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Preliminary communication

Early apoptosis in peripheral blood mononuclear cells from patients with bipolar disorder

Gabriel Rodrigo Fries [a,b,*], Mirela Paiva Vasconcelos-Moreno [a,c], Carolina Gubert [a,b], Barbara Tietböhl Martins Quadros dos Santos [a], André Luiz Schuh Teixeira da Rosa [a], Bárbara Eisele [a], Juliana Sartori [a], Bianca Pfaffenseller [a,b], Flávio Kapczinski [a,b,c], Márcia Kauer-Sant'Anna [a,b,c]

[a] Laboratory of Molecular Psychiatry, Centro de Pesquisas Experimentais, Hospital de Clínicas de Porto Alegre, and INCT for Translational Medicine, Brazil
[b] Programa de Pós-Graduação em Ciências Biológicas: Bioquímica, Universidade Federal do Rio Grande do Sul, UFRGS, Brazil
[c] Programa de Pós-Graduação em Medicina: Psiquiatria, Universidade Federal do Rio Grande do Sul, UFRGS, Brazil

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ABSTRACT

Background: The pathophysiology of bipolar disorder (BD) includes several systemic alterations, such as inflammatory markers, oxidative stress, and DNA damage. Most of these parameters may be related to dysfunctions in cellular resilience mechanisms reported in patients, such as endoplasmic reticulum stress and mitochondrial damage. As a consequence, these impairments can ultimately lead to cell death. Therefore, the aim of this study was to assess cell death and viability in peripheral blood mononuclear cells (PBMCs) from patients with BD and controls.
Methods: Ten euthymic patients with BD type I and seven age- and sex-matched healthy controls were recruited and had peripheral blood collected by venipuncture in heparine tubes. PBMCs were isolated from total blood, followed by measurement of cell viability by trypan blue exclusion, and apoptosis and necrosis by anexin V/propidium iodide (PI) staining.
Results: Cell viability did not significantly differ between groups, as well as the percentage of cells in necrosis or in late apoptosis/necrosis. However, the percentage of cells in early apoptosis was higher in patients when compared with controls ($p=0.002$).
Limitations: This is a preliminary study with relatively small sample size.
Conclusions: The systemic toxicity along with dysfunctional cell resilience mechanisms reported in patients with BD may be inducing apoptosis in PBMCs. A deeper look into the clinical relevance of such findings is warranted.

1. Introduction

Bipolar disorder (BD) is a severe and chronic psychiatric disorder associated with increased morbidity and mortality due to general medical conditions, including obesity, metabolic syndrome, and cardiovascular diseases, among others (Kupfer, 2005; Roshanaei-Moghaddam and Katon, 2009). The pathophysiology of BD includes several systemic alterations, such as increased inflammatory markers, reduced neurotrophic factors, oxidative stress, and DNA damage (O'Brien et al., 2006; Andreazza et al., 2007; Andreazza et al., 2008; Fernandes et al., 2011), which characterize a so-called systemic toxicity (Kapczinski et al., 2010, 2011). Most of these systemic alterations may be related to dysfunctions in cellular resilience mechanisms reported in patients, such as endoplasmic reticulum stress and mitochondrial damage (Hayashi et al., 2009; Clay et al., 2011).

Cellular resilience is defined as the ability of a given cell to handle and adapt to a certain stimulus, mostly by activating protective and adaptive mechanisms. Therefore, impaired cellular resilience mechanisms would make cells more vulnerable to stressful situations, ultimately leading to cell death in toxic and stressful environments. Based on the stimulus, different types of cell death can take place, namely apoptosis, necrosis, autophagy, or associated with mitosis (Kroemer et al., 2009). These can be experimentally identified by their morphology, enzymological criteria, functional aspects, or immunological features (Kroemer et al., 2009). Necrosis, for instance, is characterized by a gain in cell volume (oncosis), swelling of organelles, plasma membrane rupture and subsequent loss of intracellular

* Corresponding author at: Laboratory of Molecular Psychiatry, Centro de Pesquisas Experimentais, Hospital de Clínicas de Porto Alegre, and INCT for Translational Medicine, 2350, CEP 90035903, Porto Alegre-RS, Brazil. Tel.: +55 51 335 98845; fax: +55 51 33598 846.
E-mail address: gabrielrfries@gmail.com (G.R. Fries).

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contents (Kroemer et al., 2009). Contrary to the previous concept of necrosis as a merely accidental cell death mechanism, it is currently considered to occur in a regulated manner, as well (Vandenabeele et al., 2010). Apoptosis, on the other hand, is typically characterized by rounding-up of the cell, reduction of cellular size (pyknosis), chromatin condensation, little or no ultrastructural modifications of cytoplasmic organelles, nuclear fragmentation, plasma membrane blebbing, and engulfment by resident phagocytes *in vivo* (Kroemer et al., 2009). Evidence has suggested that apoptotic factors are altered in BD, including increased DNA damage in peripheral blood of patients (Andreazza et al., 2007), increased apoptotic serum activity (Politi et al., 2008), altered expression of molecules involved in cell death/survival pathways in peripheral blood mononuclear cells (PBMCs) from patients (Herberth et al., 2011), as well as mitochondrial dysfunction (Shao et al., 2008). Altogether, these studies implicate the involvement of cell death in BD.

To further characterize the involvement of cellular resilience and death in BD, we aimed to assess cell viability, necrosis and apoptosis in PBMCs from patients with BD and controls. Moreover, we sought to correlate clinical features from patients with such cellular parameters, aiming at identifying the relevance of peripheral cell death in BD pathophysiology.

2. Methods

2.1. Patients and controls

The present study was approved by the Ethical and Research Committee of Hospital de Clínicas de Porto Alegre, Brazil, protocol number 12-0102. Ten euthymic patients with BD type I were recruited at the Bipolar Disorders Program (PROTAHBI), an outpatient program of Hospital de Clínicas de Porto Alegre, Brazil. Seven age- and sex-matched healthy controls without history of psychiatric illness and history of psychiatric or neurologic disorders in first-degree relatives were enrolled at the Blood Bank from the same hospital. Written informed consent was obtained from all participants after receiving a complete description of the study. All participants were at least 18 years old. Patients with BD were diagnosed according to DSM-IV Axis I (SCID-I) criteria. Euthymia was confirmed by the Hamilton Depression Rating Scale (HDRS) and Young Mania Rating Scale (YMRS). Exclusion criteria for both patients and controls included history of autoimmune diseases or chronic infection/inflammatory disorders, as well as any severe systemic disease or use of immunosuppressive therapy.

2.2. Analysis of cell death

Ten milliliters of peripheral blood were collected from all participants by venipuncture in heparine tubes. PBMCs were isolated from total blood with Ficoll-Hypaque (GE Healthcare) density gradient centrifugation, followed by cell counting and measurement of cell viability by trypan blue exclusion. Afterwards, one hundred cells were pelleted and submitted to the analysis of apoptosis and necrosis by annexin V/propidium iodide (PI) staining, according to manufacturer's instructions (BD Biosciences, USA). Analysis of stained cells was performed on a BD FACScalibur flow cytometer (BD Biosciences), by assessing the fluorescence median intensity of samples on a FL1 × FL2 plot. The percentage of cells in early apoptosis (annexin V+/PI−), necrosis (annexin V−/PI+), and late apoptosis/necrosis (annexin V+/PI+) was collected.

2.3. Statistical analyses

Data were fitted into a normal standard distribution and analyses were therefore performed by independent samples *t*-tests. Percentage of cell on necrosis did not fit a normal standard distribution and was analyzed by Mann–Whitney test. Sex difference between patients and controls was assessed by chi-square test, whereas age and scale scores were analyzed by independent samples *t*-test. Correlations were analyzed by Pearson's correlation test. *P* values lower than 0.05 were considered to indicate statistical significance.

3. Results

Patients and controls did not differ regarding sex and age ($p > 0.05$ for both comparisons, Table 1). Even though patients were euthymic, HDRS and YMRS scores were higher in patients when compared to controls (Table 1). Cell viability assessed by trypan blue exclusion did not significantly differ between groups (controls – 96.7% ± 2.09; patients – 93.8% ± 5.08, $t(14) = 1.552$, $p = 0.143$), as well as the percentage of cells in necrosis (controls – 0.6% ± 0.7; patients – 0.68% ± 0.47, $U = 33$, $Z = -0.195$, $p = 0.775$) or in late apoptosis/necrosis (controls – 6.99% ± 5.49; patients – 10.7% ± 5.92, $t(15)$`=-1.309, $p = 0.21$). However, the percentage of cells in early apoptosis was significantly higher in patients when compared with controls (controls – 20.11% ± 5.23; patients = 31.56% ± 7.05; $t(15) = -3.64$, $p = 0.002$; Fig. 1). Since typical antipsychotics and benzodiazepines have been shown to induce apoptosis, two patients that were on these medications were removed from the analysis. Even so, the difference between patients and controls remained significant ($t(13) = -3.806$, $p = 0.002$). No correlations were found between the cellular parameters and YMRS and HDRS scores, number of manic and depressive episodes, number of hospitalizations or number of suicide attempts ($p > 0.05$ for all analyses).

Table 1
Clinical and demographic characteristics of patients and controls.

Characteristic	Patients ($n = 10$)	Controls ($n = 7$)	*P*
Age (years)[a]	49.7 (6.1)	51.7 (5.1)	0.488
Gender (male/female)	3/7	2/5	0.949
HDRS[a]	3.8 (1.9)	0.57 (1.1)	0.002
YMRS[a]	0.57 (1.1)	0	0.044
Medications			
Mood stabilizers	70%	n/a	
Antidepressants	20%	n/a	
Atypical antipsychotics	60%	n/a	
Typical antipsychotics	10%	n/a	
Benzodiazepines	20%	n/a	

[a] Mean (SD).

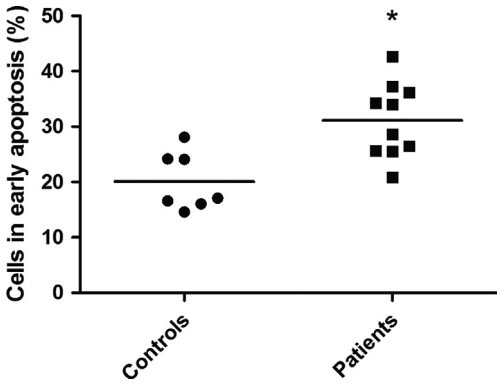

Fig. 1. Early apoptosis in BD patients and controls. *$P = 0.002$, independent *t*-test.