Pharmacological Reports 68 (2016) 1042–1045

Contents lists available at ScienceDirect

Pharmacological Reports

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

Short communication

Peripheral mRNA expression of pluripotency markers in bipolar disorder and the effect of long-term lithium treatment

Ewa Ferensztajn-Rochowiak[a], Maciej Tarnowski[b], Jerzy Samochowiec[c], Michal Michalak[d], Mariusz Z. Ratajczak[b,e], Janusz K. Rybakowski[a,*]

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

ARTICLE INFO

Article history:
Received 4 May 2016
Received in revised form 6 June 2016
Accepted 8 June 2016
Available online xxx

Keywords:
bipolar disorder
lithium
Oct-4
Sox 2
Nanog

ABSTRACT

Background: The aim was to evaluate the peripheral mRNA expression of pluripotency master transcriptional factors such as octamer-binding transcription factor 4 (Oct4), sex-determining region Y-box 2 (Sox2) and homeobox protein Nanog, in patients with bipolar disorder (BD), and the effect of long-term lithium treatment.

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. Assessment of the mRNA levels of pluripotency markers (Oct-4, Sox 2 and Nanog) was performed, using the Real-time quantitative reverse transcription PCR (RQ-PCR) procedure, and the number of CD34+ very small embryonic-like stem cells (VSELs) was measured by flow cytometric analysis.

Results: In those BD patients not treated with lithium the expression of all three pluripotency genes was significantly higher than that in the control subjects. Oct-4, Sox2 and Nanog also positively correlated with the number of CD34+ VSELs/[ul] in this group. In the lithium-treated patients the mRNA levels of Nanog were significantly higher than in the control individuals and correlated with the number and % of CD34+ VSELs.

Conclusions: The overexpression of the pluripotency master transcriptional factors in patients with a long duration of BD not treated with lithium, may contribute to the pathogenesis of the illness and make them potential biological markers of BD. Long-term lithium treatment may attenuate these excessive regenerative processes, especially in relation to the transcription factors Oct-4 and Sox2.

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 more than half a century the illness has been the subject of intense neurobiological studies. In recent years, processes connected with the production and function of stem cells have also been implicated in its pathogenesis. 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. A review on the effect of lithium on different stem cell populations has been published recently [1]. In our previous study, we demonstrated that in BD subjects not taking lithium, the number of CD34+ very small embrional-like stem cells (VSELs) in peripheral blood was increased and correlated with the duration of illness. On the other hand, among long-term lithium-treated bipolar patients these values were similar to controls which might suggest that lithium can suppress the activation of regenerative processes by reducing the number of CD34 + VSELs [2].

Important elements in stem cell production and differentiation are 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. TFs are central regulators, controlling the early stages of embryogenesis, and in the maintenance of the

* Corresponding author.
E-mail address: janusz.rybakowski@gmail.com (J.K. Rybakowski).

http://dx.doi.org/10.1016/j.pharep.2016.06.006
1734-1140X/

pluripotent state (self-renewal, differentiation capability, *etc.*) in some pluripotent stem cells occurring in the adult organism [3]. 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 [4]. Recently, an expression of Oct4 was described on VSELs, which migrated and localized in an injured spinal cord [5].

In the present study we investigated mRNA expression of the master pluripotency transcription factors (Oct-4, Sox2, Nanog) and their relationship with VSELs in BD patients. We also assessed the effect of long-term lithium treatment on these parameters.

Subjects and methods

Subjects studied

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

Among the BD patients, the BD Li(−) group comprised 15 persons (5 male, 10 female), aged 53 ± 7 years with a duration of illness of at least 10 years (mean 20 ± 9 years, range 10–44 years) who had never been exposed to lithium. Their mood-stabilizing treatment consisted of valproic acid (7 persons), lamotrigine (6 persons) or carbamazepine (3 persons). Other psychotropic drugs used included antidepressants (9 persons) and antipsychotics (7 persons).

The BD Li(+) group 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). Within this subgroup, 6 persons were additionally treated with carbamazepine. Treatment with other psychotropic drugs included antidepressant drugs (8 persons) and quetiapine (3 persons).

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

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.

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

Laboratory methods

Assessment of the mRNA expression of the master pluripotency transcription factors: Oct-4, Sox 2 and Nanog 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). The primers were as follows:

1. Oct-4 (forward) 5-CCCCTGGTGCCGTGAA-3
(reverse) 5-GCAAATTGCTCGAGTTCTTTCTG-3
2. Sox2 (forward) 5-CACACTGCCCCTCTCACACA-3
(reverse) 5-TTTTGAGCGTACCGGGTTTT-3
3. Nanog (forward) 5-GCAGAAGGCCTCAGCACCTA-3
(reverse) 5-AGGTTCCCAGTCGGGTTCA-3

Quantitative assessment of mRNA levels 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 product was determined by the comparative Ct method. The mRNA expression of all the genes investigated was normalized by the non-regulated reference gene β-2microglobulin (β–2 M). β–2 M expression correlated with the amount of RNA used for reverse transcription. Data were presented as target gene expression with respect to the housekeeping gene ß2 M [6].

The evaluation of CD34 + VSELs number was performed by flow cytometric analysis, according to the methods employed by Zuba-Surma and Ratajczak [7] and described in a previous paper [2].

Statistical methods

Calculations were performed using the Statistica (StatSoft-Poland), version 10 statistical package. The level of statistical significance was determined at $p < 0.05$. The study parameters were compared between the three groups: Bipolar Li (−), Bipolar Li (+) and control subjects. The Shapiro-Wilk test was used to check the distributions. As the data were not consistent with a normal distribution, we used the Kruskal-Wallis test, with Dunn's *post-hoc* test. Correlations with CD34 VSEL were calculated using Spearman's rank correlation.

Results

The mRNA expression of pluripotency markers Oct-4, Sox2 and Nanog is presented in Table 1.

Table 1 In patients not treated with lithium the expression of the Oct4, Sox2 and Nanog genes was significantly higher than in the control subjects (14.24 *vs.* 0.92, $p = 0.003$ for Oct-4; 6.71 *vs.* 0.09, $p < 0.001$ for Sox2; 18.17 *vs.* 0.2, $p < 0.001$ for Nanog). In the lithium-treated group the mRNA levels of Nanog were significantly higher than in the control individuals (5.11 *vs.* 0.2, $p = 0.024$).

Correlations between the mRNA levels of stem cell pluripotency markers and CD34+ VSELs number in the bipolar patients and the control subjects are presented in Table 2.

Table 2 The positive correlation was found between the mRNA expression of Oct-4, Sox2 and Nanog, and the number of CD34+ VSELs/[ul] accordingly in patients not treated with lithium $p = 0.045$, $p = 0.0065$ and $p = 0.011$. In the lithium-treated patients, positive correlations were found between Nanog expression, the number ($p = 0.011$) and the % of CD34+ VSELs ($p = 0.018$).

In the BD group, the values of Oct-4, Sox2 and Nanog did not correlate with the duration of illness. In the lithium-treated bipolar patients these values were negatively correlated with the duration of lithium treatment and serum lithium concentration, the levels of

Table 1
Real-time PCR for mRNA expression of stem cell pluripotency markers (mean ± SD) in bipolar patients not treated with lithium Li (−), patients treated with lithium Li (+), and control subjects. Values are given as target gene expression with respect to the housekeeping gene β-2 microglobulin (ß2 M)

	Bipolar Li (−)	Bipolar Li (+)	Control
Oct4	14.24 ± 16.32 * $p = 0.003$	6.73 ± 12.2	0.92 ± 0.84
Sox2	6.71 ± 8.78 * $p < 0.001$	4.46 ± 7.45	0.09 ± 0.13
Nanog	18.17 ± 20.65 * $p < 0.001$	5.11 ± 8.14 # $p = 0.024$	0.2 ± 0.28

$p < 0.05$—statistical significance.
Oct-4—octamer-binding transcription factor 4; Sox 2—sex determining region Y-box 2; Nanog—homeobox protein.
* Difference between Bipolar Li (−) and control group.
# Difference between Bipolar Li (+) and control group.