Schizophrenia Research xxx (2017) xxx–xxx

Contents lists available at ScienceDirect

Schizophrenia Research

journal homepage: www.elsevier.com/locate/schres

Increased platelet glycogen sysnthase kinase 3beta in first-episode psychosis

Helena P.G. Joaquim [a], Marcus V. Zanetti [a,b,c], Mauricio H. Serpa [a,b,c], Martinus T. Van de Bilt [a], Paulo C. Sallet [a], Tiffany M. Chaim [b,c], Geraldo F. Busatto [b,c], Wagner F. Gattaz [a,b], Leda L. Talib [a,b,*]

[a] Laboratory of Neuroscience (LIM-27), Department and Institute of Psychiatry, University of Sao Paulo, Brazil
[b] Center for Interdisciplinary Research on Applied Neurosciences (NAPNA), University of São Paulo, Brazil
[c] Laboratory of Psychiatric Neuroimaging, LIM-21, Department and Institute of Psychiatry, University of Sao Paulo, Brazil

ARTICLE INFO

Article history:
Received 10 October 2016
Received in revised form 31 August 2017
Accepted 31 August 2017
Available online xxxx

Keywords:
First-episode psychosis
Non-affective psychosis
Schizophrenia
GSK3B
Antipsychotic treatment

ABSTRACT

Past studies have linked intracellular pathways related to psychotic disorders to the GSK3B enzyme. This study aimed to investigate GSK3B protein expression and phosphorylation in drug-naïve first-episode psychosis patients ($n = 43$) at baseline and following symptom remission, and in healthy controls ($n = 77$). At baseline GSK3B total level was higher in patients ($p < 0.001$). In schizophrenia spectrum patients ($n = 25$) GSK3B total and phosphorylated levels were higher than in controls and patients with other non-affective psychotic disorders ($n = 18$) ($p < 0.001$; $p = 0.027$; $p = 0.05$ respectively). No enzyme changes were found after clinical remission. The implication of this finding for the biology of psychoses warrants further studies to clarify whether increased GSK3B may be useful as a biomarker for psychosis in general, and schizophrenia in particular.

1. Introduction

The enzyme glycogen synthase kinase 3-Beta (GSK3B) plays a major role in cytoskeletal organization and remodeling. It is thereby involved in mechanisms of synaptic plasticity, neurogenesis and resilience to neuronal injury (Grimes and Jope 2001; Gould and Manji 2005; Jope et al. 2007; Muyllaert et al. 2008; Peineau et al. 2008). GSK3B has also been linked to dopamine D2 neuronal pathways (Lovestone et al. 2007). The main regulatory mechanism of GSK3B activity is the phosphorylation of the Ser-9 residue (pGSK3B), which inhibits the enzymatic activity (Wang et al. 1994).

In the last decade several lines of inquiry indicate abnormal GSK3B metabolism in the pathogenesis of schizophrenia (Emamian et al. 2004; Koros and Dorner-Ciossek 2007). Some genomic studies reported genes that are associated both with schizophrenia and GSK3B regulation, such as: catechol-*O*-methyl transferase (COMT), neuregulin1 (NRG1), v-AKT murine thymoma viral oncogene homolog 1 (AKT1), glutamate decarboxylase 1 (GAD1), dystrobrevin-binding protein 1 (DTNBP1) (Lovestone et al. 2007).

Alterations in GSK3B protein expression and activity were reported in different tissues from schizophrenia samples (Emamian et al. 2004; Koros and Dorner-Ciossek 2007; Bousman et al. 2013; Wesseling et al. 2014; Chen et al. 2015; Levchenko et al. 2015). Several studies linked D2 receptor activation with a decrease of pGSK3B in neurons and consequent increase in GSK3B activity, which is believed to be neurotoxic (Beaulieu et al. 2004, 2005, 2009; Beaulieu 2012; Bibb 2005). There are, however, no studies of GSK3B with drug naïve first-episode psychosis (FEP) patients. The aim of this study is to evaluate the GSK3B and pGSK3B levels in patients with non-affective FEP and healthy controls, and to investigate the effects of antipsychotic drug treatment.

2. Materials and methods

2.1. Participants and clinical assessment

Forty-three drug naïve non-affective FEP patients referred to our inpatient and outpatient units at the Institute of Psychiatry, University of São Paulo, and 77 age and gender-matched, healthy individuals were enrolled in this study.

At baseline, the subjects carried out a clinical interview and the Structured Clinical Interview (SCID) (First et al. 1995) for Diagnostic and Statistical Manual for Mental Disorders, 4th edition (DSM-IV), as well as the Alcohol Use Disorders Identification Test (AUDIT) (Saunders et al. 1993). Symptom severity was assessed with the Positive and Negative Syndrome Scale (PANSS; Kay et al. 1987).

Exclusion criteria for both groups were: previous psychopharmacological drugs use; presence of neurological or any organic disorders that could affect the central nervous system; history of head trauma with

* Corresponding author at: Laboratório de Neurociências LIM27, Rua Dr. Ovídio Pires de Campos, 785, 3° andar, 05403-010 São Paulo, SP, Brazil.
E-mail address: leda.talib@hc.fm.usp.br (L.L. Talib).

http://dx.doi.org/10.1016/j.schres.2017.08.062
0920-9964/

loss of consciousness; use of drugs that have a significant effect on GSK3B protein expression or activity. Local ethics committees approved the study and all subjects provided informed written consent.

2.2. Study design

After blood collection, patients started a semi-naturalistic antipsychotic treatment regimen with haloperidol (15%), olanzapine (25%) or risperidone (60%) based on the recommendations from the International Psychopharmacology Algorithm Project (IPAP http://www.ipap.org/).

FEP subjects were then clinically evaluated on a weekly basis by an experienced psychiatrist using the PANSS until achieving clinical remission - defined as a PANSS item score < 4 (Andreasen et al. 2005). The mean interval between baseline and remission was 96 ± 75 days. After remission, FEP patients were evaluated weekly until completing a month of sustained remission. At this point, a second blood collection was made. The socio-demographic data are described in Table 1.

2.3. Laboratory analysis

Forty milliliters of blood were collected in citrate tubes at baseline (drug naïve) and after remission. Platelets were isolated from the peripheral blood of patients and controls and processed as described before (Joaquim et al. 2012). Protein levels were determined by a modified Lowry method (Bio-Rad DC Protein Assay) and protein concentration was normalized to 0.1 mg/ml with buffer plus protease inhibitor as provided by the manufacturer (Assay Designs, Inc., MI, USA). GSK3B concentrations was determined by a specific Enzyme Immunometric Assay to assess total GSK3B (tGSK3B) and pGSK3B (TiterZyme EIA – Assay Designs, Inc., MI, USA) according to the manufactures instructions. As GSK3B is regulated predominantly in an inhibitory manner by phosphorylation, its activity was indirectly estimated by the ratio pGSK3B/tGSK3B (rGSK3B), in which lower ratios reflect higher enzymatic activity (Joaquim et al. 2012).

2.4. Statistical analysis

Statistical analysis was performed using SPSS v.22. We conducted the statistical comparison between FEP and controls with a T-student test. The comparisons between subgroups were analyzed using an Oneway ANOVA and Turkey HSD for post-hoc analyses. For the longitudinal comparisons, we used a *t*-test for paired samples for each diagnosis subgroup separately. All the tests were two-tailed and statistical significance was set at 0.05.

Table 1
Demographic data of patients and health control.

Diagnostic	Schizophrenia (*n* = 25)	Other non-affective psychosis (n = 18)	Control (n = 77)	p-value
Gender. women (%)	10 (40%)	6 (33%)	32 (44%)	0.705[a]
Age in years (sd)	24.3 (3.9)	28.5 (9.6)	26.2 (3.9)	0.045[b]
Scholarship in years (sd)	11.8 (3.7)	9.8 (3.0)	14.7 (2.3)	<0.001[b]
Illness duration in days (sd)	58.7 (59.3)	38.2 (33.8)	–	0.136[c]
PANSS ratings				
Total (sd)	68 (28)	52 (17)	–	0.027[c]
Positive (sd)	16 (8)	13 (5)	–	0.195[c]
Negative (sd)	17 (9)	11(5)	–	0.004[c]
General (sd)	35 (14)	28 (10)	–	0.052[c]

M = male; F = female; sd = Standard deviation; SCZ = Schizophrenia; BD = Bipolar disorder. PANSS = Positive and Negative Syndrome Scale.
[a] Chi-square test.
[b] ANOVA test.
[c] *t*-Test.

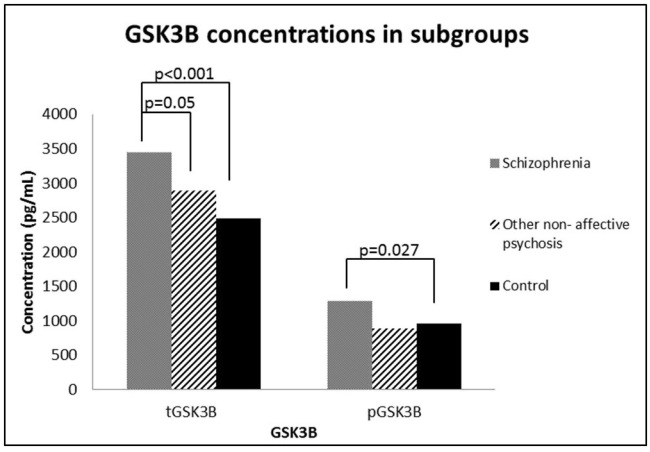

Fig. 1. Total and phosphorylated glycogen synthase kinase 3B (tGSK3B and pGSK3B) concentrations in diagnosis subgroups. Legend: tGSK3B = total glycogen synthase kinase 3 beta; pGSK3B = phosphorylated glycogen synthase kinase 3 beta; rGSK3B = glycogen synthase kinase 3 beta ratio; p value generated using post-hoc Tukey test.

3. Results

Total baseline GSK3B levels were higher in FEP than in control ($p < 0.001$). No significant differences were observed regarding pGSK3B or rGSK3B between patients and controls (Fig. 1). When we separate the FEP group into schizophrenia spectrum and other non-affective psychosis (58% psychosis not otherwise specified; 25% delusional disorder; 17% acute and transient psychotic disorders), we found that tGSK3B levels where higher in schizophrenic patients than in non-affective psychosis ($p < 0.05$) or than controls ($p < 0.001$). pGSK3B was significantly higher in schizophrenia patients than in controls ($p < 0.05$). No differences in rGSK3B were found among the three groups (Table 2).

There were no significant changes in tGSK3B, pGSK3B or rGSK3B levels before and after antipsychotic treatment. The antipsychotic drugs used in this trial had a similar effect on GSK3B concentrations. There was a positive correlation between tGSK3B pre and post remission ($r = 0.474$; $p < 0.001$), pGSK3B pre and post remission ($r = 0.490$; $p < 0.001$); pGSK3B pre and post remission ($r = 0.252$; $p = 0.020$). Considering the subgroups, we found no significant differences between time response of schizophrenic patients and other non-affective psychoses (77 ± 35 days versus 105 ± 93 days) ($p = 0.228$).

No statistically significant correlations between GSK3B concentrations, age, sex, duration of psychosis before treatment, time needed to remission and PANSS scores exist in the data.

4. Discussion

In this study we investigated the concentrations of GSK3B total and phosphorylated in platelets of drug-naïve FEP patients. Concentrations of tGSK3B in platelets were higher in patients than in controls, with the highest values in schizophrenic patients. On the other hand, pGSK3B was increased only in schizophrenic patients as compared to controls and to non-schizophrenic psychoses. To our knowledge, this is the first study of GSK3B concentrations in drug naïve psychotic patients.

Our findings contrast with reports of decreased tGSK3B and pGSK3B in lymphocytes (Nadri et al. 2002), cerebrospinal fluid (Kozlovsky et al. 2004) and different post mortem brain areas of schizophrenic patients (Kozlovsky et al. 2000, 2004, 2005; Emamian et al. 2004). However, some studies in schizophrenia also describe no differences from controls in GSK3B protein concentrations in post mortem brain (Beasley et al. 2002; Kozlovsky et al. 2005) and in lymphocytes (Emamian et al. 2004). Moreover, we failed to find any correlation between GSK3B

Please cite this article as: Joaquim, H.P.G., et al., Increased platelet glycogen sysnthase kinase 3beta in first-episode psychosis, Schizophr. Res. (2017), http://dx.doi.org/10.1016/j.schres.2017.08.062