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Electroconvulsive Therapy (ECT) increases serum Brain Derived Neurotrophic Factor (BDNF) in drug resistant depressed patients

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Abstract Several findings have suggested that the neurotrophin BDNF could contribute to clinical efficacy of antidepressant treatments. The purpose of this study was to analyse if ECT operates a modulation of serum BDNF levels in a sample of drug resistant depressed patients. The results obtained show significantly higher serum levels of BDNF following ECT. More specifically, while no change occurred in the whole sample between T0 (baseline) and T1 (after ECT) ($p=0.543$) a significant increase has been identified at T2, one month after the end of ECT ($p=0.002$). However, the BDNF augmentation was evident even between T0 and T1 in a subgroup of patients who has low baseline BDNF levels. Although future researches are needed, the results herein presented show for the first time that ECT is associated with changes in serum BDNF and further support the possible involvement of BDNF in antidepressant therapies.

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1. Introduction

Major depression is a severe and life-threatening illness which represents one of the most important causes of disability world-wide. In particular, about 50% of the affected patients experience a chronic course and up to 20% of them shows an insufficient response to drug treatments (Fava et al., 2003; Hussain and Cochrane, 2004). As other chronic diseases, major depression is a complex disorder caused by the interaction between environmental and biological/genetic risk factors. Although the molecular alterations underlying the pathogenesis remains to be clearly established, recent preclinical and clinical studies have suggested an involvement of the neurotrophin Brain Derived Neurotrophic Factor (BDNF) in the aetiology of major depression as well as in the antidepressant drug treatment (Angelucci et al., 2005; Castren, 2004; Duman, 2004; Hashimoto et al., 2004). BDNF is a neurotrophic factor widely expressed in the Central Nervous System (CNS) that plays a major role in brain development, survival and maintenance of neuronal functions and synaptic plasticity. Studies of brain imaging suggest that depressed patients have neuronal atrophy and cell loss in discrete brain regions (Bremner et al., 2000; Krishnan et al., 1993; Kumar et al., 1998, 2000, 2004; Sheline et al., 1996), which are suggestive of a reduction in neuron plasticity. In line with these observations, different studies reported a reduction of BDNF expression in post-mortem brain of depressed subjects (Dwivedi et al., 2001, 2003; Molnar et al., 2003). Extensive research in rodents has shown that stress-related behaviours can alter the expression of BDNF in the limbic system and cortex (Roceri et al., 2002, 2004; Smith and Cizza, 1996; Vollmayr et al., 2001). Furthermore, antidepressant drug treatment enhances BDNF expression and production (Altar, 1999; Castren, 2004; Duman et al., 1997; Nibuya et al., 1995) and the neurotrophin signalling appears to be required for antidepressant activity in animal models of depression (Castren, 2004; Saarelainen et al., 2003; Sairanen et al., 2005). The involvement of BDNF in major depression and antidepressant treatment has gained further support from a series of biochemical studies in humans. A decrease in BDNF serum levels has been associated with major depression (Karege et al., 2002a), an effect that is normalized by antidepressant drug therapies (Aydemir et al., 2005; Shimizu et al., 2003). Moreover a significant correlation between BDNF serum levels and depressive personality traits in a healthy subject was also found (Lang et al., 2004).

Electroconvulsive Therapy (ECT) is one of the eligible therapies for the treatment of major depression (American Psychiatry Association, 2000; UK ECT Review Group, 2003). Preclinical studies have shown that electroconvulsive seizures (ECS) produces a robust increase in BDNF mRNA (Altar et al., 2004; Chen et al., 2001; Duman et al., 1997; Nibuya et al., 1995; Zetterstrom et al., 1997) and BDNF protein (Altar et al., 2003) in different rat brain areas. However, whether or not ECT could affect the peripheral levels of BDNF in depressed patients is still an open question. In order to address this issue, we investigated, in the present study, serum levels of BDNF in a group of drug resistant depressed patients before and after ECT.

2. Experimental procedures

2.1. Subjects

Twenty three patients from the "Villa S. Chiara" Private Clinic (16 females, 7 males; mean age $53,0 \pm 17,39$ years, range 18–79 years) with major depression diagnosis (ICD-10) and planning to receive ECT treatment were recruited after written informed consent approved by the local Ethic Committee. Diagnosis of unipolar depression was confirmed by the diagnostic scale SCAN-IGC. In particular, ten patients met the criteria for major depression without psychotic symptoms and thirteen patients for major depression with psychotic symptoms. Cognitive deficits were evaluated with Mini Mental State examination (mean value $27,9 \pm 2,00$). An independent psychiatrist recommended ECT according to clinical judgement because of the patients' drug resistance. Drug resistance was defined as a failure to respond to at least three courses of antidepressant medications with adequate dose and duration (stage III definition of Thase and Rush, 1997). Patients were maintained on the same drug treatment for at least 3 weeks before ECT treatment and during the entire study period (for the antidepressant treatment: 7 patients were treated with SSRIs, 15 patients were treated with other drug classes and one patient was drug-free). Illness severity and the outcome of ECT treatment was assessed with the Montgomery and Asberg Depression Rating Scale (MADRS) (Montgomery and Asberg, 1979).

Longitudinal blood sampling and the concomitant MADRS evaluations were performed at T0 (Baseline: the day before ECT treatment), T1 (the day after the last ECT treatment) and T2 (1 month after the end of the ECT treatment). Complete remission was considered when MADRS score after treatment were ≤ 8 at T1 and T2.

2.2. ECT Treatment

A medical history and a physical examination together with blood and urine routine examinations, electrocardiogram (ECG), cerebral CAT scan, and a chest film were requested to screen the general medical conditions. Premedication included atropine sulphate (0,5 mg IV), succinylcholine (0,7 mg/kg IV), thiopental (3,0 mg/kg IV for males, 2,5 mg/kg IV for females).

ECT was performed between 7:00 and 9:00 a.m. using a Thymatron™ DG (Somatix, Inc., Lake Bluff, IL, USA) with standard settings (Abrams and Swartz, 1989) with a bipolar brief pulse square wave. The patients were treated with bilateral ECT. Two stimulus electrodes were placed over the left and right frontotemporal scalp. For each patient, ECT treatment conditions have been set up (charge delivered max 504 mC, current 0.9 A, frequency 30–70 Hz, pulse width 1 ms, duration max 8 s). During ECT, the motor convulsions, the electroencephalogram (EEG), the induced tachycardia (ECG) and, if necessary, the electromyogram (EMG) were monitored. Treatment was given three times a week. The mean number of treatments received was $7,0 \pm 1,97$ (range 3–10) and ECT treatment was completed on the basis of the clinical judgment of the treating physicians.

2.3. BDNF serum determination and statistical analysis

Venous blood samples were collected in the morning after an overnight fast (between 8:00 and 9:00 a.m.) in anticoagulant-free tubes. They were kept at room temperature for 1 h followed by 1 h at 4 °C before serum separation by centrifugation at (3000 rpm for 15 min at 4 °C). Serum samples were stored at –80 °C till the time of assay. BDNF levels were measured by the ELISA method using the human BDNF Quantikine Kit (R D System, Minneapolis, USA), according to the manufacturer's instructions. The BDNF

content was expressed as equivalent of human recombinant BDNF protein. The detection limit of the assay was 1 pg/ml and the data were expressed as ng of BDNF protein/ml of serum.

The clinical and biological changes occurring after ECT were analysed by means of General Linear Model according to a repeated measures design with Time (T_0 , T_1 , T_2) as within-subjects factor. Greenhouse–Geisser correction was applied to degrees of freedom when sphericity assumption was violated. In order to take into account the baseline levels, this procedure was applied also entering T_0 evaluation as a covariate (Vickers, 2001). Pearson coefficient was used to evaluate bivariate correlations and Student's t -test for comparing means when patients were categorised in two groups. Statistical evaluations were performed using the SPSS version 11.0 software package.

3. Results

ECT treatment improved depression symptomatology measured with MADRS (MADRS score mean values \pm standard deviations: $T_0=34.21 \pm 7.91$; $T_1=8.86 \pm 5.37$; $T_2=7.74 \pm 7.32$): analysing data by ANOVA with Time (T_0 , T_1 and T_2) as within-subjects factor, MADRS scores significantly decrease [MADRS Greenhouse–Geisser (G–G) correction $F(1.50, 32.99)=98.64$ $p=2.69 \times 10^{-13}$] with a medium percentage improvement at T_2 of 73.06%. In particular, planned “repeated” contrasts indicated that a significant improvement in symptoms between T_1 and T_0 ($p=2.05 \times 10^{-13}$) and between T_2 and T_0 ($p=1.33 \times 10^{-9}$). Complete remission was reached by 8 patients.

No significant differences (with unpaired and two-sided T -tests) have been found in BDNF baseline (T_0) levels stratifying patients for gender ($T=-0.15$, $p=0.882$), presence of psychotic symptoms ($T=1.39$, $p=0.178$), antidepressant treatment (SSRIs vs. other agents in monotherapy or in combination with SSRIs) ($T=0.24$, $p=0.814$) and no correlations have been evidenced with age ($R=-0.288$, $p=0.182$) and Mini Mental Examination Scores ($R=0.310$,

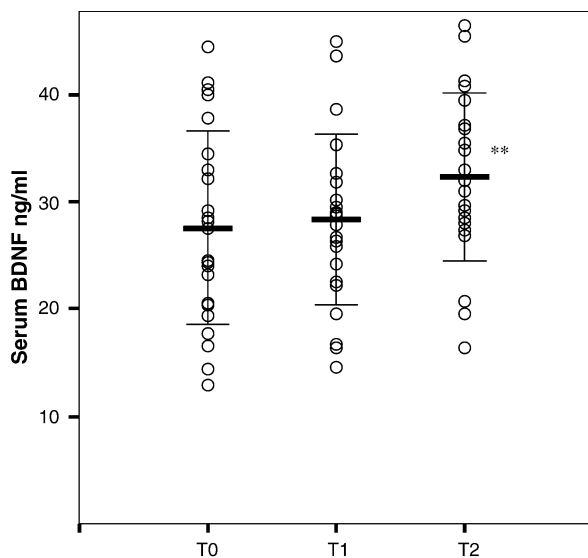


Figure 1 BDNF serum levels at three different sampling times: T_0 =Baseline; T_1 =the day after last ECT treatment; T_2 =1 month after the end of ECT treatment (Mean values \pm standard deviations: $T_0=27.64 \pm 9.13$ ng/ml; $T_1=28.38 \pm 7.95$ ng/ml; $T_2=32.30 \pm 7.76$ ng/ml) (** $p < 0.01$).

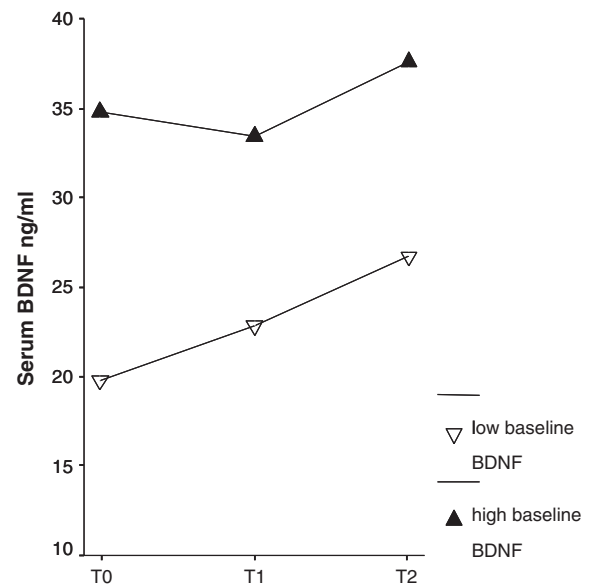


Figure 2 Longitudinal changes in serum BDNF mean values in patients with BDNF baseline levels below (group 1) or above the median (group 2).

$p=0.150$). Moreover no correlation was found between baseline BDNF levels and T_0 MADRS scores ($R=-0.058$, $p=0.793$).

According to ANOVA with time (T_0 , T_1 and T_2) as within-subjects factor, a significant increase of BDNF was found [(G–G) correction: $F(1.96, 43.05)=7.64$; $p=0.002$]. Planned “repeated” contrasts (T_0 vs. T_1 , T_1 vs. T_2) indicated that no change occurred between T_0 and T_1 ($p=.543$) while a steep increase occurred between T_1 and T_2 ($p=0.006$) (see Fig. 1). When baseline BDNF was entered in the model as a covariate (Vickers, 2001), ANOVA confirmed the changes across time [G–G correction: $F(1.85, 38.91)=8.27$; $p=0.001$] but also revealed a significant interaction Baseline*Time [G–G correction: $F(1.85, 38.91)=4.94$; $p=0.014$], indicating that changes across time were dependent on baseline levels of BDNF. Planned contrasts indicated that baseline BDNF interfered with changes between T_0 and T_1 ($p=0.014$) and not between T_1 and T_2 ($p=0.615$). To further address this effect, we divided patients in two groups, below or above the median value of baseline BDNF. As shown in Fig. 2, the BDNF increase in the “low baseline BDNF” group (group 1) was linear between T_0 and T_2 , whilst in patients with “high baseline BDNF” (group 2) a non-linear pattern was observed: no significant change was evident between T_0 and T_1 , whereas a significant increase was evident between T_1 and T_2 , with a profile that is similar to the one displayed by the other group (low baseline BDNF levels). It is worth noticing that these effects could not be ascribed to the regression-to-the-mean effect, that was computed and resulted not significant for both regressions (T_1 on T_0 , $p=0.723$ and T_2 on T_0 , $p=0.275$). These two groups are similar for demographic, clinical characteristics and drug treatments except for differences in age at onset (mean values: group 1–48.08 \pm 17.92 years; group 2–32.09 \pm 11.46 years; T -test: $T=-2.52$, $p=0.020$).

No correlation was evidenced between baseline BDNF concentrations (BDNF_{T0}) or percentage increases in BDNF levels [$\Delta\text{BDNF} = (\text{BDNF}_{T2} - \text{BDNF}_{T0}) \times 100 / \text{BDNF}_{T0}$] and ECT efficacy measured as percentage differences in MADRS scores (BDNF baseline $R=0.100$ $p=0.65$; ΔBDNF $R=-0.04$ $p=0.869$). Moreover, when patients were stratified for remission after treatment (8 subject versus 15), no significant differences were found for BDNF_{T0} ($T=-0.785$, $p=0.441$) neither for longitudinal changes ($F=0.256$, $p=0.775$).

4. Discussion

The results presented in the current investigation show that drug resistant depressed patients have significantly higher serum levels of BDNF following ECT. More specifically, while no change occurred in the whole sample between T0 (baseline) and T1 (after ECT) a significant increase has been identified at T2, one month after the end of ECT. However, the increase in serum levels of BDNF was evident even between T0 and T1 in a subgroup of patients who had low baseline BDNF levels.

To our knowledge this is the first study showing a regulation of peripheral BDNF levels in patients following ECT, providing further support to the possible role of this neurotrophin in the action of antidepressant therapies (Shimizu et al., 2003). However, these findings should be confirmed in other and/or larger studies, because of the relatively small sample size.

Previous studies have reported that the serum levels of BDNF are reduced in untreated depressed patients (Karege et al., 2002a). Moreover, it was found that depressed patients treated chronically with antidepressants displayed normal levels of BDNF, thus suggesting that the pharmacological treatment could normalize serum BDNF levels (Shimizu et al., 2003). More recently, longitudinal studies (Aydemir et al., 2005; Gonul et al., 2005; Gervasoni et al., 2005) observed an increase of BDNF serum levels following antidepressant therapy.

Preclinical studies, indicate that BDNF could play a critical role in the pathophysiology of depression as well as in the therapeutic action of antidepressant treatments. Consonant with these investigations, it has also been reported that the expression of this neurotrophin was consistently increased also following ECS in rodents (Altar et al., 2004). Overall these findings, together with the present results, strongly suggest a relationship between BDNF and antidepressant effects of both pharmacological and non-pharmacological therapies.

Unlike previous studies (Karege et al., 2002a; Karege et al., 2005; Gervasoni et al., 2005), we did not find a correlation between BDNF levels and depressive symptoms, as measured by MADRS. In this regard, it must be mentioned that the design of our study included resistant depressed patients who were under pharmacological treatment, thus explaining, at least in part, the lack of correlation between BDNF and MADRS scores. Moreover, it should be mentioned that the baseline BDNF levels observed in our sample were higher than those observed in drug-free patients (Karege et al., 2002a; Shimizu et al., 2003; Gonul et al., 2005; Aydemir et al., 2005), but similar

to those reported in previous studies in which BDNF levels were measured in depressed treated patients (Shimizu et al., 2003). This observation may explain the lapse of time between the end of ECT and the changes in BDNF levels. Consonant with this hypothesis, it is interesting to note that those patients who had low baseline BDNF levels displayed increased levels of BDNF between T0 and T1. While the time-related changes of BDNF in the treatment of depression seems to be potentially clear, future research is needed to elucidate this matter in resistant depressed patients who are treated with both pharmacological and non-pharmacological approaches.

Although the involvement of BDNF in depression is well-accepted, we are aware that the data presented must be interpreted with caution because of the limitation inherent in working with peripheral BDNF levels that could not correspond to brain BDNF. The origin and the functional role of peripheral BDNF is poorly understood even though beyond brain production, alternative sources can be visceral epithelial cells, endothelial cells, muscle cells, activated macrophages and lymphocytes. Blood platelets, rather than synthesize BDNF, sequester, store and release it after stimulation (Karege et al., 2002b). It was suggested that circulating levels of BDNF might reflect the central neurotrophin activity: in fact, BDNF can cross the blood-brain barrier through a high-capacity, saturable transport system (Pan et al., 1998) and serum BDNF parallels brain protein modulations during postnatal neurodevelopment, maturation and aging in rat models. Alternatively, differences in BDNF serum levels in depression might represent a 'peripheral' phenomenon: indeed recent evidences have suggested alterations in the release of platelet BDNF in depressed patients (Karege et al., 2005), while antidepressant drugs are able to influence platelet activation (Markovitz et al., 2000).

It remains to be established whether the effect of ECT on serum BDNF reflects a primary condition or a secondary response to neuro-hormonal perturbations. We observed a time lag between the end of ECT and the changes in BDNF levels suggesting that, rather than being a direct consequence of convulsions, the elevation of peripheral BDNF levels might represent the consequence of adaptive processes. At this regard, the time of onset of these changes could be different depending on some clinical features of our patients as age at onset. Alternatively, the observed modifications might also relate to adaptive response and/or normalization of neuro-hormonal or psychoimmune systems that are altered in depressed patients. Moreover, as mentioned above this changes could be related to the influence of antidepressant treatments on platelets function. All these observations suggest that a more prolonged follow up study could clarify if serum BDNF may be a long term marker of ECT effectiveness.

In conclusion, we provide evidence that ECT treatment is associated with changes in BDNF levels in a group of drug resistant depressed patients. Further information will be required to understand the biological mechanisms underlying this phenomenon and to clarify the usefulness in the clinical practice of serum BDNF as a biomarker for the treatment of depression.

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