



Mitochondrial activity and oxidative stress markers in peripheral blood mononuclear cells of patients with bipolar disorder, schizophrenia, and healthy subjects



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ABSTRACT

Evidence suggests that mitochondrial dysfunction is involved in the pathophysiology of psychiatric disorders such as schizophrenia (SZ) and bipolar disorder (BD). However, the exact mechanisms underlying this dysfunction are not well understood. Impaired activity of electron transport chain (ETC) complexes has been described in these disorders and may reflect changes in mitochondrial metabolism and oxidative stress markers. The objective of this study was to compare ETC complex activity and protein and lipid oxidation markers in 12 euthymic patients with BD type I, in 18 patients with stable chronic SZ, and in 30 matched healthy volunteers. Activity of complexes I, II, and III was determined by enzyme kinetics of mitochondria isolated from peripheral blood mononuclear cells (PBMCs). Protein oxidation was evaluated using the protein carbonyl content (PCC) method, and lipid peroxidation, the thiobarbituric acid reactive substances (TBARS) assay kit. A significant decrease in complex I activity was observed ($p = 0.02$), as well as an increase in plasma levels of TBARS ($p = 0.00617$) in patients with SZ when compared to matched controls. Conversely, no significant differences were found in complex I activity ($p = 0.17$) or in plasma TBARS levels ($p = 0.26$) in patients with BD vs. matched controls. Our results suggest that mitochondrial complex I dysfunction and oxidative stress play important roles in the pathophysiology of SZ and may be used in potential novel adjunctive therapy for SZ, focusing primarily on cognitive impairment and disorder progression.

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1. Introduction

Mitochondria are responsible for many essential processes, e.g., energy production, apoptosis, intracellular calcium buffering, and reactive oxygen species (ROS) production, all potentially leading to either synaptic plasticity or cell death (Ben-Shachar, 2009). Cell energy is mainly obtained through oxidative phosphorylation.

Mitochondrial adenosine-triphosphate (ATP) is produced as electrons flow through electron transport chain (ETC) complexes (I to IV), which are located in the inner mitochondrial membrane. The resulting energy, in the form of protons, reenters the mitochondrial matrix through ATP synthase (complex V), producing ATP. Mitochondria and cytosolic ROS are formed as a consequence of ETC activity, possibly affecting redox status and inducing lipid peroxidation (Blanchet et al., 2011).

Noteworthy, mitochondrial functions become less effective, including impairment of complex I and, to a lesser extent, complex III activity. The reduced activity of ETC complexes in turn leads to enhanced ROS production, reduced Ca^{2+} buffering capacity, and mitochondrial DNA mutations (Mattson, 2006). During the transfer

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along ETC complexes, single electrons may escape, encounter molecular oxygen, and eventually reduce it to form a superoxide anion (O_2^-), especially in complex I (Green and Kroemer, 2004). Also, complex I deficiency has been associated with increased ROS levels and aberrant Ca^{2+} /ATP homeostasis (Blanchet et al., 2011).

Oxidative stress plays an important role in the pathogenesis of several diseases, especially neurological and psychiatric disorders such as bipolar disorder (BD) and schizophrenia (SZ). Studies have described dysfunction in ETC complex activity in patients with BD and SZ, but most of the findings available derive from postmortem tissue analysis or animal models, with controversial results (Andreazza et al., 2010; Karry et al., 2004; Maurer and Möller, 1997; Valvassori et al., 2010).

As previously suggested, changes associated with psychiatric disorders are not exclusively observed in the brain, but also in peripheral markers (Kunz et al., 2011). Lymphocytes, for example, have been shown to be a suitable cell model for studies of mitochondrial dysfunction (Leuner et al., 2012). Notwithstanding, to the authors' knowledge, no previous study has examined both mitochondrial activity and oxidative stress markers in peripheral blood mononuclear cells (PBMCs) obtained from patients with SZ and BD and from healthy controls. It is our hypothesis that substantial mitochondrial dysfunction and increased ROS in peripheral tissues could be strong indicators of an important role played by these markers in the pathophysiology of the disorders assessed.

Therefore, the objective of the present study was to assess plasma oxidative stress markers, based on thiobarbituric acid reactive substances (TBARS) and protein carbonyl contents (PCC), as well as ETC complex activity (complexes I, II, and III), in PBMCs obtained from patients with chronic SZ and BD type I and from healthy controls.

2. Materials and methods

2.1. Subjects

This study was approved by the Research Ethics Committee of Hospital de Clínicas de Porto Alegre (HCPA), southern Brazil. All procedures were performed in accordance with the ethical standards set forth in the Declaration of Helsinki. Moreover, all subjects provided written informed consent before their inclusion in the study.

We recruited 18 stabilized outpatients with SZ, all chronically medicated, among subjects attending the HCPA Schizophrenia Program, plus 12 euthymic outpatients with BD type I, among those attending the HCPA Bipolar Disorder Program. All subjects underwent a comprehensive clinical interview by a trained psychiatrist. Diagnoses of BD and SZ were confirmed according to the Diagnostic and Statistical Manual of Mental Disorders, 4th edition (DSM-IV). Psychiatric status in patients with SZ was assessed using the Brief Psychiatric Rating Scale (BPRS). Only patients with BPRS scores ≤ 25 and no history of psychiatric hospitalization over the last two years were included. Mood symptoms in patients with BD were assessed using the Young Mania Rating Scale (YMRS) (Young et al., 1978) and the Hamilton Rating Scale for Depression (HAM-D) (Hamilton, 1960). Patients were considered euthymic, and thus able to participate in the study, when both YMRS and HAM-D scores were ≤ 7 . All patients reported adherence to treatment and were in remission at the time of blood collection. The control group comprised 30 drug-free healthy volunteers, all selected at the hospital's blood bank, with no current or previous history and no first-degree family history of major psychiatric disorder, including dementia or mental retardation. Patients and controls were matched individually for age and sex.

2.2. Isolation of mitochondria from peripheral blood mononuclear cells

Approximately 20 mL of heparinized peripheral blood were collected from each subject. Initial centrifugation (1000 g/15 min) was performed to separate the plasma for further biochemical quantification of oxidative stress markers. This plasma fraction was again centrifuged at 4000 g/10 min for purification.

The other fraction was used for PBMC extraction. Briefly, the total blood volume remaining from the initial centrifugation was diluted 1:2 in phosphate buffered saline (PBS) (0.10 g/L $CaCl_2$, 0.20 g/L KCl, 0.20 g/L KH_2PO_4 , 0.10 g/L $MgCl_2 \cdot 6H_2O$, 8 g/L NaCl, pH 7.4). Subsequently, equal parts of blood and Ficoll–Paque reagent (GE HealthCare, Sweden) were mixed and centrifuged at 1300 g/35 min. After centrifugation, the ring of PBMCs at the Ficoll/plasma interface was collected and washed twice with 10 mL of PBS. After the last centrifugation, at 1500 g/5 min, the pellet was submitted to the mitochondrial isolation protocol.

Mitochondrial isolation was adapted from Chua et al. (2003). Briefly, the cell pellet ($\sim 1.4 \times 10^7$ cells) was lysed by the addition of lysis buffer (250 mM mannitol, 70 mM sucrose, 5 mM Tris pH 7.5, 1 mM ethylenediaminetetraacetic acid [EDTA] pH 7.5, and 1% protease inhibitor cocktail [Sigma, St. Louis, USA]), followed by three sections of heat shock with N_2 (L) and dry bath set to 37 °C. After two centrifugations, at 1000 g/5 min and 1400 g/15 min, both at 0 °C and with successive disposal of the supernatant, the mitochondrial pellet was separated after one last centrifugation (17,000 g/30 min at 0 °C) and eluted with SETH buffer (250 mM sucrose, 2 mM EDTA, 10 mM Trizma base, 50 U/mL heparin, 1 M HCl). Finally, the sample was stored for up to seven days at –20 °C to measure the activity of ETC enzymes. Total protein was measured using the Coomassie Blue staining method (Bradford, 1976) using bovine serum albumin as standard.

Mitochondrial isolation was performed immediately after blood collection. Subsequently, the mitochondrial pellet was stored frozen at –20 °C for up to three days for the assessment of ETC complex activity, in all groups. Maximum storage time of mitochondrial pellets was determined using a curve of time vs. mitochondrial viability (data not shown).

2.3. Activity of electron transport chain enzymes

A small aliquot (50 μ L) of the mitochondrial pellet (~ 0.05 mg protein/mL) was used to determine the activity of enzymes of the ETC (complexes I, II, and III). NADH dehydrogenase (complex I) was assessed using an adapted version of the method described by Cassina and Radi (1996), which measures the rate of reduction of NADH-dependent ferricyanide at 420 nm for 3 min after addition of 100 mM potassium phosphate buffer pH 7.4, 1 mM rotenone, 10 mM FeCN, and 14 mM NADH. The activity of succinate dehydrogenase (complex II) and succinate:cytochrome c oxidoreductase (complexes II–III) was measured using an adaptation of the method proposed by Fischer et al. (1985). Briefly, complex II activity was measured by reduction of 2,6-dichloroindofenol (DCIP) at 600 nm for 5 min after addition of 62.5 mM potassium phosphate buffer pH 7.4, 250 mM sodium succinate, 0.5 mM DCIP, 100 mM sodium azide, and 1 mM rotenone. Complex II–III activity was measured by reduction of cytochrome c from succinate at 550 nm for 5 min after addition of 62.5 mM potassium phosphate buffer, 250 mM sodium succinate, 100 mM sodium azide, 0.5 mM rotenone, and 0.5% cytochrome c.

The results of the assessment of ETC complex activity were expressed as nmol/mg protein/minute.

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