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BRIEF COMMUNICATION

Higher proportion of inactive Gsk3β in platelets of elderly patients with bipolar disorder: an effect of treatment?

Rodolfo Braga Ladeira, Helena Passarelli Giroud Joaquim, Leda Leme Talib, Paula Villela Nunes, Orestes Vicente Forlenza

Laboratory of Neuroscience (LIM-27), Department and Institute of Psychiatry, School of Medicine, Universidade de São Paulo (USP), São Paulo, SP, Brazil.

Objective: It has been postulated that mood stabilizers inhibit glycogen synthase kinase 3-beta (Gsk3β) activity, mainly through its phosphorylation on serine-9 (Ser9). However, in vivo studies addressing Gsk3β activity in patients with bipolar disorder are scarce. Here, we compare Gsk3β inactivation (as indicated by Ser9-phosphorylation) in platelets of elderly patients with bipolar disorder undergoing clinical treatment and healthy elderly adults not taking medication.
Methods: Platelet samples were obtained from 37 elderly adults (bipolar disorder = 19, controls = 18). Relative changes in Gsk3β inactivation was estimated by comparing the ratios of phosphorylated Gsk3β to total Gsk3β (p-Gsk3β Ser9/Gsk3β) between the disease and control groups.
Results: Phosphorylated-Gsk3β ($p < 0.001$) and the p-Gsk3β Ser9/Gsk3β ratio ($p = 0.006$) were elevated in bipolar patients. In the bipolar disorder group, p-Gsk3β Ser9/Gsk3β was positively correlated with serum lithium levels ($r = 0.478$, $p = 0.039$).
Conclusions: Gsk3β inactivation is higher in this group of elderly adults undergoing treatment for bipolar disorder. However, whether the treatment or the disease causes Gsk3β inactivation was confounded by the lack of an unmedicated, bipolar control group and the non-uniform treatment regimens of the bipolar disorder group. Thus, further studies should help distinguish whether Gsk3β inactivation is an effect of drug treatment or an intrinsic characteristic of bipolar disorder.

Keywords: Glycogen synthase kinase 3; bipolar disorder; aged; lithium; blood platelets

Introduction

Glycogen synthase kinase-3 (Gsk3) is an important enzyme that is widely distributed in various biological systems. Gsk3 modulates critical intracellular signaling pathways, protein synthesis, cell proliferation, differentiation, adhesion, and apoptosis.[1] Gsk3β, the most studied of the Gsk3 isoforms, is abundantly expressed in the central nervous system.[2] The major physiological mechanism that regulates Gsk3β activity is phosphorylation of its n-terminal serine-9 (p-Gsk3β Ser9). This serine phosphorylation inhibits Gsk3β activity.[3]

Evidence linking Gsk3β activity to the pathophysiology of many neuropsychiatric disorders is growing. Although the exact contribution of Gsk3β to the pathophysiology of bipolar disorder is difficult to address, a combination of pharmacological and genetic evidence supports its involvement in mood regulation and in the action of several drugs used for the management of bipolar disorder.[4] However, these studies rely on in vitro or animal-based systems and only a few studies have been conducted in humans.[4,5] Pandey et al.[6] showed that total Gsk3 levels in platelets of drug-free, symptomatic bipolar patients are decreased. The abnormality was rectified upon treatment, yielding levels that were similar to those found in healthy controls. Conversely, Li et al.[7] showed that total Gsk3 levels were higher in peripheral blood mononuclear cells (PBMCs) of manic bipolar patients compared to healthy controls. Phosphorylated Gsk3 levels were lower in PBMCs of bipolar disorder patients than in healthy controls,[8] but antimanic treatment increased p-Gsk3.[7] Moreover, p-Gsk3β Ser9 levels were higher in PBMCs of bipolar disorder patients treated with lithium when compared to healthy controls.[9]

Given the growing interest on the role of Gsk3β in the pathophysiology of bipolar illness, and the paucity of data obtained from samples of elderly adults, the aim of this study was to evaluate Gsk3β inactivation (as indicated by increased proportion of Ser9 phosphorylated forms) in platelets of elderly patients with bipolar disorder treated with mood stabilizers. We hypothesized that these patients would have higher proportion of p-Gsk3β Ser9 to total Gsk3β (p-Gsk3β Ser9/Gsk3β) compared to our healthy comparison group.

Methods

Patients and clinical evaluation

Subjects, age 60 years or older, with a diagnosis of DSM-IV[10] bipolar disorder were followed up at the Institute of

Correspondence: Orestes V Forlenza, Laboratory of Neuroscience (LIM-27), Department and Institute of Psychiatry, School of Medicine, Universidade de São Paulo (USP), Rua Dr. Ovídio Pires de Campos, 785, 3° andar, CEP 05403-010, São Paulo, SP, Brazil.
E-mail: forlenza@usp.br
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Psychiatry, University of Sao Paulo, Brazil. The comparison group was composed of elderly adults with no current or past psychiatric disorders. They were recruited from an ongoing clinical cohort aimed at investigating health and cognition.[11] Psychiatric history, Structured Clinical Interview for DSM-IV disorders (SCID),[12] medical history, and physical and neurological examinations were collected prior to enrollment.

Subjects were excluded if they: i) were illiterate; ii) met the criteria for any other major DSM-IV Axis I diagnoses; iii) had any acute or major unstable medical illness or organic brain syndromes including dementia; iv) were taking any clinical medications that could possibly affect Gsk3β test results (such as non-steroidal anti-inflammatories, insulin or other anti-diabetic drugs); and v) refused to sign the informed consent (previously approved by the local ethics committee).

Clinical symptoms were assessed using the Young Mania Rating Scale (YMRS) for mania[13] and the 21-item Hamilton Depression Scale (HAM-D) for depression.[14]

Euthymia was defined as a score below 7 in those scales. Cognitive state was ascertained with the Cambridge Examination for Mental Disorders in the Elderly (CAMDEX) semi-structured interview yielding the Cambridge Cognitive Test (CAMCOG),[15] which includes the Mini-Mental State Examination. All assessments were completed during the week of blood collection. Vital signs, laboratory tests for chemistry, blood cell count, thyroid function, liver function, and metabolic profile were recorded.

Preparation of human platelets and measurements of Gsk3β and serum lithium levels

Blood samples were collected from a peripheral vein in the forearm the morning after a 10-hour fast and intake of oral psychiatric medications. Serum lithium levels were measured by lithium ion-selective electrode (ISE) using a 9180 Electrolyte analyzer (Roche). Platelets were isolated from plasma with anticoagulant citrate, and levels of p-Gsk3β Ser9 and total Gsk3β were determined by enzyme immunometric assay (TiterZyme EIA-Assay Designs, Inc, MI, USA) as previously described by our group.[11] Enzymatic inactivation was estimated by means of the ratio between the levels of the p-Gsk3β Ser9 and total levels (active and inactive) of Gsk3β (p-Gsk3β Ser9/Gsk3β).

Statistical analysis

When comparing the two groups, continuous variables were analyzed using Student's *t* test or whenever necessary Mann-Whitney U test. Categorical variables were analyzed using Pearson's chi-squared test or, whenever necessary, Fisher's exact test. Spearman's correlation analysis was conducted to evaluate the correlation between Gsk3β (total, p-Gsk3β Ser9 or p-Gsk3β Ser9/Gsk3β) in platelets from bipolar patients and serum lithium levels, scores on the HAM-D, YMRS, or CAMCOG. Linear regression analysis (backward variable selection method) was performed to identify predictors of Gsk3β phosphorylation and p-Gsk3β Ser9/Gsk3β. The statistical analyses above were conducted using SPSS, and α was set at 5%. Numerical variables were expressed as mean and standard deviation, whereas categorical variables were expressed as frequency.

Results

Demographics, clinical characteristics and medication status are summarized in Table 1. The sample comprised 19 bipolar patients and 18 healthy controls. All bipolar patients were using either one or more psychotropic medications.

Bipolar patients were younger than the healthy group (mean ± standard deviation = 68.3±4.9 vs. 74.2±6.5 years, p = 0.004), and their cognitive performance in the CAMCOG was worse (84.0±11.3 vs. 94.1±7.4, p = 0.003). There were no significant differences in gender (male: 26 vs. 33%) or years of education (8.4±5.1 vs. 10.7±4.3 years) between the bipolar disorder and healthy groups, respectively. In the bipolar group, the mean age of the first mood episode was 38.3±15.5 years, 74% had depression as the index episode, and 53% had been hospitalized for mood-related disturbances in the past.

The medical comorbidities detected in 10% or more of all bipolar patients or controls were hypertension, hyperlipidemia, and hypothyroidism (Table 1). No statistically significant differences between the two groups were observed for the occurrence of general medical conditions, except for a higher prevalence of hypothyroidism among patients with bipolar disorder (47 vs. 11%, p = 0.016).

No significant differences were observed in total protein expression of Gsk3β in platelets between the two groups (Figure 1). Bipolar patients had higher p-Gsk3β Ser9 levels (1117.1±437.0 vs. 667.7±185.4 pg/mL, $p < 0.001$, Student's *t* test), yielding a higher p-Gsk3β Ser9/Gsk3β ratio (0.25±0.06 vs. 0.19±0.05, p = 0.006, Student's *t* test). This indicates that the bipolar group had a higher proportion of inactive (p-Gsk3β Ser9) Gsk3β.

Linear regression analyses (backward method) were performed to identify predictors of higher Gsk3β phosphorylation and p-Gsk3β Ser9/Gsk3β ratio. The independent variables, selected because they were different between the groups, were psychiatric diagnosis (patients with bipolar disorder or comparison group), age, CAMCOG score, and presence of hypothyroidism. The dependent variables were p-Gsk3β Ser9 and p-Gsk3β Ser9/Gsk3β. Psychiatric diagnosis had the highest influence on the mean values of p-Gsk3β Ser9 (beta = 611.2, $p < 0.001$) and p-Gsk3β Ser9/Gsk3β (beta = 0.081, p = 0.001). The presence of hypothyroidism attenuated the effect of the psychiatric diagnosis (bipolar disorder) on the phosphorylation state of Gsk3β and p-Gsk3β Ser9/Gsk3β ratio (beta = -373.6, p = 0.007 and beta = -0.055, p = 0.031, respectively) and thus, this interference was conservative.

A moderate but significant correlation was found between p-Gsk3β Ser9/Gsk3β and serum lithium levels

Table 1 Characteristics of bipolar patients and comparison group*

	Bipolar patients (n=19)	Comparison group (n=18)	p-value
Age (years), mean (SD)	68.3 (4.9)	74.2 (6.5)	0.004
Male sex	5 (26)	6 (33)	0.641
Education (years), mean (SD)	8.4 (5.1)	10.7 (4.3)	0.166
CAMCOG, mean (SD)	84.0 (11.3)	94.1 (7.4)	0.003
Age at onset of illness (years), mean (SD)	38.3 (15.5)	-	
Depression as index episode	14 (74)	-	
Ever hospitalized for mood-related disturbances	10 (53)	-	
Mood			
Euthymic	6 (32)	-	
Depressed	9 (47)	-	
Manic	2 (11)	-	
Mixed	2 (11)	-	
Psychotropic medication			
Lithium carbonate	14 (74)	-	
Carbamazepine	1 (5)	-	
Valproate sodium	7 (37)	-	
Lamotrigine	4 (21)	-	
Olanzapine	6 (32)	-	
Quetiapine	2 (11)	-	
Aripiprazole	1 (5)	-	
Sertraline	5 (26)	-	
Clonazepam	4 (21)	-	
Current medical comorbidities			
Hypertension	7 (37)	10 (56)	0.254
Hyperlipidemia	4 (21)	7 (39)	0.235
Hypothyroidism	9 (47)	2 (11)	0.016

CAMCOG = Cambridge Cognitive Test; SD = standard deviation.
* Data expressed as number (percentage) of subjects, unless otherwise indicated.

in the bipolar group (Spearman's $r = 0.478$, two-tailed p = 0.039). No significant correlations were found between Gsk3β (total, p-Gsk3β Ser9 or p-Gsk3β Ser9/Gsk3β) and HAM-D, YMRS, and CAMCOG total scores. To evaluate the relationship between the type of treatment and Gsk3β inactivation, data were further plotted across two subsamples of bipolar disorder (medicated or not with a certain drug) and controls. ANOVAs followed by post-hoc analyses indicated that p-Gsk3β Ser9 and p-Gsk3β Ser9/Gsk3β were higher in bipolar patients undergoing lithium treatment as compared to healthy controls (Dunnett, p = 0.006 and Tukey, p = 0.016, respectively), but not when compared to bipolar patients treated with other drugs (clonazepam, olanzapine, sertraline, and valproate) and controls. We stratified the bipolar patients according to prior history of hospitalization for mood-related disturbance, type of index episode (mania or depression), and the most recent type of mood episode (depression vs. euthymia). These factors were not significantly associated with differences between total Gsk3β, p-Gsk3β Ser9, or p-Gsk3β Ser9/Gsk3β. However, the interpretation of these sub-analyses must take into account the fact that the non-lithium group and the subgroups stratified according to clinical characteristics were very small.

Discussion

Higher p-Gsk3β Ser9/Gsk3β ratio was detected in elderly bipolar patients who were medicated, suggesting that Gsk3β was proportionally inactivated in these patients. No significant differences were observed in total Gsk3β levels between the bipolar disorder and healthy groups. Thus, the higher p-Gsk3β Ser9/Gsk3β ratio was due to a significant increase in the p-Gsk3β Ser9 levels. In the bipolar disorder group, there was a moderate but significant positive correlation between p-Gsk3β Ser9/Gsk3β and serum lithium levels. Although lithium and other drugs currently used for the treatment of unipolar depression and bipolar disorder are known to inhibit Gsk3β,[3] this is the first study, to the best of our knowledge, to report correlation between serum lithium levels and Gsk3β inactivation in vivo. These results are in line with the findings of higher p-Gsk3β Ser9 levels and, therefore, increased Gsk3β inactivation in PBMCs of medicated patients with bipolar disorder.[7,9] Moreover, lithium-induced Ser9 phosphorylation has been demonstrated in nonhuman animal and in vitro studies.[16] However, increased Gsk3β inactivation has not been correlated with the severity of manic symptoms, depressive symptoms or cognitive deficits in elderly bipolar patients.

Although our data are consistent with previous studies, a few methodological caveats apply. Namely, our sample size is relatively small, and there is a possibility of recruitment bias given that the study was conducted in a specialized psychogeriatric setting. Despite the potential presence of confounding factors due to age (e.g., cognitive changes, clinical comorbidities, and medications), our sample comprises a homogeneous diagnostic group because these patients have long-standing diagnosis of bipolar disorder (on average 40 years). Furthermore, all recruited patients were using either one or more psychotropic medications (e.g., anticonvulsants, atypical antipsychotics, or antidepressants), which may also affect Gsk3β

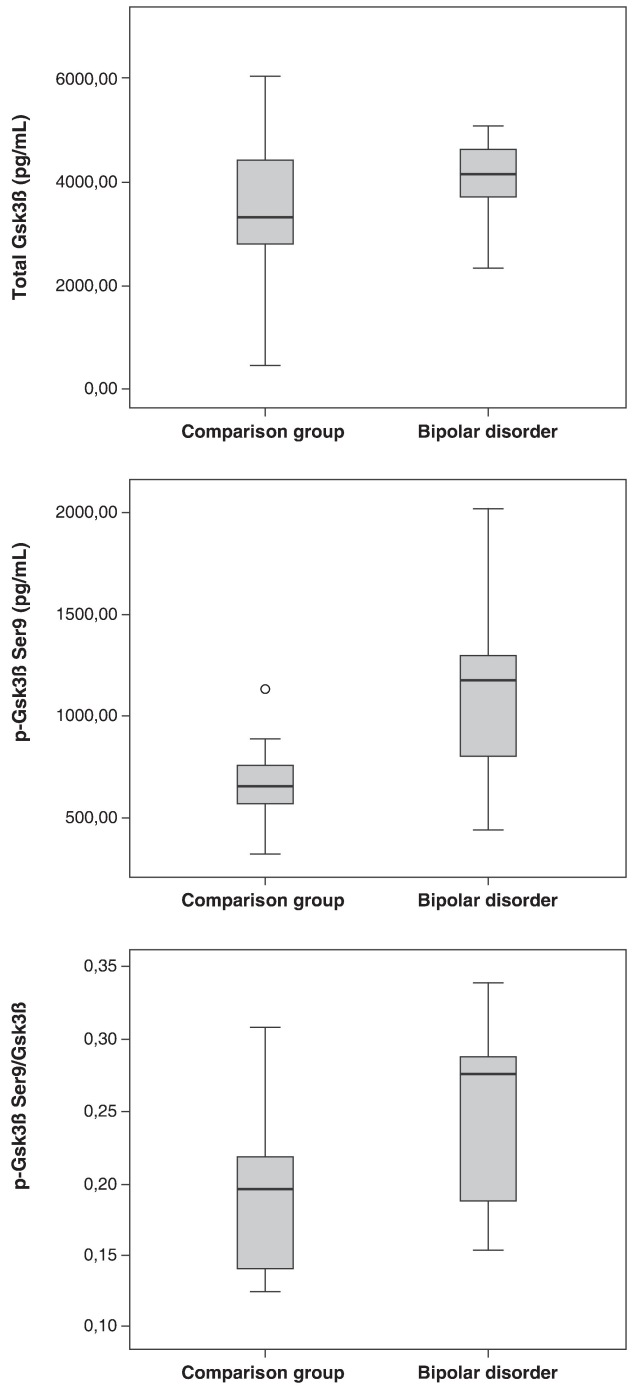


Figure 1 Levels of total Gsk3β, p-Gsk3β Ser9, and p-Gsk3β Ser9/Gsk3β in platelets of older adults with bipolar disorder undergoing pharmacological treatment and comparison group. ° represents an outlier subject. No significant differences were observed in total Gsk3β in platelets between older adults with bipolar disorder undergoing pharmacological treatment and comparison group ($p = 0.171$, Student's *t* test). Bipolar patients had higher p-Gsk3β Ser9 levels ($p < 0.001$, Student's *t* test) and higher p-Gsk3β Ser9/Gsk3β ($p = 0.006$, Student's *t* test)

regulation. The absence of a control group with unmedicated bipolar patients and the non-uniform pattern of treatments in our bipolar disorder group do not allow us to conclude whether Gsk3β inactivation is due to properties of bipolar illness itself or an effect of the therapeutic drugs in use.

In spite of the fact that the platelets share many biochemical similarities with neurons and that Gsk3β is abundant in platelets, these results should be viewed with caution. It is not yet clear to what extent platelet Gsk3β activation actually reflects neuronal Gsk3β activation. The normalization of p-Gsk3β Ser9 by total Gsk3β levels was necessary to evaluate whether the change in p-Gsk3β was due to a variation of total Gsk3β among subjects. Given the fact that no significant differences were observed in total Gsk3β between groups, it is conceivable that the differences in the proportion of these forms is a consequence of the increase in the inactive form of Gsk3β.

In a previous study, we found that p-Gsk3β Ser9 and p-Gsk3β Ser9/Gsk3β are decreased in the platelets of patients with geriatric depression.[17] We argued that overactive Gsk3β could be regarded as a marker of severity of depression and cognitive impairment in these subjects. In the present study, we found an opposite effect of Gsk3β activation, i.e. older patients with bipolar disorder have increased serine-phosphorylation and inactivation of Gsk3β. We reason that methodological differences (e.g., diagnostic groups and medication status) may account for the contradiction in these findings.

Our results reinforce the evidence that Gsk3β is involved in the pathophysiology of mood disorder and psychotropic actions, particularly lithium. Inactive p-Gsk3β Ser9 levels are increased in this group of elderly adults with bipolar disorder undergoing treatment. Despite of the caveats stated above and the limitations of our controls, the statistically significant correlation between p-Gsk3β Ser9/Gsk3β and serum lithium levels suggests that this pharmacological agent contributes to Gsk3β inactivation.

Therefore, the determination of platelet Gsk3β ratio may be useful as a biological marker for the pathophysiology of bipolar disorder and perhaps other neuropsychiatric illnesses.

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Disclosure

The authors report no conflicts of interest.

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