Journal of Affective Disorders 171 (2015) 167–170

Contents lists available at ScienceDirect

Journal of Affective Disorders

journal homepage: www.elsevier.com/locate/jad

Brief report

Decreased plasma levels of brain-derived neurotrophic factor (BDNF) during mixed episodes of bipolar disorder

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ARTICLE INFO

Article history:
Received 12 March 2014
Accepted 29 August 2014
Available online 28 September 2014

Keywords:
BDNF
Bipolar disorder
Mixed state
Bipolar spectrum
DSM-5
Biomarkers

ABSTRACT

Background: Brain-derived neurotrophic factor (BDNF) is a neurotrophin involved in neurogenesis and neuroplasticity. Decreased blood levels of BDNF have been found during acute manic and depressive states. BDNF has been proposed as a biomarker in illness phases of mood disorders. No information is available regarding BDNF levels during the mixed states of bipolar disorder (BD). The aim of this study was to evaluate BDNF levels during mixed episodes of BD patients and compare them with those of healthy subjects and depressed patients.
Methods: Plasma BDNF levels were measured by an ELISA assay in 18 patients with major depressive episode (MDE), 19 patients with mixed episode (ME) and 15 healthy subjects (HS).
Results: BDNF levels were significantly higher in HS, as compared with patients' samples (HS *vs.* MDE patients: $p < 001$; HS *vs.* ME patients: $p = .022$). No significant differences were found between BDNF levels of ME and MDE patients. The severity of illness as assessed by CGI-S was significantly higher in ME than in MDE patients ($p = .01$).
Limitations: The small sample size may have weakened the power of statistical analyses. All patients received mood-stabilizing and antidepressant treatments which have been reported to influence peripheral BDNF levels.
Conclusions: Our results are consistent with previous studies showing reduced BDNF during both manic and depressive episodes. This finding supports the role of BDNF as a state-marker of mood episodes, and may represent a contribution to a unitary approach model between unipolar and BDs, as well as to the manic-depressive spectrum model.

1. Background

According to the neurotrophic hypothesis, stress and depression are likely to be associated with a neurotrophins deficit leading to neuronal atrophy and cell loss in key limbic areas and in prefrontal cortex. Antidepressant treatments can block or reverse these effects (Sapolsky, 2001; Sheline et al., 2003; Banasr et al., 2011).

A particular attention has been devoted to brain-derived neurotrophic factor (BDNF), a neurotrophin involved in differentiation and survival of neurons, as well as in modulation of synaptic plasticity (Sermasi et al., 2000; Poo, 2001; Popoli et al., 2002). Although the exact relationship between central and peripheral BDNF pools is still unclear, the permeability of the blood–brain barrier to BDNF was demonstrated (Pan et al., 1998). In addition, Karege et al. (2002) reported the twin-course of central and peripheral BDNF levels during the central nervous system (CNS) development in rats. Several recent works analyzed peripheral (serum and/or plasma) BDNF levels in samples of psychiatric patients, with the aim to assess their potential role as biomarkers for mood disorders. Peripheral BDNF levels resulted significantly decreased in patients suffering from major depressive disorder (MDD), compared with healthy subjects. Conversely, BDNF levels returned to the control levels after effective treatments and symptom remission (Piccinni et al., 2008a, 2009; Sen et al., 2008). Moreover, low BDNF levels were shown to be related both to recurrence and severity of depressive episodes (Dell'Osso et al., 2010).

Recent meta-analysis of clinical data suggest that peripheral BDNF levels are significantly reduced during manic and depressive,

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http://dx.doi.org/10.1016/j.jad.2014.08.058
0165-0327/

but not in euthymic phases of bipolar disorder (BD) (Lin, 2009; Fernandes et al., 2011). In particular, BDNF levels increased after treatments leading to clinical recovery of acute mania or depression. In addition, Fernandes et al. (2011) found that the differences between euthymic and non-euthymic states in BD become less evident with aging and duration of illness. These data suggest peripheral BDNF levels as potential biomarkers of mood (depressive and manic) episodes, as well as predictors of antidepressant effectiveness and evaluation of disease progression.

However, the current literature is lacking regarding BDNF levels during the mixed episode of BD patients. The aim of the present study was, therefore, to assess BDNF plasma levels in a sample of patients with mixed episode, and compare them with those of depressed patients and healthy subjects.

2. Methods

2.1. Methods: subjects

Eighteen inpatients (9 men and 9 women, mean age ± SD: 44.9 ± 17 years) suffering from a current major depressive episode (MDE) (16 BD, 2 MDD), and 19 inpatients (10 men and 9 women, mean age ± SD: 38.2 ± 9.96 years) from mixed episode (ME), all with or without psychotic features, were consecutively recruited at the Psychiatric Unit of the Department of Clinical and Experimental Medicine, University of Pisa. Diagnoses were assessed according to DSM-IV-TR (American Psychiatric Association, 2000) criteria. Exclusion criteria for patients were: age lower than 18 years, presence of a major neurological or medical illness, diagnosis of substance abuse in the last 6 months, pregnancy, inability to sign informed consent or presence of neurological disorders. Both MDE and ME patients were maintained on the same drug treatment for at least four weeks before the blood collection to avoid a potential effect of treatment changes on central and peripheral BDNF levels.

Fifteen healthy subjects (HS, 3 men and 12 women, mean age ± SD: 36.9 ± 9.2 years) were recruited as the control group. Exclusion criteria for healthy subjects were: age lower than 18 years, history of past or current major medical or mental disorders, heavy cigarette smoking, regular medication and drug abuse. Written informed consent was obtained from each subject to participate in the study. The study was approved by the Ethics Committee of the University of Pisa in accordance with the Declaration of Helsinki (1996).

2.2. Methods: clinical assessment

Clinical diagnosis was confirmed by means of the Mini International Neuropsychiatric Interview (MINI) (Sheehan et al., 1998).

The severity of depressive symptoms was assessed by means of the 21-item Hamilton Rating Scale for Depression (HRSD-21) (Hamilton, 1960). The severity of manic symptoms was assessed by means of the Young Mania Rating Scale (YMRS) (Young et al., 1978). Moreover, Clinical Global Impressions-Severity of Illness scale (CGI-S) (Guy, 1976) was administered. Comorbid diagnoses of anxiety disorders were assessed by means of the MINI. Scale administration was performed by trained raters.

2.3. Methods: BDNF assay

To avoid a potential bias due to the presence of a diurnal rhythm of plasma BDNF levels (Piccinni et al., 2008b), venous blood samples were drawn in the morning (between 8:00 and 10:00 a.m.). Blood was collected into EDTA-coated tubes that were kept on ice, centrifuged at 2000 × *g* for 10 min at 4 °C and refrigerated at −20 °C. (Lommatzsch et al., 2005; Begliuomini et al., 2007).

According to the manufacturer's instructions, acidification and subsequent neutralization of the samples were performed before carrying out the ELISA assay (BDNF Emax Immunoassay system, Promega, Madison, WI, USA). Ninety-six-well plates were coated with anti-BDNF monoclonal antibody and incubated at 4 °C for 18 h. The plates were incubated in a blocking buffer for 1 h at room temperature before samples were added. The samples and BDNF standards were maintained at room temperature under shaking for 2 h, followed by washing with the appropriate buffer. The plates were then incubated with anti-human BDNF polyclonal antibody at room temperature for 2 h, washed and incubated with anti-IgG antibody conjugated to horseradish peroxidase for 1 h at room temperature. The plates were then incubated in peroxidase substrate and tetramethylbenzidine solution to produce a color reaction. The reaction was stopped with 1 M HCl. The absorbance at 450 nm was measured with a microplate reader (Model 550, Bio Rad Laboratories) to determine BDNF values that are expressed as ng/ml.

2.4. Methods: statistical analysis

Data were recorded into a digital database and elaborated by using SPSS software, 17th version. Since BDNF levels and rating scales scores were normally distributed, parametric tests were performed. In particular, the one-way ANOVA, followed by the Bonferroni *post-hoc* test, was used to compare the mean BDNF plasma levels amongst the three groups (HS, MDE, ME). ANOVA was also used to test the homogeneity of the independent variable age amongst the same groups. Rating scales scores, number of episodes and the onset age amongst the two patients' groups were compared by means of the *t*-test for independent samples. The distribution of categorical variables (gender, co-morbidities) was analyzed by means of 2 × 2 cross tables and the chi-squared test. A *p*-value $< .05$ was judged as statistically significant.

3. Results

The three groups of subjects (HS, MDE, ME) resulted homogeneous for age [$F(2, 51) = 1.98$, $p = .149$] and gender ($\chi^2 = 4.324$, $p = .115$).

The severity of illness, as assessed by the CGI-S, was significantly higher in ME patients, compared with MDE ones (6.05 *vs.* 5.56, $t = -2.853$, $p = .01$), while no significant differences for the number of episodes and onset age emerged between the two patients groups.

The variance test (ANOVA) showed that the mean plasma BDNF values were significantly different between the three groups [$F(2, 51) = 9.847$, $p < .001$]. According to the Bonferroni *post-hoc* test, BDNF levels were significantly higher in HS, as compared with both patients' samples (HS *vs.* MDE patients: $p < .001$; HS *vs.* ME patients: $p < .025$). MDE patients presented lower BDNF levels than ME patients, but the difference was not statistically significant ($p = .257$).

No significant differences in psychiatric comorbid disorders rate resulted between MDE and ME patients, excepting for social anxiety disorder (SAD) which was diagnosed only amongst MDE patients ($\chi^2 = 9.113$, $p = .003$) (see Fig. 1 and Table 1 for the summary of results).

4. Discussion, limitations and conclusions

The main finding of the present work is that the plasma BDNF levels were significantly decreased in the course of mixed episodes of BD. This is consistent with previous studies showing lower plasma BDNF concentrations in both depressed and manic patients, and further supports the consistency of BDNF as a