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Oxidative stress in early stage Bipolar Disorder and the association with response to lithium

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Abstract

Background—Several studies have described increased oxidative stress (OxS) parameters and imbalance of antioxidant enzymes in Bipolar Disorder (BD) but few is know about the impact of treatment at these targets. However, no study has evaluated OxS parameters in unmedicated early stage BD and their association with lithium treatment in bipolar depression.

Methods—Patients with BD I or II ($n = 29$) in a depressive episode were treated for 6 weeks with lithium. Plasma samples were collected at baseline and endpoint, and were also compared to age-matched controls ($n = 28$). The thiobarbituric acid reactive substances (TBARS), and the antioxidant enzymes superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GPx) activities were measured.

Results—Subjects with BD depression at baseline presented a significant increase in CAT ($p = 0.005$) and GPx ($p < 0.001$) levels, with lower SOD/CAT ratio ($p = 0.001$) and no changes on SOD or TBARS compared to healthy controls. Regarding therapeutics, lithium only induced a decrease in TBARS ($p = 0.023$) and SOD ($p = 0.029$) levels, especially in BDII. Finally, TBARS levels were significantly lower at endpoint in lithium responders compared to non-responders ($p = 0.018$) with no difference in any biomarker regarding remission.

Conclusion—The present findings suggest a reactive increase in antioxidant enzymes levels during depressive episodes in early stage BD with minimal prior treatment. Also, decreased lipid peroxidation (TBARS) levels were observed, associated with lithium's clinical efficacy. Overall, these results reinforce the role for altered oxidative stress in the pathophysiology of BD and the

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Conflict of interest CAZ is listed as co-inventor on a patent for the use of ketamine in major depression and have assigned their patent rights on ketamine to the US government. The other authors report no conflict of interest.

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presence of antioxidant effects of lithium in the prevention of illness progression and clinical efficacy.

Keywords

Oxidative stress; Lithium; Bipolar Disorder; Depression; Neuroprotection

1. Introduction

It has been proposed a progressive course of Bipolar Disorder (BD) associated with longer illness duration, cognitive decline, decreased functioning and impaired cellular resilience leading to deleterious consequences on signal transduction and synaptic plasticity (Machado-Vieira et al., 2013). Thus, intervention in the early stage of BD may be a valuable tool to improve the course of the illness and provide a better prognosis (Berk et al., 2013).

Increased Oxidative Stress (OxS) generates deleterious consequences on signal transduction, synaptic plasticity, and cellular resilience, especially by inducing lipid peroxidation in membranes, proteins and DNA (Grintzalis et al., 2013; Mahadik et al., 2001; Soeiro-de-Souza et al., 2013). DNA damage, which can be induced by oxidative stress, has been found to be associated with the severity of depressive symptoms in BD (Andreazza et al., 2007b).

Thiobarbituric acid reactive substances (TBARS) levels are a direct index of cell lipid peroxidation whereas antioxidant system involves coordinated effects of antioxidant enzymes superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GPx) (Reddy et al., 1991).

Imbalance in antioxidant enzymes has been reported in several studies of BD and is consistently associated with increased OxS (Andreazza et al., 2008). Only one study evaluated OxS parameters in BD patients with short duration of illness and found increased activity of antioxidant system in mania (Machado-Vieira et al., 2007). No study, however, has evaluated OxS in recent-onset BD patients in unmedicated depressive episodes.

Also, consistent evidences support the role of a subtle mitochondrial compromise in BD (Clay et al., 2011; Manji et al., 2012). Initially, magnetic resonance spectroscopy studies showed increased lactate levels in BD, suggesting a metabolic shift (reviewed in Stork and Renshaw, 2005). Post-mortem studies using brains of BD patients have shown decreased expression of mitochondrial electron transport chain genes (Konradi et al., 2004; Sun et al., 2006). Mitochondrial dysfunction increases production of reactive oxygen species, leading to enhanced OxS. OxS parameters have been found to be increased in BD post-mortem brains (Andreazza et al., 2010) and also in the peripheral blood of subjects with BD (Andreazza et al., 2008). Only one study evaluated subjects with BD during a depressive episode in a smaller sample, and found a slight increase in CAT levels compared to controls (Andreazza et al., 2007a).

Evidence suggests that the presence of OxS might be associated with the consistently found hyperactivation of the glutamatergic and dopaminergic systems in BD (Berk et al., 2011). Glutamatergic hyperactivity leads to increased calcium influx (Plein and Berk, 2001) which

increases OxS (Shao et al., 2005). On the other hand, enhanced OxS has been suggested to increase glutamate (Lovell et al., 2000; Volterra et al., 1994). The excessive dopamine production increases OxS due to the production of reactive oxygen species in dopamine metabolism (Miyazaki and Asanuma, 2008). Moreover, the opposite mechanism also happens when OxS induces dopamine uptake, thus increasing dopamine activity (Kim and Andreazza, 2012) in a vicious cycle.

Other systems associated with BD are γ -Aminobutyric acid (Brambilla et al., 2003) and serotonergic systems (Fountoulakis et al., 2012), which are found to show decreased activity. Similar to other systems, OxS is associated with decreased γ -Aminobutyric acid release (Palmeira et al., 1993). Increased metabolism of serotonin, which decreases serotonergic function, is found to be associated with increased oxidative stress (Bianchi et al., 2005; Nocito et al., 2007). Consistent with these findings, evidences suggest that treatment with antidepressants can decrease OxS (Bilici et al., 2001).

Lithium is recommended as a first-line treatment for bipolar depression (Haeberle et al., 2012; Yatham et al., 2013) and it has shown several neuroprotective and neurotrophic actions (Machado-Vieira et al., 2009). Lithium has been shown to increase brain-derived neurotrophic factor (BDNF) (de Sousa et al., 2011), regulate intracellular Ca^{+2} (Wasserman et al., 2004), activate CREB (Ozaki and Chuang, 1997), increase Akt (Yazlovitskaya et al., 2006), and inhibit apoptotic caspase-3 (Ghribi et al., 2002). Similarly, lithium has been shown to protect against glutamatergic excitotoxicity (Nonaka et al., 1998). This agent has shown to decrease OxS in preclinical models (Schäfer et al., 2004), in bipolar mania (Machado-Vieira et al., 2007) and healthy volunteers (Khairova et al., 2012). Nonetheless, the effects of lithium on OxS parameters specifically in bipolar depression have never been studied.

The present study evaluated OxS parameters (TBARS, SOD, CAT and GPx) in unmedicated bipolar depression versus controls as well as the potential antioxidant effects of lithium in a therapeutically relevant paradigm. Our hypothesis was that subjects in a bipolar depression episode would present increased OxS (TBARS) and imbalance of antioxidant enzymes during depressive episodes and that lithium would lower OxS levels and enhance antioxidant enzymes levels.

2. Methods

2.1. Subjects

Subjects were evaluated between August 2010 and June 2012 at the Institute of Psychiatry, University of Sao Paulo, Brazil. Twenty-nine patients, 21 (72.4%) women, with 28.4 (± 5.5) years of age and diagnosis of BD I (38%) or BD II (62%) in a depressive episode were included, as diagnosed by the Structured Clinical Interview for Axis I DSM-IV-TR Disorders (SCID) (First et al., 1995). Patients who had a score ≥ 18 on the 21-item Hamilton Depression Scale (HAM-D) (HAMILTON, 1960) were eligible for the study. Also, 26 (89.6%) patients were drug-free for at least 6 weeks prior to their enrollment and 21 (72.4%) subjects were treatment-naïve at baseline. Exclusion criteria included presence of chronic medical illness, comorbid substance abuse or dependence in the past year, rapid cycling in

the past 12 months, previous head trauma, current major axis I psychiatric disorder, subjects submitted to electro-convulsive therapy and current significant abnormal laboratory tests.

The comparison group was constituted by 28 age-matched healthy controls (within 3 years of difference of BD patients); these 16 men (57.1%) and 12 women (42.8%) (age = 28.0 ± 7.2) were recruited through advertisement in the local community. Controls were excluded if they had lifetime history of any mental disorder (by SCID), including substance abuse or dependence, or if they had any disease with central nervous system involvement or any first-degree relative with a mental disorder. This study was approved by the local institutional review board, and all participants provided written informed consent before study entry.

2.2. Study design

Patients had blood samples collected at baseline and at endpoint (week 6), while healthy controls had only one-point sample collection. At baseline, patients were started on open-label lithium carbonate at 450 mg/day, with flexible doses increase according to clinical improvement, controlling plasma lithium levels to ensure compliance and avoid toxicity (<1.2 mEq/L).

Most patients were on lithium monotherapy, although hypnotic (benzodiazepine or zolpidem) use as needed was allowed and 4 patients were also in use of antipsychotics or mood stabilizer. Psychometric assessments were made at baseline and on week 1, week 2, week 4, and week 6 (endpoint). Assessment of symptoms was performed with the HAM-D, Young Mania Rating Scale (YMRS) (Young et al., 1978), and Clinical Global Impression. Clinical response was defined as a decrease of 50% or more in the HAM-D at endpoint and remission as HAM-D < 8 and YMRS < 8 at endpoint.

2.3. Assays

Blood samples were collected from 8:00 to 10:00 AM using vacutainer tubes. All subjects were in 8-h fasting. Samples were centrifuged at 20 °C and 1620 g for 15 min. Plasma was obtained, frozen, and stored at -80 °C. Given the complexity of the study, not all the patients and controls had samples available to be included in all analyses. All samples were assessed in duplicate. TBARS levels (malondialdehyde – thiobarbituric acid adduct) and SOD, CAT, and GPx activities were determined using spectrophotometry according to commercially available kits from Cayman Chemical Company®. Since SOD and CAT act sequentially, the results are also expressed as SOD/CAT ratio. CAT and GPx levels are presented as nM/min/mL, SOD as U/mL and TBARS as nM/mL.

2.4. Statistics

Student's *t* test and Mann–Whitney test were used for intragroup comparisons with normal and non-normal distributions of variables, respectively. Changes in OxS measures and enzyme activities before and after lithium treatment in the BD group were compared using paired student's *t* test and Wilcoxon signed ranks test. Kruskal–Wallis and ANOVA were used to compare two subgroups of patients with controls. Significance level was set at <0.05 (two-tailed). Statistical analysis was performed using the SPSS 14.0 and last observation carried forward was used in one patient who discontinued treatment.

3. Results

3.1. Clinical and demographical data

Demographic and clinical data are summarized in Table 1; patients and controls showed similar age, but a trend for different gender distribution ($p = 0.05$). Patients had a significant decrease in depressive symptoms measured by HAM-D from baseline (22.5 ± 3.5) to endpoint (7.3 ± 5.9) ($z = -4.68$, $p < 0.001$). Twenty-five (86.2%) patients responded to treatment and 18 (62.1%) achieved symptomatic remission at week 6. Mean duration of illness was 3.0 years (± 1.6).

3.2. Antioxidant enzymes are imbalanced in drug-free bipolar depression compared to controls

TBARS levels in BD patients at baseline ($n = 29$) and controls ($n = 22$) were not different ($p = 0.95$) (Fig. 1A) (Table 2). Baseline SOD levels in BD patients ($n = 25$) and controls ($n = 28$) were similar ($p = 0.56$) (Fig. 1B). CAT was increased in BD patients ($n = 29$) in comparison to controls ($n = 22$) ($p = 0.005$) (Fig. 1C). SOD/CAT ratio ($n = 25$) in bipolar depression = was decreased compared to controls ($n = 22$) ($p = 0.001$) (Fig. 1D). Finally, baseline GPx in subjects with BD ($n = 25$) was increased in comparison to controls ($n = 27$) ($t = 4.19$, $p < 0.001$) (Fig. 1E).

Since the BD and control groups had a trend for unbalance in gender, we compared Oxs parameters of males versus females in patients and then in controls to assure that the gender difference did not influence the results. After analyses (data not shown), the effects of gender difference in the sample did not influence in the results.

3.3. Lithium treatment decreases TBARS and SOD in bipolar depression

There was a significant decrease in TBARS from baseline to endpoint ($n = 28$, $p = 0.023$) (Fig. 1A) (Table 2). SOD activity decreased after lithium treatment ($n = 24$, $p = 0.029$) (Fig. 1B), while CAT levels did not show difference ($n = 28$, $p = 0.85$) (Fig. 1C). SOD/CAT ratio at endpoint showed no difference from baseline ($n = 24$, $p = 0.48$) (Fig. 1D). GPx levels were not different from baseline after lithium treatment ($n = 24$, $t = 0.47$, $p = 0.64$) (Fig. 1E).

3.4. TBARS at endpoint was decreased in responders compared to non-responders at endpoint

At endpoint, TBARS was decreased in responders (31.4 ± 32.9) compared to non-responders (76.7 ± 30.2) ($p = 0.018$) (Fig. 2A), although endpoint TBARS values from remitters ($n = 18$, 30.5 ± 32.7) and non-remitters ($n = 10$, 51.2 ± 39.1) showed significant difference ($p = 0.14$). There was no association between any other biological measure with response or remission (data not shown). Finally, lithium levels showed no association with any marker at endpoint (data not shown).

3.5. BD II but not BD I patients showed decrease in TBARS after lithium treatment

A selective decrease of TBARS in BD II (58.63 ± 46.23 nM/mL) to endpoint (29.14 ± 31.86 nM/mL) was observed ($n = 18$, $p = 0.039$), whereas in BD I, TBARS showed no alteration from baseline (67.86 ± 46.06 nM/mL) to endpoint (53.62 ± 38.87 nM/mL) ($n = 10$, $t = 0.71$,

$p = 0.50$). Endpoint TBARS in BD II was significantly decreased compared to endpoint TBARS in BD I ($p = 0.04$) (Fig. 2B). Other OxS parameters were not associated with BD subtype (data not shown).

4. Discussion

The present findings showed an imbalance of antioxidant enzymes in bipolar depression, with increased CAT and GPx and decreased SOD/CAT ratio in recent-onset drug-free subjects compared to healthy controls. Also, lithium significantly decreased plasma TBARS and SOD activity after 6 weeks of lithium treatment; importantly, TBARS levels at endpoint were associated with clinical response. TBARS decrease was only relevant in subjects with BD II. A number of different factors might contribute to the heterogeneity of results observed in the literature of OxS enzymes in BD (Kuloglu et al., 2002; Montero et al., 2012). Nevertheless, it has been suggested that enzymes imbalance is more associated with overall changes on OxS parameters than a specific alteration affecting a single protein (Andrades et al., 2005; Andreazza et al., 2007a).

We found increased CAT and GPx, and decreased SOD/CAT ratio in bipolar depression, which is consistent with imbalance and increased OxS found in other studies. Previously, our group found increased CAT activity in BD patients during drug naïve mania (Machado-Vieira et al., 2007). Only one study evaluated BD patients during a depressive episode (Andreazza et al., 2007a), but subjects were under diverse treatments and compared with other mood states. Other analyses comprised patients without depression or samples including symptomatic and asymptomatic patients and found decreased CAT levels (Andreazza et al., 2007a; Ozcan et al., 2004; Raffa et al., 2012; Ranjekar et al., 2003) or unaltered (Fontoura et al., 2012) in BD versus healthy subjects. Also, our finding of increased GPx levels in bipolar depression is in line with Andreazza et al. (2007a), while other studies did not find a significant difference in different mood states (Abdalla et al., 1986; Andreazza et al., 2009; Kuloglu et al., 2002; Raffa et al., 2012; Ranjekar et al., 2003). In spite of the fact that imbalance of anti-oxidant enzymes is an indicative of OxS present across mania, depression, and euthymia, BD studies do not suggest a specific antioxidant enzyme alteration associated with any mood phase.

Although the mechanisms underlying CAT and GPx balance are unclear, increases in CAT and GPx found here in early stage BD are compatible with a compensatory mechanism, proposed to be present in early stages of BD (Berk et al., 2013). Loss of neurotrophic support, increased oxidative stress and inflammation are associated with illness progression in BD (Berk et al., 2013). Oxidative stress and loss of neurotrophic support play key roles in the development of several neuropsychiatric disorders, including BD. Oxidative stress can down-regulate neurotrophic factors, while neurotrophic factors promote the expression of antioxidant proteins, also limiting inflammation.

The increases in CAT and GPx found in our study might explain the unaltered TBARS values found in our BD patients at baseline, in contrast with increased TBARS in most BD studies (Andreazza et al., 2008). Increases in CAT were also found in early stage BD during mania (Machado-Vieira et al., 2007). Importantly, CAT has shown efficacy against

dopamine-induced OxS in mitochondria (isolated from) rat brain (Berman and Hastings, 1999) and in one study in SH-SY5Y neuroblastoma human cells (Lai and Yu, 1997) although not in another study performed in the same cells (Emdadul Haque et al., 2003). Moreover, an elevation in GPx activity was the response to oxygen reactive species exposure in mice (Esposito et al., 1999). Glutathione, which antioxidant reactions are catalyzed by the enzyme GPx, was protective from dopamine-induced OxS in vitro (Kuhn et al., 1999), in SH-SY5Y neuroblastoma human cells (Emdadul Haque et al., 2003; Kuhn et al., 1999), and in rat striatum (Hastings et al., 1996). Regarding the glutamatergic system, glutathione decreased glutamatergic activity through reducing glutamate binding to glutamate receptors (Ogita et al., 1986; Varga et al., 1997). Also, our results showing decreased SOD/CAT ratio in BD patients relative to controls might be seen as compensatory, since decreased SOD/CAT ratio as well as increased CAT and GPx favors scavenge of the reactive oxygen species H_2O_2 (Gsell et al., 1995; Khairova et al., 2012). The role of CAT and GPx as markers of early stage bipolar depression warrants further investigation.

Regarding the effects of lithium on OxS parameters, it was observed a clinically relevant decrease in lipid peroxidation (TBARS) levels. Our findings are in line with previous studies showing lower TBARS levels in lithium treated BD after mania (Machado-Vieira et al., 2007) and euthymia (Banerjee et al., 2012). In the study of Banerjee et al. (2012), lithium's antioxidant effect was correlated with the activity of $Na^+-K^+-ATPase$ enzyme, pointing to a possible antioxidant mechanism of the drug. Thus, our results provide further support to the idea that lithium possesses an antioxidant effect.

Importantly, the response to lithium treatment was associated with decrease in TBARS levels. This finding reinforces the presence of an association of central and peripheral levels of OxS markers, which also was previously suggested by studies in acute neurological disorders such as stroke (Polidori et al., 1998). The decrease in TBARS levels in responders compared to non-responders warrants further research to confirm TBARS as a marker of treatment efficacy.

Interestingly, TBARS decreased after lithium treatment only in subjects with BD II. Although BD I and BD II are associated with different clinical course and response to treatment, neurobiological differences between the two types of BD remain understudied (D'Addario et al., 2012; Huang et al., 2012). Lithium treatment also decreased SOD activity in our sample. A decrease in SOD was also observed in healthy subjects taking lithium (Khairova et al., 2012), reinforcing that its antioxidant profile may involve SOD activity, independently of disorder and treatment outcome. Since SOD catalyzes a dismutation reaction that converts superoxide in hydrogen peroxide (Liochev and Fridovich, 1999), SOD decrease could potentially lower hydrogen peroxide formation.

The study is limited by the lack of a randomized double-blind design, also lacking a second point blood collection for controls, gender unbalance between BD and control groups, and the relatively small sample size. Also, the absence of patients studied during mania and euthymia in the sample may limit the generalization of the findings to all phases of BD, thus not addressing the specificity of our findings for bipolar depression. The younger age, short

length of illness and minimal prior treatment (most drug-free/naïve patients) are major strengths of this investigation and also a novelty in OxS research in BD during depression.

Overall, the present findings suggest a compensatory elevation in antioxidant enzymes CAT and GPx during depressive episodes in early stage of BD, with a decrease in lipid peroxidation (TBARS) associated with lithium's antidepressant actions. The present results reinforce the role for altered oxidative stress in the pathophysiology of BD and potential clinically relevant antioxidant effects of lithium in the prevention of illness progression.

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Fig. 1.

OxS parameters in patients with bipolar disorder in a depressive episode before (black bar) and after lithium treatment (grey bar) compared to healthy controls (white bar): A) TBARS–Thiobarbituric Acid Reactive Substances; B) SOD – Superoxide Dismutase; C) CAT – Catalase; D) SOD/CAT ratio, and E) GPx – Glutathione Peroxidase; * $p < 0.05$, ** $p < 0.01$.

Fig. 2.

A) Endpoint thiobarbituric acid reactive substances (TBARS) levels in responders compared with non-responders to lithium treatment. B) Endpoint TBARS levels in Bipolar Disorder I compared to Bipolar Disorder II; $*p < 0.05$.

Table 1

Demographic and clinical characteristics of bipolar disorder patients and healthy controls.

	Controls (<i>n</i> = 28)	Bipolar (<i>n</i> = 29)	<i>p</i>
<i>Gender</i>			
Male/Female, <i>n</i> (%)	16 (57.1)/12 (42.9)	8 (27.6)/21 (72.4)	0.05 ^{*a}
Age, years	28.0 (±7.2)	28.4 (±5.5)	0.60 ^b
<i>Bipolar Disorder type</i>			
Type I/Type II, <i>n</i> (%)		11 (37.9)/18 (62.1)	
<i>Mood Phase</i>			
Depressive/mixed		27 (93.1)/2 (6.9)	
Duration of illness, years		3.0 (±1.6)	
Drug-naïve, <i>n</i> (%)		21 (72.4)	
Drug-free, <i>n</i> (%)		26 (89.6)	
History of psychosis, <i>n</i> (%)		4 (13.8)	
<i>HAM-D</i>			
Baseline/endpoint		22.6 (±3.6)/7.3 (±5.9)	
<i>YMRS</i>			
Baseline/endpoint		6.1 (±5.6)/3.8 (±8.6)	
Response, <i>n</i> (%)		25 (86.2)	
Remission, <i>n</i> (%)		18 (62.1)	
Dropout, <i>n</i> (%)		1 (3.4)	
Endpoint serum lithium, mEq/L		0.49 (±0.20)	

HAM-D – Hamilton Depression Scale, YMRS – Young Mania Rating Scale.

* Significantly different.

^a Chi-square,^b Student's *t* test.

Table 2

OxS parameters in bipolar disorder patients in a depressive episode before and after lithium treatment compared to healthy controls.

	Before treatment (<i>n</i> = 29)	After treatment (<i>n</i> = 28)	Healthy controls (<i>n</i> = 22)	Patients vs. controls <i>p</i>	Before vs. after treatment <i>p</i>
TBARS (nM/mL)	60.77 ± 45.14	37.88 ± 35.85	62.74 ± 37.58	0.95	0.023 [*]
SOD (U/mL)	1.52 ± 0.23	1.38 ± 0.30	1.46 ± 0.45	0.56	0.029 [*]
CAT (nM/min/mL)	21.59 ± 9.49	22.17 ± 12.70	5.96 ± 2.67	0.005 ^{**}	0.85
SOD/CAT ratio	0.11 ± 0.11	0.15 ± 0.22	0.31 ± 0.17	0.001 ^{**}	0.48
GPx (nM/min/mL)	70.32 ± 15.62	68.76 ± 19.53	49.91 ± 19.10	<0.001 ^{**}	0.64

TBARS – Thiobarbituric Acid Reactive Substances, SOD – Superoxide Dismutase, CAT – Catalase, GPx – Glutathione Peroxidase,

^{*}
p < 0.05,

^{**}
p < 0.01.