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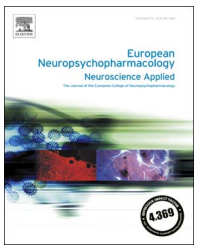

Distinct lithium-induced gene expression effects in lymphoblastoid cell lines from patients with bipolar disorder

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Abstract
Lithium is the most commonly prescribed medication for the treatment of bipolar disorder (BD), yet the mechanisms underlying its beneficial effects are still unclear. We aimed to compare the effects of lithium treatment in lymphoblastoid cell lines (LCLs) from BD patients and controls. LCLs were generated from sixty-two BD patients (based on DSM-IV) and seventeen healthy controls matched for age, sex, and ethnicity. Patients were recruited from outpatient clinics from February 2012 to October 2014. LCLs were treated with 1 mM lithium for 7 days followed by microarray gene expression assay and validation by real-time quantitative PCR. Baseline differences between groups, as well as differences between vehicle- and lithium-treated cells within each group were analyzed. The biological significance of differentially expressed genes was examined by pathway enrichment analysis. No significant differences in baseline gene expression (adjusted p-value < 0.05) were detected between groups. Lithium treatment of LCLs from controls did not lead to any significant differences. However, lithium altered the

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expression of 236 genes in LCLs from patients; those genes were enriched for signaling pathways related to apoptosis. Among those genes, the alterations in the expression of *PIK3CG*, *SERP1* and *UPP1* were validated by real-time PCR. A significant correlation was also found between circadian functioning and *CEBPG* and *FGF2* expression levels. In summary, our results suggest that lithium treatment induces expression changes in genes associated with the apoptosis pathway in BD LCLs. The more pronounced effects of lithium in patients compared to controls suggest a disease-specific effect of this drug.

1. Introduction

Bipolar disorder (BD) is a chronic and severe psychiatric disorder with a prevalence of approximately 1% in global population (Grande et al., 2016). It is a lifelong episodic illness with a progressive course characterized by the occurrence of at least one manic episode (BD I) or one hypomanic and one major depressive episode (BD II). Specifically, manic or hypomanic episodes are states of elevated mood and increased motor drive that impair social or occupational functioning, and a comprehensive biological, social, and psychological approach is required for its treatment and investigation (Grande et al., 2016). Biological rhythms and circadian functioning are also tightly linked to the pathophysiology of BD and have been shown to be significantly impaired in patients (Murray and Harvey, 2010). So far, no biomarker has yet been approved for BD's diagnosis, prognosis, or treatment response (Carvalho et al., 2016; Frey et al., 2013).

Mood stabilizers, including lithium, constitute the most common category of medications used in the management of BD. The mood stabilizing properties of lithium were first discovered in 1949. Since then, it has been considered the first line of treatment for BD (Pisanu et al., 2016b). High variability is generally observed in patient response to treatment, which has led to several investigations of lithium's mechanisms of action and the biological underpinnings of this response heterogeneity. Accordingly, the responsiveness to lithium has been particularly investigated in genomic studies and point to the existence of a distinct, more homogenous phenotype within BD patients (Alda et al., 2005; Grof et al., 2002; Scott et al., 2017) that appears to be stable across life (Berghofer et al., 2013). In addition, as seen in other diseases (Brunner-La Rocca et al., 2001; Potze et al., 2014), lithium might induce different effects in patients compared to controls, and understanding these diagnosis-specific effects might shed light into the genetic make-up of patients and its interaction with lithium.

Different models have been developed for the assessment of the effects of medications *in vitro* and *in vivo*. Among cellular models, lymphoblastoid cell lines (LCLs) derived from the transformation of patients' lymphocytes represent a valid and useful experimental tool (Breen et al., 2016; Hunsberger et al., 2015; Kittel-Schneider et al., 2015; Sugawara et al., 2010). Accordingly, alterations in the expression of specific genes in LCLs might help understand which genes might be linked to lithium treatment, thus shedding light into its mechanisms of action and clarifying the beneficial effects of lithium in patients. With this scenario, in this study we aimed to investigate the effects of lithium treatment on genome-wide gene expression in LCLs from BD I patients and healthy controls.

2. Experimental procedures

2.1. Subjects

Sixty-two patients with diagnosis of BD I and 17 healthy controls were recruited at The University of Texas Health Science Center at San Antonio. All patients were recruited from out-patient clinics from February 2012 to October 2014 and diagnosed according to the DSM-IV criteria. Healthy controls were recruited based on the absence of any current or past diagnosis of psychiatric disorders using the M.I.N.I version 6.0 (Sheehan et al., 1997). Written informed consent was obtained from each subject after a complete description of the study, which was approved by the local ethics committee. Mood symptoms were assessed by the 15-item Bipolar Inventory of Symptoms Scale (BISS-15) (Bowden et al., 2007). Subjects were also administered the 13-item Composite Scale of Morningness (CSM) (Smith et al., 1989) for the assessment of circadian functioning, in which scores can range from 13 (extreme eveningness) to 55 (extreme morningness).

2.2. Cell culture

Peripheral blood was collected from each fasting subject and LCLs were generated from leukocytes using LeucoPREP brand cell separation tubes (Becton Dickinson Labware, Bedford, MA, USA), followed by their transformation using Epstein-Barr virus (EBV) under standard procedures. After reaching the confluence state, each cell line was adequately prepared for storage in liquid nitrogen. For the actual experiment, cells were thawed and regrown in RPMI-1640 medium (Sigma-Aldrich, St. Louis, MO, USA) supplemented with 15% fetal bovine serum (Gibco, Carlsbad, CA, USA) and 1% penicillin/streptomycin (Gibco). After 72 hours of stabilization in culture, LCLs were counted and plated in 6-well plates at a confluence of 0.5×10^6 cells/well and treated with medium containing either 1 mM lithium acetoacetate (Sigma-Aldrich) or vehicle (medium) for 7 days (same number of cells/well for all subjects from both groups). The 7-day treatment scheme with 1 mM lithium is suggested to mimic a chronic treatment with lithium in patients and has been consistently used in previous studies (Breen et al., 2016; Cruceanu et al., 2012; Emamghoreishi et al., 2015b; Sun et al., 2004). Cells were cultured at 37 °C in a humidified incubator with 5% CO_2 for 7 days, and medium was changed after 96 h in culture. After 1 week of treatment, in which continuous presence of 1 mM lithium was warranted, cells were harvested for RNA isolation.

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