) rapid cycling; (b) current substance abuse or dependence; and (c) previous electroconvulsive therapy.

Twenty-eight healthy volunteers were recruited through advertisement in the local community and constituted our control group. Controls were excluded if they had a positive lifetime history for any mental disorder (as assessed with the SCID), including substance abuse or dependence, or any first-degree relative with an axis I mental disorder.

The exclusion criteria common for both patients and controls were presence of neurological disorders or any medical disorder that could affect the central nervous system, and mental retardation. The study was approved by the local institutional ethics committee and all patients provided written consent before study entry.

2.2. Clinical procedures

Patients collected blood at baseline and after lithium treatment, while controls had only one-point collection. BD subjects were submitted to clinical evaluation by experienced psychiatrists, who conducted the SCID and clinical assessments with the following clinical instruments: the aforementioned HAM-D, the Young Mania Rating Scale (YMRS) and the Clinical Global Impression (CGI). Inter-rater reliability was over 0.9 for all of them.

All BD patients were then started on treatment with oral lithium (450 mg/day) and a systematic follow-up was carried out for 6 weeks. Subsequent dosage adjustments were permitted in a flexible manner to a dose of ≤ 900 mg/day, based on the clinical efficacy of individual patients and the level of lithium in their plasma (in order to ensure compliance and prevent risk of intoxication). After 6 weeks of treatment clinical assessment and blood collection were repeated.

2.3. Assays

Blood samples were collected from 8:00 to 10:00 AM using vacutainer tubes. All subjects were in 8-hour fasting. Samples were centrifuged at 20 °C and 1620 $\times g$ for 15 min. Plasma was obtained, frozen, and stored at −80 °C. NT-3 and NT-4/5 levels were determined using ELISA according to the procedures provided by the manufactures (R&D Systems, Minnesota, MN). All the samples were assayed in duplicate. The lower level of detection was 10 pg/ml. NT-3 and NT-4/5 levels are presented as pg/ml.

2.4. Statistical analyses

Analysis of variance (ANOVA) was used to determine age difference between patients and controls; Chi-square test was used to determine between-samples gender differences. Backwards stepwise logistic regression models were used to assess NT differences between patients and controls. Group (patients versus controls) was set as the dependent variable, and NT levels and gender were set as independent variables. Gender was included in the model as an independent variable because it significantly differed between patients and controls, and once it might affect NT levels (Begliuomini et al., 2007). Possible differences in NT-3 and NT-4/5 levels between controls and each subtype of BD were tested by repeating the analyses with BD subtype I (BD-I) or BD subtype II (BD-II) diagnosis separately included as dependent variables.

Also, as associations between NT levels, tobacco smoking (Bus et al., 2011) and the season in which blood have been collected (Molendijk et al., 2012) have been reported in the literature, these factors were also assessed in our sample.

In order to evaluate which factors were related to NT levels within the BD group, Mann–Whitney tests were used for categorical variables, whereas Spearman correlations were used for continuous variables. NT-3 and NT-4/5 levels pre and post lithium therapy were compared using paired samples Student's t test. Linear mixed models searched for possible correlations between NT levels variance and HAM-D score improvement. Level of significance (p-value) was set at 0.05 for all tests. All statistical analyses were conducted in the SPSS 18.0 software.

3. Results

Sample characteristics are presented in Table 1. Subjects with BD and controls had a similar age distribution ($F = 0.025$, $p = 0.87$). BD group had significantly more males than the control group ($\text{chi}^2 = 7.07$, $df = 1$, $p = 0.01$). Most of the patients were treatment naïve (82.6%) and illness duration varied from 1 to 60 months (mean = 36.5 months). Eight patients (34.8%) had a diagnosis of BD-I and 15 (65.2%) had the diagnosis of BD-II.

Table 1
Clinical and demographic characteristics.

Sociodemographic and clinical measures	BD patients $n = 23$	Controls $n = 28$	p
Age (range, mean; years)	18–43 (28.0)	17–51 (28.3)	0.87
Gender (male; %)	4 (17.4%)	13 (46.4%)	0.01
Treatment-naïve (yes; %)	19 (82.6%)	–	
Duration of illness (range, mean; months)	1–60 (36.5)	–	
Bipolar disorder type I (n; %)	8 (34.8%)	–	
Psychotic symptoms (yes; %)	3 (13.0%)	–	
Baseline HAM-D (range; mean)	18–28 (22.5)	–	
Endpoint HAM-D (range; mean)	0–30 (8.6)	–	
Tobacco use (yes; %)	4 (17.4%)	0 (0.0%)	0.02