

Short communication

Increased oxidative stress and DNA damage in bipolar disorder: A twin-case report

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Abstract

Objective: There is an emerging body of data suggesting that oxidative stress may be associated with the pathophysiology of bipolar disorder (BD). In the present study we investigated the oxidative stress profile in two monozygotic twins during a manic episode.

Methods: Two monozygotic twins diagnosed as currently manic by the Structured Clinical Interview for DSM-IV were studied. Serum thiobarbituric acid reactive substances (TBARS), superoxide dismutase (SOD) and catalase (CAT) were measured as parameters of oxidative stress. DNA damage was assessed using the single cell gel electrophoresis technique (Comet Assay). All biochemical measures were conducted at baseline and after a 6-week treatment.

Results: Bipolar twins had higher TBARS, SOD and DNA damage, and lower CAT than the healthy control. TBARS and SOD were normalized after mood stabilization, whereas CAT and DNA damage remained altered at week 6.

Conclusions: These findings support that oxidative stress may play a role in the pathophysiology of BD and that pharmacological treatment may exert antioxidant effects. Studies with larger samples are warranted to further clarify this issue.

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Keywords: Bipolar disorder; DNA damage; Oxidative stress; Pathophysiology

1. Introduction

Studies have consistently reported increased lipid peroxidation and changes in the major antioxidant enzymes in individuals with bipolar disorder (BD) (Ozcan et al., 2004; Ranjekar et al., 2003; Kuloglu et al., 2002), suggesting that oxidative stress may play a role in the pathophysiology of BD. The excessive generation of reactive oxygen species, such as hydroxyl radicals, can lead to lipid and protein oxidation, with consequent membrane and DNA damage. Moreover, there is recent evidence that the mood stabilizing

agents lithium and valproate exert robust antioxidant effects *in vitro* (Shao et al., 2005). We have recently demonstrated that BD subjects have increased DNA damage, possibly due to increased oxidative stress (Andreazza et al., *in press*). As an extension of this latter study, we prospectively investigated the oxidative stress profile and DNA damage in two medication-free monozygotic twins during a manic episode. Because genetic inheritance increases the risk for the development of BD, we hypothesized that the BD twins would present increased oxidative stress and DNA damage and these changes would be reversed after mood stabilization.

2. Case report

Two identical female twins, 59 years old, were assessed. One subject (patient 1) was admitted in the hospital for inpatient treatment, while the other (patient 2) refused treatment. The

Abbreviations: BD, bipolar disorder; CAT, catalase; DSM-IV, Diagnostic and Statistical Manual of Mental Disorders – Fourth Edition; SOD, superoxide dismutase; TBARS, thiobarbituric acid reactive substances.

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diagnosis of Bipolar I Disorder, current manic episode was carried out using the Structured Clinical Interview for DSM-IV-Axis I (First et al., 1998), and the severity of manic and depressive symptoms were assessed using the Young Mania Rating Scale (Young et al., 1978) and the Hamilton Depression Rating Scale (Hamilton, 1960), respectively. A healthy 58 year-old female was used as control. Blood samples were collected from each subject by venipuncture at baseline (time 0) and after 6 weeks (time 1). During this period, patient 1 used lithium carbonate 1500 mg/day (serum levels=0.9 mEq/l)+chlorpromazine 300 mg/day for 2 weeks. Then, chlorpromazine was switched to haloperidol 10 mg/day until the discharge. As parameters of oxidative stress, serum thiobarbituric acid reactive substances (TBARS), a marker of lipid peroxidation, and superoxide dismutase (SOD) and catalase (CAT), two major antioxidant enzymes were measured. All biochemical procedures were run in duplicate. To assess DNA damage we used the single cell gel electrophoresis technique, also known as the Comet Assay (CA), as previously described (Andreazza et al., in press). Under alkaline conditions the CA detects DNA single- and double-strand breaks and alkali-labile sites (Tice et al., 2000). Negative and positive controls were used for each electrophoresis assay in order to ensure the reliability of the procedure. All subjects provided written informed consent and this study was approved by the local ethics committee (Hospital de Clínicas de Porto Alegre, Porto Alegre, Brazil).

3. Results and discussion

At baseline (time 0), bipolar subjects had higher SOD and TBARS, and lower CAT levels than the healthy control (see Table 1). These findings are in accordance with previous studies (Ozcan et al., 2004; Ranjekar et al., 2003; Kuloglu et al., 2002), and indicate that the oxidative stress status was increased in the bipolar twins. It is intriguing that patient 1 had similar TBARS and SOD levels as the control after mood stabilization (time 1), whereas these oxidative stress parameters remained altered in the untreated twin (patient 2). These findings suggest that SOD and TBARS may be normalized by pharmacological treatment. CAT activity remained lower in both patients. Under normal conditions SOD metabolizes the excess of superoxide anion

(O_2^-) producing hydrogen peroxide (H_2O_2), which can spontaneously generate highly reactant hydroxyl radicals (OH^-). CAT and glutathione peroxidase detoxify the excess of H_2O_2 to prevent OH^- generation. Therefore, an imbalance within this physiological antioxidant system may increase the production of OH^- and, consequently, lead to lipid and protein damage (Mahadik and Mukherjee, 1996). In this context, lithium has been shown to possess antioxidant properties (Shao et al., 2005), haloperidol seems to increase oxidative stress (Pillai et al., in press), and studies conducted with chlorpromazine have reported controversial results (Pillai et al., in press; Roy et al., 1984). Thus, it is not clear which of these medications are associated with the partial improvement of the parameters of oxidative stress observed in the treated patient.

We also found that DNA damage was markedly higher in BD twins as compared with the healthy control at both time points, suggesting that DNA damage may be a trait rather than a state in BD. Interestingly, a recent study found that genes encoding the DNA repair enzyme, PARP, and several antioxidant enzymes, including SOD and CAT were decreased in the hippocampus of BD subjects (Benes et al., 2006). These results are in line with the present report, suggesting that the generation of ROS associated with the oxidative stress may induce lipid and protein oxidation, and consequently increasing DNA damage. Although the only study that directly assessed DNA fragmentation in BD showed no changes in the anterior cingulate cortex, the authors did not rule out the possibility that oxidative stress might be present (Benes et al., 2003). While the present study should be interpreted with the limitations of a case report, further studies are warranted to better determine the role of oxidative stress in the pathophysiology of BD.

4. Conclusion

These findings further substantiate that oxidative stress may play a role in the pathophysiology of BD and suggest that some measures of oxidative stress might be corrected by pharmacological treatment. Prospective studies with larger samples are warranted to investigate the effects of mood stabilizers on oxidative stress, as well as the clinical impact of oxidative stress in BD patients.

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Table 1
Clinical and oxidative stress parameters in patients and control

	YMRS score	HAMD score	TBARS (nmol/ml)	SOD (U/mg protein)	CAT (U/mg protein)	DNA damage (arbitrary units)
Healthy control	N/A	N/A	3.12±0.25	3.01±0.14	1.54±0.19	35.77±5.4
Patient 1						
Time 0	36	0	4.39±0.20	5.64±0.15	0.31±0.05	108.0±6.5
Time 1	4	3	3.45±0.31	2.62±0.14	0.31±0.06	118.6±6.8
Patient 2						
Time 0	19	6	6.36±0.38	4.98±0.21	0.55±0.05	106.8±4.6
Time 1	15	6	5.78±0.25	3.98±0.18	0.62±0.02	129.0±7.5

YMRS=Young Mania Rating Scale; HAMD=Hamilton Depression Rating Scale; TBARS=thiobarbituric acid reactive substances; SOD=superoxide dismutase; CAT=catalase.

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