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Sertraline reduces glutamate uptake in human platelets

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ABSTRACT

Mitochondrial damage and declines in ATP levels have been recently attributed to sertraline. The effects of sertraline on different parameters were investigated in washed platelets from 18 healthy male volunteers, after 24 h of drug exposure. Sertraline toxicity was observed only at the highest concentrations, 30 and 100 μM, which significantly reduced platelet viability to 76 ± 3% and 20 ± 2%, respectively. The same concentrations significantly decreased total ATP to 73 ± 3% and 13 ± 2%, respectively. Basal values of glycogen were not significantly affected by sertraline treatment. Glutamate uptake was significantly reduced after treatment with 3, 30 and 100 μM, by 28 ± 6%, 32 ± 5% and 54 ± 4%, respectively. Our data showed that sertraline at therapeutic concentrations does not compromise platelet viability and ATP levels, but they suggest that in a situation where extracellular glutamate levels are potentially increased, sertraline might aggravate an excitotoxic condition.

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1. Introduction

Sertraline (Zoloft) is a selective serotonin reuptake inhibitor (SSRI) that is widely prescribed as antidepressant (Sheehan and Kamijima, 2009), and is also approved for the treatment of panic, obsessive-compulsive, and post-traumatic stress disorders (Li et al., 2012). Although sertraline is generally considered a safe drug, numerous cases associating this SSRI and liver toxicity have been reported (Collados et al., 2010; Fartoux-Heymann et al., 2001; Persky and Reinus, 2003). Recently, deleterious effects of sertraline on hepatic cells have been described, including the induction of mitochondrial dysfunction (Li et al., 2012), apoptosis (Chen et al., 2014a) and endoplasmic-reticulum stress (Chen et al., 2014b).

Human platelets display functional similarities to neural cells, which include storing, releasing and taking up neurotransmitters, besides expressing their specific receptors and/or transporters (Da Prada et al., 1988). For this reason, platelets have been studied as peripheral markers in several neurodegenerative diseases such as Huntington's disease (Mangano and Schwarcz, 1981a), Parkinson's disease (Ferrarese et al., 1999), amyotrophic lateral sclerosis (Ferrarese et al., 2001), epilepsy (Rainesalo et al., 2003) and Alzheimer's disease (Di Luca et al., 2000; Ferrarese et al., 2000; Zoia et al., 2004). Human platelets contain a high-affinity Na^+-dependent glutamate uptake system, with kinetic and pharmacological properties similar to neurons and glia (Mangano and Schwarcz, 1981b; Begni et al., 2005; Hoogland et al., 2005), and are used as a peripheral model to investigate the imbalance of glutamatergic homeostasis that is present in several of these neurological diseases. The reduced glutamate uptake capacity, due to malfunctioning or decreased expression of glutamate transporters, has been implicated in the pathogenesis of various neurological diseases (Rothstein et al., 1995; Rossi et al., 2000; Proper et al., 2002). Another resemblance shared between platelets and glial cells, more specifically astrocytes, is the large amounts of glycogen, which in astrocytes seems to be important to maintain glutamate uptake and avoid excitotoxicity mediated by the extracellular glutamate (Rocha et al., 2014; Brown and Ransom, 2015).

In view of the recent data showing that sertraline causes mitochondrial damage and depletes cellular ATP (Li et al., 2012), we decided to investigate if this SSRI could affect the platelet metabolism, studying their energy resources and functioning, specifically glutamate uptake, which is energy-dependent.

2. Methods

2.1. Subjects

Human blood was collected from 18 healthy male volunteers registered as donors in the Hemotherapy Service of the Clinical Hospital of Porto Alegre, Rio Grande do Sul, Brazil. The study was carried out in accordance with the Code of Ethics of the World

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Medical Association (Declaration of Helsinki) for experiments involving humans. Informed consent was obtained from the donors, and their privacy rights were observed.

2.2. Procedures

Two samples of 4 mL were taken from the antecubital vein of each donor and placed in vacutainers (BD, Franklin Lakes, USA) containing K_3-EDTA. Immediately, the vacutainers were gently inverted 10 times and placed in an ABX Micros ES 60 hematology analyzer (HORIBA ABX SAS, Japan) to determine the number of platelets in each blood sample. Platelets were isolated as previously described by Mangano and Schwarcz (1981b). The vacutainers were then centrifuged at 300 × *g* for 5 min at 4 °C and the platelet-rich plasma (PRP) was obtained. The supernatant (PRP) was carefully removed with a plastic pipette, with care not to disturb the leukocyte layer. The volume of PRP collected from each sample was recorded and the PRP transferred to a microcentrifuge tube. The PRP was then centrifuged at 7000 × *g* for 10 min at 4 °C. The plasma was discarded and the pellet resuspended in 0.5 mL of 0.32 M phosphate-buffered sucrose (pH 7.4 at 4 °C). The suspension, hereafter referred to as the platelet concentrate (PC), was repeatedly passed through a 1-mL plastic pipette tip until the visible platelet aggregates were eliminated. An additional 0.5 mL of buffered sucrose was added to the suspension, and the solution was mixed with 5 gentle inversions. The PC was again centrifuged at 7000 × *g* for 5 min at 4 °C. The supernatant was discarded, and the pellet was resuspended in a volume of 0.32 M phosphate-buffered sucrose (pH 7.4 at 4 °C), equal to one-fifth of the initial volume of PRP obtained. Next, the two PC suspensions obtained from each subject were blended and the number of platelets was determined again. As previously observed by our group, the platelets should be counted in the PC after they are washed (Rocha et al., 2014). The mean platelet volume (MPV) and platelet distribution width (PDW) were also determined in both the whole blood and the PC, to evaluate potential variations in the platelets during the processing. The incubation was performed in a 96-well plate; to each well were added 20×10^6 platelets, and the final volume was 150 μL of Tris–citrate buffer (112.8 mM NaCl, 4.5 mM KCl, 1.1 mM KH_2PO_4, 1.1 mM $MgSO_4$, 11 mM Na_3–citrate, 25 mM Tris–HCl and 10.2 mM glucose), pH 6.5. Sertraline was diluted in Tris–citrate buffer and the final concentrations used were 0.1, 0.3, 3, 30 and 100 μM.

2.2.1. MTT assay

Thiazolyl blue tetrazolium bromide (MTT, Sigma, USA) was utilized to determine the effect of sertraline on platelet viability. After 20 h of incubation at room temperature, 20 μL MTT (5 mg/mL) was added to each well, and the plate was incubated for an additional 4 h. Subsequently, the incubation medium was gently discarded and 150 μL of DMSO was added to dissolve the formazan, which was detected using a microplate reader (EZ Read 400, Biochrom, UK). The absorption was read at 570 nm; the value obtained (UAbs) was expressed as a percentage of the control, % of control (UAbs/20×10^6 platelets).

2.2.2. Glycogen determination

The assay to determine the glycogen content was previously described by Rocha et al. (2014) and comprised the following steps: (1) cell lysis and glycogen extraction, (2) precipitation of glycogen and its washing, (3) conversion of glycogen to free glucose, (4) neutralization, and (5) detection of free glucose. In each experiment, a standard curve was established from a stock solution (1 mg/mL) of glycogen type III (Sigma, USA). Free glucose was determined using a glucose-oxidase kit (Laborclin Bio Liquid, Brazil). Absorbance was read at 505 nm, and levels of glycogen were expressed as a percentage of the control, % of control (μg/20×10^6 platelets).

2.2.3. ATP assay

After 24 h at room temperature, the incubation medium was gently discarded and 100 μL of ice-cold lysis buffer (in PBS containing 2 mM sodium azide and 1% Triton X-100 at pH 7.8) was added to each well. The lysis buffer containing the cells was repeatedly passed through a 1-mL plastic pipette tip (15 times), and the plate was kept on an ice surface during this procedure. The samples were transferred to mini-tubes for centrifugation (3 min at 10,000 × *g* at 4 °C) and the supernatant from each sample was transferred to another mini-tube kept in ice. The ATP was measured using the luciferin-luciferase bioluminescence assay (Molecular Probes ATP Determination Kit, USA), and an aliquot of 10 μL was used to determine ATP, according to the datasheet description. The luminescence was read using a SpectraMax M5 Microplate Reader (Molecular Devices, USA) at 560 nm, and the values obtained were expressed as μM of ATP.

2.2.4. Glutamate uptake assay

In each experiment, platelets from the same donor (20×10^6 platelets) were incubated in 0.5 mL sodium–Tris citrate buffer for 24 h at room temperature, without the drug (controls) or containing different concentrations of sertraline. Specific Na^+-dependent [^{3}H]-glutamate uptake was measured as described by Mangano and Schwarcz (1981b). Briefly, platelets were incubated for 15 min at 37 °C and the uptake assay was initiated by the addition of [^{3}H]-glutamate (specific activity 49.6 Ci/mmol PerkinElmer, USA) at a final concentration of 10 μM (0.2 μCi per tube). After 10 min, the uptake reaction was stopped by adding ice-cold Tris–citrate buffer containing 1 mM cold glutamate, followed by centrifugation for 10 min at 6340 × *g*. The tubes were rinsed three times with 3 mL ice-cold Tris–citrate buffer containing 1 mM L-glutamate. After rinsing, the platelet pellets were dissolved overnight with 0.5 N NaOH, liquid scintillation cocktail (Optiphase HiSafe 3, PerkinElmer, USA) was added, and the radioactivity was measured in a β-counter. Non-specific glutamate uptake was obtained using Tris–citrate buffer in which the sodium chloride and sodium citrate was replaced with equimolar choline chloride and potassium citrate. Net high-affinity glutamate uptake was calculated by subtracting the sodium-free control measurements from the uptake assays performed in the presence of sodium. The glutamate transport rate was expressed as a percentage of the control, % of control (pmol/20×10^6 platelets/10 min).

2.3. Statistical methods

Values are reported as mean ± SEM, and the statistical analysis was conducted using SPSS. The data were analyzed through one-way ANOVA followed by Tukey's multiple comparisons test, $p < 0.05$ (two-tailed).

3. Results

We used washed platelets to study sertraline effects after 24 h of drug exposure. Initially, the platelet indices (cell count, MPV and PDW) were determined in whole blood and again after obtaining the PC, to evaluate if these parameters changed during the processing. Comparison of these indices before and after the platelets were collected revealed no differences due to the procedures used to separate and wash the cells. Then, the platelet number quantified in each PC was used to normalize the cell density for each experiment. As previously described (Türck and Frizzo, 2015), the cell number was reduced during the process (yield 44 ± 7%); contaminant cells were absent; and despite the loss

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