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Short Communication

Abnormalities in serum chemokine levels in euthymic patients with Bipolar Disorder

Elisa Brietzke [b], Márcia Kauer-Sant'Anna [a,b], Antônio Lúcio Teixeira [c], Flávio Kapczinski [a,b,*]

[a] National Institute for Translational Medicine (INCT), Rua Ramiro Barcelos, 2350 CEP 90035003 Porto Alegre, RS Brazil
[b] Bipolar Disorder Program and Molecular Psychiatry Unit, Hospital de Clínicas de Porto Alegre, Universidade Federal do Rio Grande do Sul (UFRGS), Porto Alegre, Brazil
[c] Laboratory of Immunopharmacology, Department of Biochemistry and Immunology, Institute of Biological Sciences, Universidade Federal de Minas Gerais (UFMG), Belo Horizonte, Brazil

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ABSTRACT

The pathophysiology of bipolar disorder (BD) includes, among other processes, changes in the neuroplasticity and regulation of apoptosis, which could potentially be influenced by inflammatory mediators such as chemokines. The objectives of this study were to investigate serum chemokine levels in patients with BD and to compare results with those obtained with healthy subjects. Here, serum chemokine levels of 30 euthymic patients with BD type I and 30 healthy volunteers were investigated and compared. The chemokines assessed were CCL2, CCL3, CCL8, CCL 9, CCL10, CCL11, and CCL24. Patients with BD showed significant differences in chemokine levels when compared with healthy subjects. While serum levels of CXCL10 were increased ($p = .018$), CCL24 levels were lower in bipolar patients ($p = .025$) when compared with controls. There was no statistical difference in the serum levels of CCL2, CCL3, CCL24, CXCL9, and CXCL11 between patients and controls. The presence of chemokine abnormalities in patients with BD during euthymia suggests that these inflammatory mediators should be further investigated with regard to their potential role as longstanding markers of the disorder.

1. Introduction

Although bipolar disorder (BD) is a highly prevalent and severe disorder, its pathophysiology has not yet been completely understood. Evidence has emerged showing an association of BD with changes in neuroplasticity and neuronal survival (Schloesser et al., 2008; Kapczinski et al., 2008a). These processes are influenced by several factors, including the orchestrated action of neurotransmitters, hormones, neurotrophins (Post, 2007; Kapczinski et al., 2008b), and inflammatory mediators such as cytokines (Brietzke and Kapczinski, 2008).

Recent evidence suggests the involvement of inflammatory cytokines in BD (Brietzke and Kapczinski, 2008; Kauer-Sant'anna et al., 2008; Soczynska et al., in press). Chemokines, a particular type of cytokines with chemoattractive properties, have received less attention in what concerns their role in BD. Nevertheless, these inflammatory mediators may be of particular interest in these patients, given their effect on the amplification of inflammatory response, possibly resulting in increased neuronal and glial death (Takahashi et al., 2008). For instance, peripheral chemokine levels seem to be altered in neurodegenerative disorders, such as multiple sclerosis (Moreira et al., 2006) and Alzheimer's disease (Galimberti et al., 2006; Reale et al., 2008; Kim et al., 2008), and in neuropsychiatric disorders such as schizophrenia (Drexhage et al., 2008; Teixeira et al., 2008) and major depressive disorder (Merendino et al., 2004; Sutcigil et al., 2007; Simon et al., 2008). Despite a growing body of evidence suggesting involvement of chemokines in psychiatric and neurodegenerative disorders, only one study so far has shown significantly increased levels of CXCL8 (IL-8) in patients with mania when compared with healthy subjects (O'Brien et al., 2006).

To date, more than 50 chemokines and approximately 20 chemokine receptors – especially G-protein-coupled receptors (Rostène et al., 2007) – have been identified. The two largest families of chemokines, CCL and CXCL, attract mononuclear cells to sites of chronic inflammation. The binding of a chemokine to its receptor activates signaling cascades that lead to cell shape rearrangement and movement (Charo and Ransohoff, 2006; Mackay, 2003; Murdoch and Finn, 2000). Activation of these signaling pathways results in increased calcium concentrations and activation of mitogen-activated protein kinases, and also has a role in synaptic plasticity (Rostène et al., 2007). For instance, CCL and CXCL chemokines have been shown to help in the prevention of neuronal apoptosis

* Corresponding author. Address: Hospital de Clínicas de Porto Alegre, Laboratório de Psiquiatria Molecular, Rua Ramiro Barcelos 2350, CEP 90035-003, Porto Alegre, RS, Brazil. Fax: +55 51 21018846.
E-mail address: kapcz@terra.com.br (F. Kapczinski).

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(Limatola et al., 2005; Ragozzino et al., 2006; Watson and Fan, 2005).

Since the understanding of the inflammatory mechanisms involved in BD could potentially open new treatment possibilities (Brietzke and Kapczinski, 2008; Nery et al., 2008, the aim of this study was to investigate chemokine levels in BD patients compared with healthy subjects. Examples of the two largest families of chemokines were selected for assessment, namely CCL2 (MCP-1), CCL3 (MIP-1alpha), CXCL8 (IL-8), CXCL 9 (MIG-1), CXCL10 (IP-10), CCL11 (Eotaxin), and CCL24 (Eotaxin-2).

2. Methods

The present study was approved by the local ethics committee and all subjects provided written informed consent before inclusion in the study.

Thirty euthymic patients with BD type I were recruited from the outpatient clinic (Bipolar Disorders Program) at Hospital de Clínicas de Porto Alegre, Porto Alegre, Brazil. Psychiatric diagnosis was based on application of the Structured Clinical Interview for DSM-IV-Axis I Disorders (SCID-I). Manic and depressive symptoms were assessed with the Young Mania Rating Scale (YMRS) and the Hamilton Depression Rating Scale (HDRS), respectively. Patients were considered to be euthymic when DSM-IV criteria for mood episodes were not fulfilled and when HDRS and YMRS scores were below 7. Patients with any additional axis I or axis II DSM-IV diagnoses were excluded, as were those with general medical conditions associated with changes in inflammatory response, such as HIV/AIDS, rheumatologic diseases, current bacterial/viral disease, current use of more than 10 cigarettes/day, current use of corticosteroids or nonsteroidal anti-inflammatory medications, acetylsalicylic acid or immunosuppressives.

The healthy group was comprised of 30 volunteers who were screened for psychiatric disorders using the non-patient version of SCID-I. Subjects included in the study were not using any medication, and their first-degree relatives had no history of major psychiatric disorders, dementia, mental retardation, cancer, or tumor. Exclusion criteria for healthy subjects were the same as those used in the selection of euthymic patients.

Five milliliters of blood were collected from each subject by venipuncture in anticoagulant-free vacuum tubes. Samples were immediately centrifuged at 3000*g* for 5 min, and the serum was kept frozen at −80 °C until assayed.

The serum concentration of chemokines was determined using sandwich ELISA kits, following the manufacturer's protocol (DuoSet R&D Systems, Minneapolis, MN, USA). For the analysis, samples were thawed and excess proteins removed by acid/salt precipitation, as routinely performed in our laboratory (Moreira et al., 2006; Teixeira et al., 2008). Briefly, an equal volume of serum and 1.2% trifluoroacetic acid/1.35 M NaCl were mixed and left at room temperature for 10 min. Samples were then centrifuged for 5 min at 3000*g*, and the supernatants adjusted for salt content (0.14 M sodium chloride and 0.01 M sodium phosphate) and pH (7.4) for the determination of chemokine levels.

All samples were assayed in duplicate. Assay detection limits were 5 pg/ml.

2.1. Statistics

Descriptive analyses included assessment of the distribution of all variables; data are presented as means and percentages. Demographic and clinical characteristics were analyzed using the χ^2-test and analysis of variance (ANOVA), as indicated. Chemokine levels showed a non-parametric distribution and were analyzed with appropriated tests, as indicated. Differences between the two groups assessed were evaluated using the Mann–Whitney *U* test.

Statistical analyses were performed using the Statistical Package for the Social Sciences (SPSS) version 15.0 (SPSS Inc., Chicago, IL, USA). In view of the non-normal distribution observed, effect size (ES) measurements were determined with a logarithmic transformation. Statistical significance was set at $p < 0.05$.

3. Results

Demographic and clinical characteristics of the sample ($n = 60$) are presented in Table 1. Both groups were homogeneous in terms of age, sex, ethnic group and years of schooling. Median and 25th and 75th percentiles for chemokine levels in BD patients and healthy subjects are presented in Table 2.

Analysis of serum chemokine levels revealed significantly higher levels of CXCL10 (IP-10) ($U = 291.5$, $p = 0.018$, $Z = -2.369$, $ES = -0.71$) and lower levels of CCL24 (Eotaxin-2) ($U = 287.0$, $p = 0.025$, $Z = -2.244$, $ES = -0.63$) in BD patients when compared with healthy subjects; no differences were observed in the other chemokines assessed (Table 2). There was no correlation between chemokine levels and duration of disorder (years of illness).

4. Discussion

Our results revealed an increase in inflammatory chemokine CXCL10 and a reduction in CCL24 levels in euthymic BD patients when compared with healthy control subjects, suggesting an association between BD and changes in inflammatory status. Such changes are persistent during interepisodic periods and involve chemokines. Whether inflammatory abnormalities in BD persist or not in the remission of mood episodes is an important research question that has been scarcely investigated so far. Our results are in accordance with previous studies which suggest that changes in inflammatory mediators may be associated with the pathophysiology and pharmacological response of BD (Kim et al., 2007; O'Brien et al., 2006). In addition, evidence that chemokines play an important role in synaptic plasticity has been reported. CCL5, CCL22, CX3CL1, and CXCL12 have been shown to protect hippocampal neurons from amyloid-beta-peptide-induced neurotoxicity (Watson and Fan, 2005), and CXCL12 has demonstrated a potential to protect rat cerebellar neurons from apoptosis (Limatola et al., 2000). The presence of chemokine abnormalities in euthymic patients indicates that these inflammatory mediators could be long-standing markers of the disease.

Increased levels of the chemokine CXCL10, a Th1-related mediator, and, at the same time, decreased levels of Th2-related hypersensitivity-related CCL24 were found. These findings are suggestive of imbalance of Th1/Th2 cytokines rather than a simple increase in cytokines and these changes are similar to those described in a number of inflammatory conditions and also in

Table 1
Demographic characteristics of the sample. Abnormalities in serum chemokine levels in euthymic patients with bipolar disorder.

	Patients with BD ($n = 30$)	Healthy subjects ($n = 30$)	Significance level (p value)
Age (mean ± SD)	43.18 (11.9)	43.24 (14.5)	.98[a]
Female (%)	72.4	82.8	.345[b]
Caucasian (%)	83.3	85.2	.28[a]
Years of schooling (mean ± SD)	8.8 (4.63)	8.2 (5.12)	.89[a]
Years of illness (mean ± SD)	21 (8.2)	–	–
HDRS score (mean ± SD)	4.4 (1.6)	–	–
YMRS score (mean ± SD)	1.6 (2.3)	–	–

SD, standard deviation; HDRS, Hamilton Depression Rating Scale; YMRS, Young Mania Rating Scale.
[a] Student *t* test.
[b] χ^2-test.