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Lithium increases nitric oxide levels in subjects with bipolar disorder during depressive episodes

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ABSTRACT

Background: Altered nitric oxide (NO) signaling has been associated with the pathophysiology of Bipolar Disorder (BD), directly affecting neurotransmitter release and synaptic plasticity cascades. Lithium has shown to regulate NO levels in preclinical models. However, no study has addressed peripheral NO levels in unmedicated BD. Also, lithium's effects on NO levels have not been studied in humans.
Methods: Plasma NO was evaluated in subjects with BD I and II during a depressive episode ($n = 26$). Subjects had a score of $\geq$18 in the 21-item Hamilton Depression Rating Scale and were followed-up during a 6-week trial with lithium. Plasma NO levels were also compared to matched healthy controls ($n = 28$). NO was determined by chemiluminescence method.
Results: Lithium treatment significantly increased plasma NO levels after 6 weeks of treatment in comparison to baseline levels in bipolar depression ($p = 0.016$). Baseline NO levels during depressive episodes showed no difference when matching up to healthy controls ($p = 0.66$).
Conclusion: The present findings suggest that lithium upregulates NO signaling in unmedicated BD with short illness duration. Further studies with larger samples are needed to confirm the effects of lithium on NO pathway and its association with synaptic plasticity and therapeutics of BD.

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1. Introduction

Recent studies have suggested that nitric oxide (NO) signaling may represent a potential therapeutic target in mood disorders (Ghasemi and Dehpour, 2011). Post-mortem studies in MDD showed a decrease in neuronal nitric oxide synthase levels in the locus coeruleus and lower number and density of nitric oxide synthase-immunoreactive neurons in the hypothalamic nuclei compared to healthy controls (Bernstein et al., 1998, 2002, 2005; Karolewicz et al., 2004). Regarding peripheral NO levels in MDD, Suzuki et al. (2001) found increased levels, whereas other study found no alteration (Kim et al., 2006). In medication-free depression, decreased NO was found in different studies (Chrapko et al., 2004; Selley, 2004; Herken et al., 2007; García et al., 2011). Regarding investigations in BD, increased NO levels were observed during different mood states (Andreazza et al., 2008), and selectively in depressive episodes (Selek et al., 2008). However, all previous studies in BD measured NO metabolites as an index of NO activity, rather than NO levels per se. Moreover, in the only study evaluating NO in bipolar depression, patients were selected in a naturalistic setting and using diverse psychotropic drugs (Selek et al., 2008).

The NO pathway is especially relevant in neuropsychiatric disorders. NO modulation was shown to affect the release of neurotransmitters (Prast and Philippu, 2001) and synaptic plasticity (Bon and Garthwaite, 2003). NO has dose-dependent effects; at high

Abbreviations: BD, Bipolar Disorder; CGI, Clinical Global Impression; CREB, cyclic-AMP-responsive-element-binding protein; HAM-D, 21-item Hamilton Depression Scale; MDD, Major Depressive Disorder; NO, Nitric Oxide; eNOS, endothelial nitric oxide synthase; iNOS, inducible nitric oxide synthase; nNOS, neuronal nitric oxide synthase; SCID, Structured Clinical Interview for Axis I DSM-IV-TR Disorders; YMRS, Young Mania Rating Scale.

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concentrations it has neurotoxic properties and when at physiological concentrations it has a neuromodulator and a neuroprotective role (Calabrese et al., 2007). Its role in neuroprotection has been associated with decreased Ca^{2+} influx and consequent inhibition of cell death (Liu and Stamler, 1999). Also, NO increases the neuroprotective proteins Akt (Ciani et al., 2002) and cyclic-AMP-responsive-element-binding protein (CREB) (Riccio et al., 2006), also inducing the production of bilirubin, a potent antioxidant (Sergent et al., 1997).

NO effects are consistent with the neuroprotective and neurotrophic actions of lithium (Machado-Vieira et al., 2009; de Sousa et al., 2011). Lithium is a standard treatment for BD and used as first option treatment for bipolar depression (Bschor and Bauer, 2006). Its mechanism of action is complex, affecting multiple intracellular signaling pathways. Several animal models showed that lithium regulates central and peripheral NO levels (Ghasemi and Dehpour, 2011). Studies in rodents have shown that lithium regulates the expression of the enzyme NO synthase Bagetta et al. (1993); (Feinstein, 1998; Anai et al., 2001; Bhalla et al., 2010) and NO activity (Harvey et al., 1994; Maruta et al., 2005), but results are mixed.

The present study evaluates plasma NO levels in unmedicated subjects with BD patients during depressive episodes in comparison to healthy controls. To date, NO levels have not been studied in drug-free patients with bipolar depression. In addition, although many studies were performed in animal models or *in vitro*, lithium's effects on NO have not been studied in humans. Thus, lithium's effects on NO levels were evaluated in bipolar depression in this 6-week trial.

2. Methods

2.1. Subjects

Between August 2010 and June 2012, 26 outpatients, 7 (26.9%) men and 19 (73.1%) women, with a mean age of 27.7 (±4.8) years and a diagnosis of BD, experiencing a major depressive episode, diagnosed by Structured Clinical Interview for Axis I DSM-IV-TR Disorders (SCID) (First et al., 1995), were enrolled in the study. Patients were recruited through media advertisement and evaluated at the Institute of Psychiatry, University of Sao Paulo, Brazil.

Patients were required to have a score ≥18 on the 21-item Hamilton Depression Scale (HAM-D) (Hamilton, 1960) when enrolled. Assessment of symptoms was also made with Young Mania Rating Scale (YMRS), and Clinical Global Impression – Severity (CGI-S) (Petkova et al., 2000). Diagnosis and psychometric assessments were performed by experienced psychiatrists. Patients were excluded if they had any medical disorder that could affect the central nervous system, substance abuse in the last year or dependence or mental retardation.

For comparison with patients, 28 age-matched (±3 years) healthy controls, 16 (57.1%) men and 12 (42.9%) women, with a mean age of 28.0 years (±7.2) were studied. Controls were excluded from the present investigation if there was a lifetime history of any mental disorder (as assessed with SCID), including substance disorders, any disease affecting central nervous system or any first-degree relative with mood or psychotic disorder.

This study had approval by the local institutional review board, and all participants provided written informed consent prior to entering the study.

2.2. Study design

Patients had blood samples collected before (at baseline) and after treatment (at endpoint), and were matched with healthy controls. At baseline, patients received lithium carbonate at 450 mg/day, with flexible increases in doses according to clinical improvement. Most patients were taking lithium monotherapy, although the use of hypnotics as needed was allowed and 2 patients who were also using antipsychotics/mood stabilizers. Psychometric assessments were made at week 0 (baseline) and then at weeks 1, 2, 4 and 6 (endpoint). Criterion for clinical response was a decrease of 50% or more in the HAM-D at endpoint and for remission a HAM-D < 8 and YMRS < 8 at endpoint.

2.3. Assays

Blood samples were collected from 8:00 to 10:00 AM in vacutainer tubes from patients and controls in 8-h fasting. Samples were centrifuged at 20 °C and 1620 × g for 15 min to obtain plasma. Plasma samples were frozen and stored at −80 °C. To avoid plasma foaming caused by proteins, we deproteinized plasma by adding $ZnSO_4$ and NaOH, and then centrifuging at 12,000 rpm for 5 min at room temperature.

Since Griess reaction has been shown not to be accurate for NO measures (Hunter et al., 2013), NO plasma samples were determined by chemiluminescence using Model 280 Nitric Oxide Analyzer (NOA™) from Sievers Instruments, Inc. (Boulder, CO, USA), which is a high-sensitive detector for measuring nitric oxide (Hunter et al., 2013), based on gas-phase chemiluminescent reaction between nitric oxide and ozone: $NO + O_3 \rightarrow NO_2^- + O_2$ and $NO_2^- \rightarrow NO_2 + hv$. The photon emission from electrically excited nitrogen dioxide was detected by a thermoelectrically cooled photomultiplier tube. Measurement of NO and its reaction products in liquid samples has a sensitivity around 1 pmol (Hampl et al., 1996) and is validated for plasma measurement (Yang et al., 1997). Three comparisons of standards and internal controls achieved high coefficients of correlation ($r > 0.99$).

2.4. Statistics

Chi-square test was used to compare gender in patients and controls. For samples with normal distribution Student's *t* test was used for comparisons and when samples had non-normal distribution, comparisons were performed with Mann–Whitney and Wilcoxon Signed Ranks tests. Correlations were evaluated with Spearman test. Statistical analysis was performed using SPSS 14.0. Significance level was set at <0.05 (two-tailed).

3. Results

3.1. Demographic and clinical data

Demographic and clinical data of BD patients and controls are summarized in Table 1. From the 26 patients enrolled, 9 (34.6%) had diagnosis of type I BD and 17 (65.4%) of type II BD; 24 (92.3%) patients were medication-free for at least 6 weeks before enrollment in the study and among those, 20 (76.9%) were drug-naïve. Patients had illness duration of mean 36.6 months (±19.4) and history of previous psychotic mood episode was present in only 3 subjects (11.5%) patients. A significant decrease in depressive symptoms measured by HAM-D was observed from baseline (22.6 ± 2.9) to endpoint (7.0 ± 6.2) ($z = -4.35$, $p < 0.001$), 22 (84.6%) patients responded to treatment and 16 (61.5%) patients achieved remission.

3.2. Lithium treatment increased NO plasma levels

Lithium treatment significantly increased NO levels from baseline (78.8 ± 20.4 μM) to endpoint (99.1 ± 53.6 μM) ($z = -2.42$, $p = 0.016$) (Fig. 1). NO at endpoint was not different in responders