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The leukocytes expressing DARPP-32 are reduced in patients with schizophrenia and bipolar disorder

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ABSTRACT

Bipolar disorder (BPD) and schizophrenia (SCZ) are severe disorders representing an enormous social, familiar and individual burden, being SCZ the most disabling psychiatric disorder characterized by psychosis and cognitive impairment. It is well known that SCZ and BPD are associated with abnormalities in dopamine signaling pathway. Recent data in the literature have demonstrated altered expression levels of some proteins involved in the modulation of this pathway in both brain and peripheral tissues. It was shown that protein and mRNA levels of dopamine and cAMP regulated phosphoprotein (DARPP-32) were downregulated in dorsolateral prefrontal cortex (DLPFC) of patients with SCZ or BPD when compared to controls. Due to the difficulty to access brain tissue and the absence of objective laboratory tests for bio-markers, we measured DARPP-32 expression in blood cell sub-populations (CD4+ T lymphocytes, CD56+ NK cells, CD19+ B lymphocytes and CD14+ monocytes) taking advantage of the close relation of nervous and immune systems. Using flow cytometry as the analytical method, our results have shown that the DARPP-32 expression was diminished in CD4+ T lymphocytes, CD19+ B lymphocytes and CD14+ monocytes of BPD patients and was also decreased in CD4+ T lymphocytes and CD56+ NK cells of SCZ patients. These results showed that DARPP-32 expression in immune cells agrees with reports of reduced DARPP-32 protein in the DLPFC of BPD or SCZ patients. Our data suggest that DARPP-32 expression in PBMC could be used as a source of bio-markers to help in the treatment response of neuropsychiatry disorders as a window to the changes in the brain of those patients.

1. Introduction

Schizophrenia (SCZ) is the most disabling psychiatry disorder characterized by psychotic positive symptoms, negative symptoms and cognitive impairment. It is a life-long disorder with a world-wide prevalence of 1% (McGurk et al., 2003). Bipolar disorder (BPD) is in turn a frequent and severe disease which affects mood, behavior, and thinking, switching between mania and depression (Goodwin and Jamison, 1990).

Evidence indicates abnormalities in the dopamine system in both SCZ and BPD (Manji and Lenox, 2000; Gao et al., 2005; Seeman et al., 2006 and Horacek et al., 2006). Recently, alterations in intracellular signal integrating proteins associated with both disorders were shown (Emamian et al., 2004; Souza et al., 2006a,b; Reis et al., 2007 and Volkow et al., 2007). It was demonstrated, in *post-mortem* brain tissue, decreased levels of *dopamine and cyclic adenosine 3′:5′-monophosphate-regulated phosphoprotein of relative molecular mass 32,000* (DARPP-32) expression in the dorsolateral prefrontal cortex (DLPFC) of individuals with schizophrenia (Albert et al., 2002 and Ishikawa et al., 2007).

DARPP-32 is a major player in the transduction of dopaminergic signaling, integrating signals from different converging pathways in neurons (Svenningsson et al., 2004). Activation of protein kinase A (PKA) results in phosphorylation of DARPP-32 at Thr-34 (Nishi et al., 1997) and (Svenningsson et al., 2000). In this phosphorylated state, DARPP-32 shows an inhibitory effect on protein phosphatase 1 (PP1) which regulates the activity of receptors, channels and transcriptional factors (Huang et al., 1999) and (Svenningsson et al., 2004). DARPP-32 effect is terminated by dephosphorylation at Thr-34 residue by protein phosphatase 2B (PP-2B, calcineurin) (Hernandez-Lopez et al., 2000). Several functions of this phosphoprotein were recently reviewed such

Abbreviations: BPD, Bipolar disorder; CD, Cluster of differentiation; CNS, Central nervous system; COMT, Catechol-O-Methyltransferase; CY, Cychrome; DARPP-32, Dopamine and cyclic adenosine 3′:5′-monophosphate-regulated phosphoprotein of relative molecular mass 32,000; DLPFC, Dorsolateral prefrontal cortex; FITC, Fluorescein isothiocyanate; IS, Immune system; LSD, Lysergic Acid Diethylamide; NCS-1, Neuronal calcium sensor 1; NK, Natural killer cell; PCP, Phencyclidine; PE, Phycoerytrin; PFC, Prefrontal cortex; PBMC, Peripheral blood mononuclear cells; SCZ, Schizophrenia.

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Table 1
Characterization of patients with schizophrenia analyzed in this study

Patient[a]	Sex	Age	Antipsychotic drugs[b]	Chlor. eq. (mg/day)	Other drugs	Total PANSS/gravity
1	M	49	Haloperidol 20 mg/d	1000 mg/d	Biperidene 2 mg/d, Diazepam 30 mg/d, Lorazepam 6 mg/d	75/mildly ill
2	M	43	Haloperidol 10 mg/d	500 mg/d	Clonazepam 2 mg/d	76/mildly ill
3	M	45	Chlorpromazine 300 mg/d, Fluphenazine 1 ampule/15d	385 mg/d	Biperidene 4 mg/d	91/moderately ill
4	M	47	–	–	Lorazepam 2 mg/d	90/moderately ill
5	M	47	Chlorpromazine 200 mg/d, Haloperidol 20 mg/d, Thioridazine 500 mg/d	1700 mg/d	Biperidene 2 mg/d, Diazepam 10 mg/d	100/markedly ill
6	M	37	Haloperidol 20 mg/d, Promethazine 75 mg/d	1000 mg/d	–	81/moderately ill
7	M	39	Haloperidol 20 mg/d	1000 mg/d	Biperidene 2 mg/d	81/moderately ill
8	M	63	Haloperidol 1 mg/d	50 mg/d	Biperidene 2 mg/d, Lorazepam 6 mg/d	77/mildly ill

[a] All patients were male and tabagists.
[b] Woods (2003).

as involvement in motor control, drug abuse, cell differentiation, tissue development, morphology and function of several organs, and anti-apoptotic effects (Souza et al., 2006a,b and Nairn et al., 2004), (Reis et al., 2007) and (Svenningson et al., 2004).

There is accumulating evidence showing the bi-directional correlation between the central nervous system (CNS) and the immune system (IS), and lymphocytes have a central role in this inter-communication (Gladkevich et al., 2004). Currently, due to the lack of specific bio-markers, the understanding of the development of mental disorders, including SCZ and BPD is still unknown. Thus, the identification of markers in cells from peripheral blood of the patients would be a convenient and accessible way to study the signaling pathways involved in these disorders as a window to the changes in the brain of those patients.

Various studies showed similarities, between the nervous and immune systems, in receptor expression and intracellular biochemical pathways of neurons, glia and leukocytes. Given that, lymphocytes are being used to identify peripheral bio-markers in neuropsychiatric disorders (Gladkevich et al., 2004; Meredith et al., 2005; Du et al., 2006 and Pellicano et al., 2007).

Then, in this work, DARPP-32 was chosen as a candidate in the search of peripheral bio-markers of SCZ and BPD. Using flow cytometry, we measured the intracellular levels of DARPP-32 in monocytes, NK cells and T and B lymphocytes. We have shown that DARPP-32 expression is decreased in immune cells of patients with SCZ and BPD. Our data suggest that DARPP-32 expression in lymphocytes and monocytes could be used as a source of bio-markers to help in the treatment response of neuropsychiatry disorders as a window to the changes in the brain of those patients.

2. Materials and methods

2.1. Materials

The following materials were purchased from indicated commercial sources: Ficoll/Hypaque (Sigma, St. Louis, MO, USA); anti-CD3 monoclonal antibodies (PharMingen-Becton Dickinson, San Diego CA, USA); anti-CD28 monoclonal antibodies (PharMingen-Becton Dickinson, San Diego CA, USA); RPMI 1640, Sigma, St. Louis, MO, USA) bovine fetal serum (GIBCO, long Island, NY, USA); L-glutamine penicillin/streptomycin (GIBCO, long Island, NY, USA); PBS (Sigma St. Louis, MO, USA). Anti-CD19, anti-CD14 and anti-CD56-PE (Caltag), anti-CD4-CY (Biosciences, San Jose, CA, USA); FACScan (Becton & Dickinson, San Diego CA, USA).

2.2. Subjects

Eight patients with schizophrenia (SCZ) and seven patients with bipolar disorder were recruited from local psychiatric clinics.

Schizophrenia inpatients met DSM-IV diagnosis of paranoid-hallucinatory or disorganized SCZ, evaluated by two psychiatrists on the basis of standardized diagnostic interviews (Centorrino et al., 2002). All SCZ patients were receiving either standard or atypical neuroleptics at the time of the interview and blood extraction (Table 1). Patients with SCZ were divided in three groups accordingly to the total PANSS score (Leucht et al., 2005): three mildly ill, four moderately ill, and one markedly ill (Table 1). Bipolar patients met DSM-IV diagnosis for Bipolar I disorder, examined by at least two psychiatrists using standardized diagnostic interviews (Kessler et al., 2005). At the moment of blood extraction, BPD patients were receiving pharmacological treatment (Table 2). BPD patients were classified into manic, depressive, mixed episode or euthimic in accordance with the Young Manic Rating Scale (YMRS) (Young et al., 1978) and Montgomery–Asberg Depression Rating Scale (MADRS) Montgomery and Asberg (1979).

As controls healthy volunteers (age 31.14 ± 11.98) of both sexes (6 male and 1 female) who had not taken any medications at the time of blood collection donated whole blood via venipuncture.

This study was approved by the Ethics Committee of Universidade Federal de Minas Gerais and all the patients and controls gave written informed consent before study entry.

2.3. Cultures

Peripheral blood mononuclear cells (PBMC) were obtained using a Ficoll/Hypaque gradient. Cells (2×10^5) were cultured with or without

Table 2
Characterization of patients with bipolar study analyzed in this study

Patient	Sex	Age	Antipsychotic drugs	Chlor. eq. (mg/day)	Li (mg/day)	Other drugs	Mood state
1	F	45	Risperidone 4 mg/d	200 mg/d	–	Valproic acid 1500 mg/d, Midazolam 30 mg/d	Mixed episode
2	F	28	Levomepromazine 25 mg/d		600 mg/d	Zuclopentixol 400 mg/m, Venlafaxine 75 mg/d, Valproic acid 2000 mg/d, Midazolam 45 mg/d	Mixed episode
3	M	56	–	–	1200 mg/d	Clonazepam 3 mg/d, Lamotrigine 250 mg/d	Euthimia
4	F	57	Olanzapine 5 mg/d	100 mg/d	1200 mg/d	Venlafaxine 150 mg/d, Imipramine 75 mg/d, Clonazepam 2 mg/d	Euthimia
5	M	47	Olanzapine 20 mg/d	400 mg/d	–	Valproic acid 1000 mg/d	Euthimia
6	M	71	Olanzapine 5 mg/d	100 mg/d	–	Valproic acid 1000 mg/d, Biperidene 2 mg/d, Clonazepan 0,5 mg/d	Manic episode
7	F	42	Haloperidol 2.5 mg/d	175 mg/d	–	Valproic acid 1500 mg/d	Manic episode