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Stem cells, pluripotency and glial cell markers in peripheral blood of bipolar patients on long-term lithium treatment

Ewa Ferensztajn-Rochowiak[a], Jolanta Kucharska-Mazur[b], Maciej Tarnowski[c], Jerzy Samochowiec[b], Mariusz Z. Ratajczak[c,d], Janusz K. Rybakowski[a,*]

[a] Department of Adult Psychiatry, Poznan University of Medical Sciences, Poznan, Poland
[b] Department of Psychiatry, Pomeranian University of Medicine, Szczecin, Poland
[c] Department of Physiology, Pomeranian University of Medicine, Szczecin, Poland
[d] Stem Cell Biology Program at the James Graham Brown Cancer Center, University of Louisville, Louisville, KY 40202, USA

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ABSTRACT

Background: We investigated the effect of long-term lithium treatment on very-small embryonic-like stem cells (VSELs) and the mRNA expression of pluripotency and glial markers, in peripheral blood, in patients with bipolar disorder (BD).

Methods: Fifteen BD patients (aged 53 ± 7 years) not treated with lithium, with duration of illness > 10 years, 15 BD patients (aged 55 ± 6 years) treated with lithium for 8–40 years (mean 16 years) and 15 control subjects (aged 50 ± 5 years) were included. The number of VSELs was measured by flow cytometric analysis. Assessment of the mRNA levels of pluripotency markers (Oct-4, Sox 2 and Nanog) and glial markers (glial fibrillary acidic protein - GFAP, Olig1 and Olig2) was performed, using the Real-time quantitative reverse transcription PCR.

Results: In BD patients not taking lithium, the number of VSELs was significantly higher than in control subjects and correlated with the duration of illness. The expression of pluripotency markers was significantly higher than in the controls and correlated with the number of VSELs. The mRNA levels of the Olig1 and Olig 2 were higher than in the controls and those of the GFAP were higher than in patients receiving lithium. In lithium-treated BD patients the number of VSELs was similar to controls and correlated negatively with the duration of lithium treatment and serum lithium concentration. The mRNA levels of Oct-4, Sox-2, GFAP and Olig1 were not different from controls. The mRNA expression of Nanog was significantly higher and correlated with the number of VSELs. The mRNA expression of Olig 2 was higher than in the BD patients not taking lithium.

Conclusion: Long-term treatment with lithium may suppress the activation of regenerative processes by reducing the number of VSELs circulating in PB. These cells, in BD patients not treated with lithium, may provide a new potential biological marker of the illness and its clinical progress. The higher expression of peripheral mRNA markers in BD patients may involve ongoing inflammatory process, compensatory mechanisms and regenerative responses. Long-term lithium treatment may attenuate these mechanisms, especially in relation to the transcription factors Oct-4, Sox-2, GFAP and Olig1.

1. Introduction

Bipolar disorder (BD) is a recurrent and often chronic condition, characterized by episodes of mania, hypomania, depression and mixed states, having a worldwide prevalence rate of 2–5%. For several decades, the illness has been the subject of intense neurobiological studies showing also that, processes connected with the production and function of stem cells may play a role in its pathogenesis (O'Shea and McInnis, 2016). Lithium remains the main mood-stabilizing drug for the long-term treatment of BD, and the mechanisms of lithium action may be related to BD pathogenesis. Recently, a review on the effect of lithium on different stem cell populations has been published (Ferensztajn-Rochowiak and Rybakowski, 2016).

Several years ago, a new human stem cell population, named very small embryonic-like stem cells (VSELs), has been discovered (Kucia et al., 2007). In subsequent research, the characterization, developmental origin and biological significance of these cells have been described (Ratajczak et al., 2008). The research group at the Pomeranian

* Corresponding author at: Department of Adult Psychiatry, Poznan University of Medical Sciences, ul.Szpitalna 27/33, 60-572 Poznan. Poland.
E-mail address: janusz.rybakowski@gmail.com (J.K. Rybakowski).

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Medical University in Szczecin published the first original report on VSELs circulating in the peripheral blood (PB) of patients with a first psychotic episode. An increased mobilization of VSELs during acute psychotic episode as compared to matched control subjects, and a non-significant decrease of VSELs mobilization during antipsychotic treatment have been found (Kucharska-Mazur et al., 2014).

Master pluripotency transcription factors (TFs) such as octamer-binding transcription factor 4 (Oct-4), sex determining region Y-box 2 (Sox2) and their downstream target, homeobox protein, Nanog, are important elements in stem cell production and differentiation. They control the early stages of embryogenesis and the maintenance of the pluripotent state in some stem cells occurring in the adult organism (Rumman et al., 2015). TFs also participate in normal cell turnover in the neural system, whereas Sox-2 is a major player in the self-renewal and differentiation of neural stem cells into neurons and astrocytes (Ellis et al., 2004). Recently, an expression of Oct4 was described on VSELs, which migrated and localized in an injured spinal cord (Golipoor et al., 2016).

Neuroinflammation, with microglial activation as an important element, plays a role in the pathogenesis of bipolar disorder (BD). Pathological changes have been also demonstrated in macroglial cells, such as astrocytes and oligodendrocytes. Postmortem brain studies of BD patients to assess glial cells, such as astrocytes and oligodendrocytes and their markers such as glial fibrillary acidic protein (GFAP), Olig1 and Olig2, produced controversial results (Toro et al., 2006; Rao et al., 2010; Hayashi et al., 2012). However, there is a lack of study of these markers in the peripheral blood (PB).

In this paper, a comprehensive report of our studies on VSELs, pluripotent and glial markers in peripheral blood of BD patients and the effect of long-term lithium treatment on these is presented. The choice of the pluripotent and glial markers was based on their possible role in the pathogenesis of psychiatric disorders, including bipolar mood disorder. Some of these results have been partly published elsewhere (Ferensztajn-Rochowiak et al., 2016a,b,c).

2. Methods

2.1. Subjects

The study comprised 30 subjects with BD during remission, treated at the outpatient clinic of the Department of Adult Psychiatry, Poznan University of Medical Sciences, and 15 healthy age-and sex matched control subjects.

The BD patients were divided into two subgroups: BD Li (+) - patients treated with lithium and BD Li (−) – lithium naïve patients. The first subgroup consisted of 15 persons (5 male, 10 female), aged 55 ± 6 years, with a duration of illness of 24 ± 9 years, treated continuously with lithium carbonate for 8–40 years (mean 16 years). The serum concentration of lithium was maintained in the range 0.5–0.8 mmol/l (mean 0.73 mmol/l). In this subgroup, 6 persons additionally received other mood-stabilizing drug, carbamazepine. Treatment with antidepressant drugs included venlafaxine (3 persons), quetiapine (3 persons), fluoxetine (2 persons), trazodone (2 persons), bupropion (1 person), tianeptine (1 person), clomipramine (1 person), escitalopram (1 person), and mirtazapine (1 person).

The second subgroup, BD Li (−), consisted of 15 persons (5 male, 10 female), aged 53 ± 7 years, with a duration of illness at least 10 years (mean 20 ± 9 years, range 10–44 years), who had never received lithium. The duration of mood-stabilizing treatment was 3–20 years (mean 9 ± 6 years) and consisted of valproic acid (7 persons), lamotrigine (6 persons) or carbamazepine (3 persons). Other psychotropic drugs used included quetiapine (5 persons), venlafaxine (3 persons), sertraline (2 persons), paroxetine (1 person), fluoxetine (1 person), escitalopram (1 person), clomipramine (1 person), trazodone (1 person), olanzapine (1 person) and clozapine (1 person).

The control group consisted of 15 healthy individuals (5 male, 10 female), aged 50 ± 5 years, without an individual or family history of psychiatric disturbances, and matched, for age, sex, BMI and ethnicity, with the bipolar patients.

A consensus diagnosis by at least two psychiatrists, according to the International Classification of Diseases (ICD-10) and Diagnostic and Statistical Manual of Mental Disorders (DSM-IV) criteria, was made for each patient using the Structured Clinical Interview for DSM-IV Axis I Disorders (SCID) (First et al., 1996). All BD patients were in remission having 7 or less points on the 17-item Hamilton Depression Rating Scale and/or on the Young Mania Rating Scale. Other medical conditions in the study group included hypothyroidism (4 persons), psoriasis (3 persons), hypertension (2 persons), chronic obstructive pulmonary disease (1 person), rheumatoid arthritis (1 person), asthma (1 person), and ischaemic heart disease (1 person). The medication used comprised l-thyroxine, indapamide, montelukast, fenofibrate and rosuvastatine. Autoimmune diseases (excluding asthma) did not require anti-inflammatory treatment during the study period.

Exclusion criteria for both patients and control subjects were a history of perinatal/developmental problems, glucose intolerance/diabetes, organic brain injuries, an acute phase of an immune disease, current infection, drug/alcohol dependence or any other serious physical condition which might interfere with the results of stem cell enumeration (Ratajczak et al., 2014a). There was no psychiatric comorbidity, alcohol abuse or excessive smoking (> 5 cig/day) within groups. Both patients and control subjects were informed that strenuous exercise and dietary restrictions were not allowed before blood sample collection.

The study was approved by the Bioethics Committee of Poznan University of Medical Sciences, and all the participants gave their informed consent, after the nature of the procedures had been fully explained to them.

2.2. Assessment of VSELs, pluripotency and glial markers

A single, fasting, venous blood sample was drawn in the morning from each individual. The blood samples were collected in the Department of Adult Psychiatry, Poznan University of Medical Sciences and transported to and evaluated in the Department of Physiology, Pomeranian University of Medicine in Szczecin within a few hours on the day of collection.

The analysis of VSELs was performed by flow cytometric analysis, according to the methods described by Zuba-Surma and Ratajczak (2010). The peripheral blood (PB) samples were lysed twice, using BD Pharm Lyse lysing buffer (BD Bioscience), at room temperature for 10 min and subsequently washed in phosphate buffered saline (PBS) with 2% fetal bovine serum (FBS; Sigma) to yield total nucleated cells (TNCs), which were stained with CD 34–allophycocyanin (APC) Abs (clone 581; BD Bioscience). The cells were subsequently washed, re-suspended, and analyzed, using a NAVIOS Flow Cytometer (Beckman Coulter). At least 10^6 events were acquired from each sample. The absolute numbers of CD34 + VSELs and the absolute number of white blood cells were calculated (individually for each patient) per 1 ml PB, based on the percentage content of these cells as detected by flow cytometry. Kaluza software (Beckman Coulter) was used for the analysis.

Assessment of the mRNA expression of the master pluripotency transcription factors and glial markers in peripheral blood was performed using the Real-time quantitative reverse transcription PCR (RQ-PCR) procedure. Total RNA was isolated from lysed blood with the RNeasy Kit (Qiagen). The RNA was reverse-transcripted with the FirstStrand cDNA synthesis kit and oligo-dT primers (Fermentas).

Quantitative assessment of mRNA levels of pluripotency markers was obtained by real-time RT-PCR on an ABI 7500 Fast instrument with Power SYBR Green PCR Master Mix reagent. The real-time conditions were as follows: 95 °C (15 s), 40 cycles at 95 °C (15 s), and 60 °C (1 min). According to melting point analysis, only one PCR product was amplified under these conditions. The relative quantity of a PCR