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Decreased brain-derived neurotrophic factor in medicated and drug-free bipolar patients

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ABSTRACT

Bipolar disorder (BD) has been associated with abnormalities in neuroplasticity and previous studies suggest an important role for BDNF in the pathophysiology of BD. The confounding effect of the use of medication in these studies has been considered a limitation. Thus, studies with both drug-free and medicated patients are necessary to assess the role of medication in serum BDNF levels. Twenty-two manic and depressed drug-free and 22 medicated BD type I patients were matched to 22 controls according to sex and age in a cross-sectional study. BDNF serum levels were assessed using sandwich-ELISA. Serum BDNF levels in drug-free (0.23 ± 0.09), and medicated (0.29 ± 0.19) BD patients were decreased when compared to controls (0.40 ± 0.12) – drug-free/medicated vs. control $p < 0.001$. The BDNF levels did not differ between medicated and drug-free BD patients. When analyzing patients according to mood states, serum BDNF levels were lower in BD patients during both manic (0.28 ± 0.11) and depressive episodes (0.22 ± 0.17), as compared with healthy controls (0.40 ± 0.12) – manic/depressed patients vs. controls $p < 0.001$. Results suggest that the association of lower serum BDNF and BD mood episodes is kept even in medicated patients, which strengthens the notion that BDNF serum levels may be considered a biomarker of mood episodes in BD.

1. Introduction

Bipolar disorder (BD) is a prevalent, highly disabling and chronic illness, characterized by the presence of manic and depressive symptoms (Yatham et al., 2009). Although genetic and familial studies strongly suggest that a neurobiological basis may underlie the pathophysiology of BD, its etiology is still poorly understood. In the last years, BD has been associated with impairments in neuroplasticity and cell survival. Brain-derived neurotrophic factor (BDNF) is a member of the nerve growth factor family, which is involved in promoting synaptic efficacy, neuronal connectivity and neuroplasticity (Post, 2007). BDNF regulates neuronal development and survival and controls the activity of many neurotransmitters, including the serotoninergic, dopaminergic and glutamatergic systems (Cotman and Berchtold, 2002). Studies support the notion that changes in BDNF levels may be involved in the pathophysiology of BD (Kapczinski et al., 2008). There is a growing body of evidence showing that serum BDNF is decreased during manic and depressive BD episodes (Cunha et al., 2006; Machado-Vieira et al., 2007; Gama et al., 2007; Monteleone et al., 2008; Tramontina et al., 2009).

A question that frequently emerges in studies about serum BDNF levels in BD is the potential bias related to the use of medication. This is considered an important limitation in most of the studies, since BDNF levels can be increased by antidepressants, mood stabilizers and atypical antipsychotics (Chen et al., 2001; Shimizu et al., 2003; Cunha et al., 2006; Sen et al., 2008). Initially in a study with medicated patients, Cunha et al. (2006) were able to show that in manic and depressive patient's serum BDNF levels were lower than in controls. In this same vein, Machado-Vieira et al. (2007) showed that plasma BDNF is decreased in drug-free manic patients. In a recent study, Monteleone et al. (2008) suggested that euthymic patients may present lower levels of BDNF regardless the medication status. However, Monteleone et al. (2008) did not separate major depression disorder (MDD) from BD types I and II in his analysis. Thus, the question whether type

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I BD patients present lower levels of BDNF during mood episodes regardless the use of medication remained unanswered.

The aim of this study is to assess whether drug-free patients have differential levels of circulating serum BDNF levels, when compared to medicated BD patients and controls. We hypothesized a priori that serum BDNF levels would be decreased in medicated and in drug-free BD patients as compared to healthy subjects, and that BDNF changes would be more pronounced in drug-free patients than in medicated patients. To this purpose, we assessed serum BDNF concentrations in medicated and drug-free BD type I during manic and depressive episodes.

2. Methods

Manic and depressed drug-free and medicated bipolar type I patients were recruited at the Bipolar Disorders Program and Psychiatry Inpatient Unit – Hospital de Clinicas de Porto Alegre, and Pronto Atendimento da Vila Cruzeiro do Sul, Porto Alegre, Brazil. Diagnosis of BD, manic and depressive episodes were established according to Structured Clinical Interview for DSM-IV-Axis I Disorders (SCID-I) (First et al., 1998). Severity of manic and depressive episodes was evaluated using the Young Mania Rating Scale (YMRS) (Young et al., 1978) and Hamilton Depression Rating Scale (HDRS) (Hamilton, 1960), respectively. To be included in the study, patients should present a manic or depressive episode according to DSM-IV and SCID-I criteria.

Twenty-two drug-free patients were included in this study. Patients did not receive any psychotropic medication for at least two weeks (five weeks if use of fluoxetine or *depot* medication) before blood withdrawn. The 22 bipolar drug-treated patients and the 22 controls included were matched by age and gender to the drug-free bipolar subjects. Patients who were drug-free and medicated presenting severe or unstable clinical illnesses were excluded by means of a clinical interview. Psychiatric assessment in controls was carried out using SCID-I, non-patient version. Control subjects were recruited in the same hospital, among relatives of patients submitted to minor surgery; controls were not on medication, and had no history of major psychiatric disorders, dementia or mental retardation in their first-degree relatives. The 'Hospital de Clinicas de Porto Alegre' Ethics Committee approved the study and all subjects provided written informed consent before entering the study.

Five milliliters of blood were withdrawn from each subject by venipuncture into a free-anticoagulant vacuum tube. The blood was immediately centrifuged at 4000*g* for 10 min, and serum was kept frozen at −80 °C until assayed. BDNF serum levels were measured with sandwich-ELISA, using a commercial kit according to the manufacturer's instructions (Chemicon, USA). Briefly, microtiter plates (96-well flat-bottom) were coated for 24 h with the samples diluted 1:25 in sample diluents and standard curve ranged from 7.8 to 500 pg of BNDF. Plates were then washed four times with wash buffer, added monoclonal anti-BNDF rabbit antibody (diluted 1:1000 with sample diluents), and incubated for 3 h at room temperature. After washing, a second incubation with anti-rabbit antibody peroxidase conjugated (diluted 1:1000) for 1 h at room temperature was carried out. After addition of streptavidin-enzyme, substrate and stop solution, the amount of BDNF was determined (absorbance set in 450 nm). The standard curve demonstrates a direct relationship between optical density (OD) and BDNF concentration. Total protein was measured by Lowry's method using bovine serum albumin (BSA) as a standard.

Statistical analysis was performed using SPSS 16.0 for Windows. Most of the BDNF values were fitted in a standard distribution curve and were therefore subjected to parametric analyses. All values are presented as mean ± standard deviation (SD), except male gender and presence of psychosis. For the comparisons between the groups, one-way analysis of variance (ANOVA) test with individual differences assessed using a Tukey post-test if the ANOVA was significant, and independent *t* tests were used. Pearson correlation coefficient was used to analyze the correlation between BDNF and YMRS and HDRS scores. *p*-Values < 0.05 two-tailed were considered statistically significant.

3. Results

Patients with BD, and controls, were recruited from January 2007 up to December 2008. The characteristics of drug-free and medicated BD patients and controls are summarized in Table 1. Medicated and drug-free patients and controls were similar in terms of gender and age; medicated and drug-free patients were similar in terms of presence of psychosis, age of first mood episode and length of illness, YMRS score, and HDRS score. Of the 22 medicated patients, 18 (81.82%) were on mood stabilizers, 15 (68.18%) on antipsychotics, and 8 (36.36%) on antidepressants. BDNF levels in serum among drug-free and medicated BD patients were decreased when compared to controls. BDNF levels did not differ between medicated and drug-free BD patients (Table 1, Fig. 1).

When analyzing patients according to mood states, there were no differences between manic ($n = 24$) and depressed ($n = 20$) patients, regarding gender, age, and length of illness (data not shown). As expected, manic patients had significantly higher rates on YMRS than depressed patients (32.21 ± 10.15 vs. 3.85 ± 3.90, respectively, $p < 0.001$) and depressed patients had significantly higher rates on HDRS than manic patients (21.45 ± 7.68 vs. 6.88 ± 6.99, respectively, $p < 0.001$). In addition, manic patients had significantly more psychotic symptoms (presence of delusions or hallucinations) than depressed BD subjects [20/24 (83.3%) vs. 7/20 (35.0%), respectively, $p = 0.001$]. Serum BDNF levels were lower in BD patients during both manic ($p < 0.001$) and depressive ($p < 0.001$) episodes, as compared with healthy controls (0.28 ± 0.11, 0.22 ± 0.17 and 0.40 ± 0.12, respectively, $p < 0.001$ for manic/depressed patients vs. controls) (Fig. 2).

A significant negative correlation between severity of manic symptoms (YMRS scores) and serum BDNF levels was observed in manic medicated and drug-free patients ($r = -0.45$, $p = 0.002$); when analyzing only the manic drug-free patients, the correlation was slightly greater ($r = -0.56$, $p = 0.001$). In the manic medicated group there was a trend towards a correlation ($r = -0.30$, $p = 0.08$). In addition, serum BDNF levels were negatively correlated to depressive symptoms (HDRS scores) in drug-free and drug-treated depressed patients ($r = -0.33$, $p = 0.036$); in the drug-free depressed patients the correlation was again slightly higher ($r = -0.50$, $p = 0.004$); in the drug-treated depressed patients the correlation was not significant ($r = -0.23$, $p = 0.21$).

4. Discussion

To the best of our knowledge, this is the first study assessing serum BDNF levels in drug-free BD patients during manic and depressive episodes as compared to medicated patients. We were able to demonstrate that serum BDNF is decreased in drug-free and drug-treated BD subjects during manic and depressive episodes. Moreover, drug-free and medicated BD patients presented similar level of serum BDNF levels.

In previous studies, our group and others showed that serum BDNF levels were lower in medicated type I BD patients (Cunha et al., 2006); such findings were supported by Machado-Vieira et al. (2007) which showed that plasma BDNF was decreased in drug-free manic patients. In a recent study, Tramontina et al. (2009) showed that serum BDNF levels increase after treatment for acute mania, which provides preliminary evidence that the

Table 1
Characteristics of the drug-free and medicated bipolar patients, and controls.

Characteristics	Group*			p-Value
	Drug-free patients	Medicated patients	Controls	
Male sex – no. (%)				
All patients	5/22 (22.7)	5/22 (22.7)	5/22 (22.7)	1.000[a]
Manic patients	4/12 (33.3)	4/12 (33.3)	4/12 (33.3)	1.000[a]
Depressive patients	1/10 (10.0)	1/10 (10.0)	1/10 (10.0)	1.000[a]
Age – years				
All patients	39.82 ± 12.85 (22)	40.00 ± 8.90 (22)	39.68 ± 10.77 (22)	0.840[b]
Manic patients	41.83 ± 13.53 (12)	43.83 ± 9.47 (12)	42.84 ± 11.46 (22)	0.679[b]
Depressive patients	35.00 ± 11.19 (10)	35.40 ± 5.68 (10)	35.24 ± 8.07 (22)	0.930[b]
Length of illness – years				
All patients	14.06 ± 8.81 (22)	13.87 ± 9.26 (22)	–	0.860[c]
Manic patients	16.00 ± 8.19 (12)	11.75 ± 9.18 (12)	–	0.290[c]
Depressive patients	12.12 ± 9.52 (10)	16.00 ± 8.37 (10)	–	0.330[c]
Presence of psychosis				
All patients	15/22 (68.2)	12/22 (54.6)	–	0.270[a]
Manic patients	11/12 (91.7)	9/12 (75.0)	–	0.290[a]
Depressive patients	4/10 (40.0)	3/10 (30.0)	–	1.000[a]
YMRS score				
Manic patients	31.92 ± 9.71 (12)	32.50 ± 11.00 (12)	–	0.890[c,d]
Depressive patients	3.00 ± 3.19 (10)	4.70 ± 7.85 (10)	–	0.530[c,d]
HDRS score				
Manic patients	8.42 ± 7.13 (12)	5.33 ± 6.81 (12)	–	0.290[c,d]
Depressive patients	23.40 ± 7.53 (10)	19.50 ± 7.71 (10)	–	0.270[c,d]
BDNF in pg/μL				
All patients	0.23 ± 0.09 (22)	0.29 ± 0.19 (22)	0.40 ± 0.12 (22)	0.001[b,e]
Manic patients	0.25 ± 0.08 (12)	0.33 ± 0.14 (12)	0.40 ± 0.12 (22)	0.003[b,e]
Depressive patients	0.21 ± 0.10 (10)	0.24 ± 0.24 (10)	0.40 ± 0.12 (22)	0.003[b,e]

Abbreviations: YMRS (Young Mania Rating Scale); HDRS (Hamilton Depression Rating Scale); BDNF (brain-derived neurotrophic factor).
* Columns show mean ± standard deviation (SD) for all categories except male sex and presence of psychosis. The number of participants in each group is shown in parenthesis.
[a] Qui-square test.
[b] One-way ANOVA test with Tukey post-test.
[c] Unpaired *t* test.
[d] YMRS and HDRS in drug-free patients = medicated patients for the manic and depressive patients.
[e] BDNF in controls > drug-free patients/medicated patients for all patients, and for the manic and depressive patients separately.

key factor for restoring BDNF serum levels may not be the medication itself, but rather, clinical response.

There are some limitations in this study. Firstly, we measured BDNF levels in serum. Although the specific cellular sources of serum BDNF are still unknown, it has been reported that platelets, vascular endothelial cells and neurons may contribute to the circulating BDNF content. However, it has been demonstrated that BDNF can cross the brain–blood barrier, and there is a high positive correlation between serum and cortical BDNF levels (Karege et al., 2005). Therefore, it has been suggested that the changes of serum BDNF levels may reflect changes in brain BDNF levels (Pan et al., 1998). Secondly, the manic and depressed drug-free patients had slightly lower serum BDNF levels, when compared to drug-treated patients (not statistically significant). Thus, lack of difference between medicated and drug-free BD patients may be a type II error.

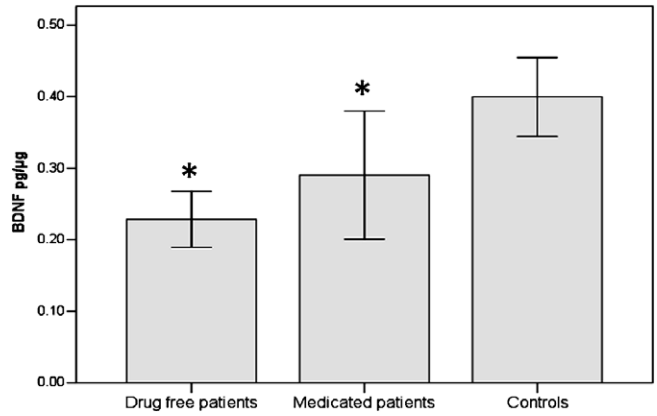

Fig. 1. Serum brain-derived neurotrophic factor (BDNF) levels in pg/μg in bipolar disorder (BD) patients during manic or depressive episodes, medicated or drug-free as compared to healthy controls. Data are expressed as mean and 95% Confidence Interval (95% CI). $^{*}p < 0.001$ for drug-free/medicated patients vs. control (one-way ANOVA with Tukey post-test).

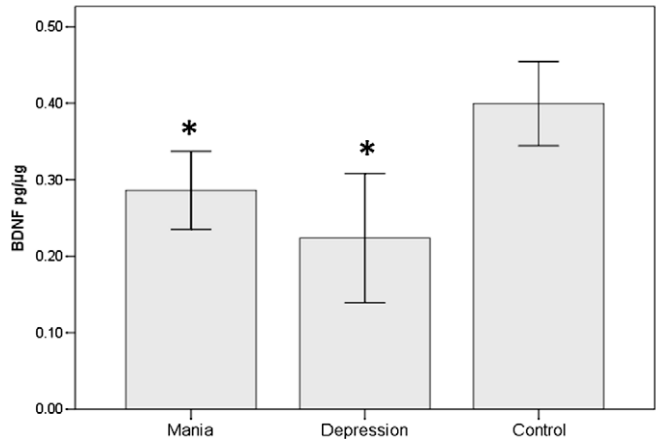

Fig. 2. Serum brain-derived neurotrophic factor (BDNF) levels in pg/μg in drug-free and in medicated bipolar disorder (BD) patients during manic or depressive episodes, and in healthy controls. Data are expressed as mean and 95% Confidence Interval (95% CI). $^{*}p < 0.001$ for drug-free/medicated patients vs. control (one-way ANOVA with Tukey post-test).

In conclusion, the present findings confirm previous studies reporting that serum BDNF levels were decreased in medicated and drug-free BD patients during manic episodes, as compared to healthy controls; Moreover, our study showed that serum BDNF levels are decreased in medicated as well as in drug-free depressed and manic patients.

Contributors

GSO designed the study, wrote the protocol, participated in data acquisition and interpretation, drafting the article and final approval of this version. BF designed the study, was responsible for the analysis and interpretation of data, drafting the article and final approval of this version. LS, GRF, BA, BP participated in study design, data acquisition and final approval of this version. KMC designed the study, wrote the protocol, participated in data acquisition and final approval of this version. FK was responsible for study design and interpretation of data, drafting the article and final approval of this version.

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Conflict of interest statement

Flavio Kapczinski has received research grants from CNPq, CAPES, SMRI, NARSAD, Lilly, AstraZeneca, and Janssen. Marcia Kauer-Sant'Anna has received research grants from AstraZenica, FIPE-HCPA, CNPq, CAPES, SMRI, NARSAD, and Lilly. Brisa Fernandes, Gislaine Oliveira, Keila Cereser, Bianca Aguiar, Bianca Pfaffenseller, Laura Stertz and Gabriel Fries have declared no conflict of interest.

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