Interdiscip Toxicol. 2018; **Vol. 11**(3): 181–188.
doi: 10.2478/intox-2018-0015

sciendo

interdisciplinary toxicology

ORIGINAL ARTICLE

In vitro cytogenotoxic evaluation of sertraline

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ITX110318A01 • Received: 03 November 2017 • Accepted: 15 May 2018

ABSTRACT

Sertraline (SRT) is an antidepressant agent used as a neuronal selective serotonin-reuptake inhibitor (SSRI). SRT blocks serotonin reuptake and increases serotonin stimulation of somatodendritic serotonin 1A receptor (5-HT1AR) and terminal autoreceptors in the brain. In the present study, the genotoxic potential of SRT was evaluated using cytokinesis-block micronucleus (CBMN) cytome assay in peripheral blood lymphocytes of healthy human subjects. DNA cleavage-protective effects of SRT were analyzed on plasmid pBR322. In addition, biochemical parameters of total oxidant status (TOS) and total antioxidant status (TAS) in blood plasma were measured to quantitate oxidative stress. Human peripheral blood lymphocytes were exposed to four different concentrations (1.25, 2.5, 3.75 and 5 µg/mL) of SRT for 24- or 48-h treatment periods. In this study, SRT was not found to induce MN formation either in 24- or 48-h treatment periods. In contrast, SRT concentration-dependently decreased the percentage of MN and MNBN ($r=-0.979$, $p<0.01$; $r=-0.930$, $p<0.05$, respectively) when it was present for the last 48 hr (48-h treatment) of the culture period. SRT neither demonstrated a cleavage activity on plasmid DNA nor conferred DNA protection against H_2O_2. The application of various concentrations of SRT significantly increased the TOS and oxidative stress index (OSI) in human peripheral blood lymphocytes for both the 24- and 48-h treatment periods. Morover, the increase in TOS was potent as the positive control MMC at both treatment times. However, SRT did not alter the TAS levels in either 24- or 48-h treatment periods when compared to control. In addition, exposing cells to SRT caused significant decreases in the nuclear division index at 1.25, 2.50 and 3.75 µg/mL in the 24-h and at the highest concentration (5 µg/mL) in the 48-h treatment periods. Our results suggest that SRT may have cytotoxic effect *via* oxidative stress on cultured human peripheral blood lymphocytes.

KEY WORDS: sertraline; micronucleus; pBR322; peripheral blood lymphocytes; oxidative stress; cytotoxicity

Introduction

Major depression is a leading problem in the society and is becoming increasingly more common. In the western world, major depression is the first-line psychological problem. Although it has become more common in all populations in all age groups, it occurs most frequently in young people, especially in adolescents. With this rate of increase, major depression is predicted to be the second most common disease after heart disease in 2020 (Seligman, 2006). Depression is a depressed state and activity that can affect a person's thoughts, actions, and health (APA 2013). Although the molecular mechanism of depression has not yet been fully elucidated, it is thought that the decline in the activities of some neurotransmitters leads to depression. In this context, according to the monoamine hypothesis, a decrease in the activity of brain monoamines, such as dopamine, serotonin and neuroepinephrine, leads to depression (Schildkraut, 1967).

The selective 5-HT (serotonin) reuptake inhibitors (SSRIs) reestablish the levels of 5-HT in the synaptic gap through binding the 5-HT reuptake transporter and thus averting the subsequent degradation of 5-HT. This reuptake inhibition leads to the rise of 5-HT concentrations in the synaptic gap and the concentration of 5-HT returns to the normal range. Mechanistically, this structural characteristic of SSRIs is believed to result in reduced symptoms of depression. In the presence of an SSRI agent, only a small amount of 5-HT is subjected to degradation in the synaptic gap (Rang *et al.*, 2001; Celada *et al.*, 2004).

Genotoxicity studies on SSRIs seem to indicate that each compound has unique properties due to its chemical structure and it is not feasible to reach a general judgement for the safety assessment of all SSRIs. Fluoxetine and paroxetine have been attributed as genotoxic drugs on blue mussel (*Mytilus edulis*) hemocytes (Lacaze *et al.*,

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2015). The mechanism proposed for the genotoxic effect of these drugs was the significant increase of intracellular reactive oxygen species (ROS) and oxidative stress leading to genotoxic and cytotoxic effects (Lacaze *et al.*, 2015). Based on computer modeling and energy calculations of drug/DNA complexes, the source of genotoxicity caused by citalopram was proposed to be an N-dialkyl group responsible for DNA intercalation (Synder *et al.*, 2006). Citalopram was also shown to induce genotoxicity *via* DNA strand breaks and mitotic recombination in *Aspergillus nidulans* and *Drosophila melanogaster* (Franco *et al.*, 2010; Gürbüzel *et al.*, 2012).

Sertraline (SRT) [(1S,4S)-4-(3,4-dichlorophenyl)-N-methyl-1,2,3,4-tetrahydronaphthalen-1-amine], the test substance of this study, is a widely prescribed serotonin reuptake inhibitor (MacQueen *et al.*, 2001). SRT blocks serotonin reuptake and increases serotonin stimulation of somatodendritic serotonin 1A receptor (5-HT1AR) and terminal autoreceptors in the brain. SRT treatment in patients with generalized anxiety disorder and major depression was shown to cause no DNA damage by the use of sister chromatid exchange (SCE) and chromosome aberration (CA) assays (Bozkurt *et al.*, 2004). More recently, SRT treatment did not induce DNA damage in the cells of peripheral blood, neither *in vivo* in patients with bipolar disorder nor *in vitro* at therapeutic drug concentrations in the comet assay (Andreazza *et al.*, 2007). Moreover, SRT failed to show any genotoxic effect in balancer heterozygous wings of *Drosophila melanogaster* by use of the somatic mutation and recombination test (Gürbüzel *et al.*, 2012). However, Battal *et al.* (2013) concluded that SRT administration in male Wistar albino rats increased the frequency of MN in chronic treatment (28 days), suggesting possible influence of the drug on some mechanisms of cell division.

The cytokinesis-block micronucleus cytome assay (CBMN assay) is a comprehensive system for measuring DNA damage, cytostasis and cytotoxicity (Fenech, 2007). Alterations in blood lymphocyte chromosomes are among the most widely used indicators of genotoxic exposure and a comparison of cancer risk with MN frequency is possible because MNs formed in peripheral blood lymphocytes have been validated as biomarkers of increased cancer risk (Bonassi *et al.*, 2011; Fenech *et al.*, 2011).

In the present study, to examine whether treatment of human peripheral lymphocytes with SRT leads to DNA damage, we performed CBMN cytome assay as the highly sensitive cytogenetic damage marker to detect DNA damage. Moreover, the effect of SRT in the context of DNA cleavage-protective (anti-oxidative) activity was evaluated on pBR322 plasmid. Cytotoxicity of SRT was evaluated by the nuclear division index (NDI). We also performed a quantitative approach using a novel spectrophotometric method to quantify the total oxidant status (TOS) and the total antioxidant status (TAS) in human peripheral lymphocytes exposed to SRT. The data obtained separately as the TOS and TAS were further evaluated to calculate the oxidative stress index (OSI) in blood plasma.

Materials and methods

Cukurova University Institutional Review Board was informed of the protocol to be used with human subjects and approved the protocol for the work described prior to the performance of the experiments (decision number:53; date: 13 May 2016). All healthy blood donors (n=4; two males and two females, all nonsmokers) gave informed consent before participation in this study. The healthy blood donors were not using any medication or dietary supplements throughout the study.

In vitro cytokinesis-block micronucleus (CBMN) cytome assay

Commercially available sertraline hydrochloride (SRT) (Lustral, manufactured by Pfizer) was used as the test substance for the *in vitro* tests. The genotoxic effect of SRT (dissolved in sterile bidistilled water) in human peripheral lymphocytes was studied at 4 different concentrations (1.25, 2.5, 3.75 and 5 µg/mL) (roughly 6.5–26 times higher than the maximum plasma level [0.19 µg/mL] of SRT achieved in patients receiving this drug) and 2 different exposure periods (24 or 48 hr). These concentrations were selected based on the previous range-finding studies which showed that the viability (mitotic index) in the highest concentration group (5 µg/mL) was reduced approximately by 50% as compared to that of control. The concentration-range-finding methodology was adapted from the OECD Guideline 487, in which at least three analysable test concentrations should be evaluated. However, to study the concentration-response relation in detail, we selected four closely spaced concentrations of SRT to be able to obtain better concentration-response data.

For the analysis of MN in binucleated lymphocytes, 0.2 mL of fresh whole blood (1/10 heparinized) was used to establish the cultures and the cultures were incubated for 68 h. The cells were treated with 1.25, 2.5, 3.75 and 5 µg/mL SRT for 24- and 48-h treatment periods. To block cytokinesis, cytochalasin B (Sigma, C6762) was added at 44 h of the incubation at a final concentration of 6 µg/mL. After an additional 24-h incubation at 37 °C, cells were initially harvested by centrifugation at 1,200 rpm for 15 min. Then, the supernatant discarded, cells were treated with prewarmed 0.4% KCl (37 °C) as the hypotonic solution and directly centrifuged (with the exception of a 5 min hypotonic treatment step). After centrifugation, the cells were fixed with cold fixative (1/5/6: glacial acetic acid/methanol/0.9% NaCl isotonic solution) for 20 min. Then, they were fixed two times with another cold fixative (1/5: glacial acetic acid/methanol) for 15 min. After each fixation process, the cells were centrifuged at 1200 rpm for 15 min. Later, the fixed cells were spread on cold glass slides, dried at room temperature and the slides were stained with 5% Giemsa (Kirsch-Volders *et al.*, 2003; Rothfuss *et al.*, 2000). In all subjects, 1,000 binucleated lymphocytes were scored from each donor (4,000 binucleated cells were scored per concentration). A total of 1,000 cells (4,000 cells for each treatment concentration) were scored to calculate the nuclear division index (NDI) for

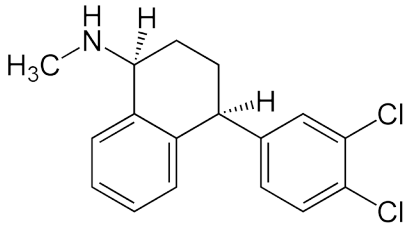

Figure 1. Chemical structure of sertraline.

the cytotoxicity of SRT using the formula: NDI = (M1) + (2 × M2) + (3 × M3) + (4 × M4)/N, where M1–M4 represent the number of cells with one to four nuclei and N is the total number of viable cells scored (excluding necrotic and apoptotic cells). NDI is a practical parameter for measuring the mitogenic response of lymphocytes and general cytotoxic effects of agents examined in the assay (Figure 1) (Fenech, 2007).

DNA cleavage activity and DNA damage protection potential

DNA is a main target of attack by cellular oxidant species and its damage can lead to mutation. A plasmid is a double-stranded small circular DNA molecule that is extrachromosomal and can replicate independently. Plasmids can appear in different forms.The prominent forms of monomeric plasmids are the supercoiled (SC), the open circular (OC) form (also called "nicked form" or relaxed form, obtained by digestion of one of the strands of double strand), and the linear form (LIN), typically obtained by digestion of both strands of plasmid. In the SC form, the plasmid migrates more rapidly than OC and LIN forms. These conformations, in order of electrophoretic mobility from slowest to fastest, are OC DNA, LIN DNA, and SC DNA (Timocin *et al.*, 2017; Fernandez *et al.*, 2011; Simandan *et al.*, 1998; Suksomtip & Pongsamart, 2008). DNA cleavage and protection activities of SRT were evaluated on pBR322 plasmid DNA. DNA cleavage activity was investigated with four concentrations of SRT and DNA protection activity was assayed with four concentrations of SRT in the presence of 35 % H_2O_2. In summary, the experiments were performed in a microcentrifuge tube containing 3 µL of pBR322 plasmid DNA and 5 µL of SRT that was dissolved in ddH_2O. This amount (8 µL) was completed to 10 µL with ddH_2O. In addition, untreated pBR322 plasmid DNA (3 µL) as a control was also used. For DNA cleavage activity, plasmid DNA (172 ng/µL) was treated with SRT for 5 min. The plasmid DNA (pBR322) was treated with the combination (SRT + 35 % H_2O_2) for 5 min at room temperature to assess possible DNA protective effect. A positive control group (plasmid DNA + 35 % H_2O_2 + dH_2O) was also included in the gel. After treatments, 2 µL loading dye was added and the reactions were loaded on 1% agarose gel. Electrophoresis was performed with 100 V for 120 min in 0.5 × TBE buffer. The gels were stained with EtBr and visualized with Vilber Lourmat gel imaging system.

Plasma TAS and TOS Measurement

Blood plasma samples (2 mL) were collected from the four healthy participant donors for measurement of serum TOS and TAS. The plasma samples were immediately collected from supernatants of centrifuged whole blood cultures at the end of the cell culture period (72-hr), stored at –80° C and analyzed within 1 month.

Reactive oxygen species (ROS) are formed in metabolic and physiological processes, and desruptive oxidation reactions may occur in organisms that remove them *via* enzymatic and non-enzymatic antioxidative mechanisms. Under certain conditions, the oxidant-antioxidant balance in the cell shows an increase in the direction of the oxidant and a decrease in antioxidant, and this imbalance can not be restored by the cell. This oxidative damage causes about 100 diseases (Halliwell & Gutteridge, 2000).

The total oxidant status (TOS) of SRT-treated human peripheral blood lymphocytes was measured using automated colorimetry method of Erel. Oxidants present in the sample oxidize the ferrous ion–chelator complex to ferric ion. The oxidation reaction is prolonged by enhancer molecules, which are abundantly present in the reaction medium. The ferric ion makes a colored complex with chromogen in an acidic medium. The color intensity, which can be measured spectrophotometrically, is related to the total amount of oxidant molecules present in the sample. The assay is calibrated with hydrogen peroxide. The results were expressed in terms of micromolar hydrogen peroxide equivalent per liter (µmol H_2O_2 Equiv./L) (Erel, 2004).

The TOS was calculated following the principle: 500 µL Reagent 1 (Assay Buffer) placed in cell and 75 µL of the prepared standard (or sample) was added. The initial absorbance was read at 530 nm for the first absorbance point. Then, 25 µL of Reagent 2 (Prochromogen solution) was added to the cell and incubated 5 min at room temperature. The absorbance was read at a second time at 530 nm. The results were calculated using the following formula:

$$\text{Result} = (\Delta\text{AbsSample}/\Delta\text{AbsStd}^2) \times 20\ (\text{Std}^2\ \text{Value}),$$

where Δsample Absorbance = (Second Absorbance of Sample – First Absorbance of Sample); Δabsorbance Standard 2 = (Second Absorbance of Std 2 – First Absorbance of Std 2); Standard2 Value = 20 µmol H_2O_2 Equiv./L; Std^2: (Stock Stabilized Standard Solution (SSSS)] (800 mM H_2O_2 Equiv./L).

Antioxidant molecules prevent or inhibit harmful reactions caused by oxidative damage (Young & Woodside, 2001). Because the measurement of different antioxidant molecules in plasma separately is not practical and the antioxidant effects are additive, the total antioxidant capacity of a sample is measured, and this is called total antioxidant status (TAS) (Erel, 2004). In this study, The TAS of SRT-treated plasma was measured using automated colorimetry method of Erel.

For the analysis of TAS, 500 µL of Reagent 1 (Assay Buffer) was placed in cell and 30 µL standard (or sample) was added. The initial absorbance was read at 660 nm for the first absorbance point. Then, 75 µL Reagent 2 (Colored

ABTS Radical Solution) was added to the cell and incubated 5 min at room temperature. The absorbance was read for the second time at 660 nm. The results were expressed using the following formula:

$$[(\Delta Abs\ Std^1) - (\Delta Abs\ Sample)]/[(\Delta Abs\ Std^1) - (\Delta Abs\ Std^2)],$$

where Δ Absorbance Standard^1=(Second Absorbance of Std^1 – First Absorbance of Std^1); Δ Absorbance Standard^2 = (Second Absorbance of Std2 – First Absorbance of Std^2); Δ Sample Absorbance = (Second Absorbance of Sample – First Absorbance of Sample); Std1: 0.0 mmol Trolox Equiv./L; Std2: 1.0 mmol Trolox Equiv./L.

Oxidative Stress Index (OSI)

The ratio of TOS to TAS was accepted as the oxidative stress index (OSI) (Harma *et al.*, 2003; Kösecik *et al.*, 2005; Yumru *et al.*, 2009). OSI value was calculated according to the following formula:

$$OSI = TOS/TAS$$

Statistics

Values of the control, positive control and the SRT-exposed groups were expressed as the mean (±SE) from four separate experiments. The comparisons between the control, positive control and the SRT-exposed groups were performed using One-Way ANOVA (LSD test) at $p \leq 0.05$. Concentration-response relationships were determined from the correlation and regression coefficients for NDI, MN, MNBN, TOS, TAS and OSI.

Results

Table 1 shows the effect of various concentrations of SRT on the formation of micronuclei (MN) in human lymphocytes. SRT was unable to induce a statistically significant increase in the formation of MN when compared with the control for 24-h and 48-h treatment periods. Interestingly at 48-h culture period, SRT significantly decreased the %MN in a concentration-dependent ($r=-0.979$, $p<0.01$) manner (Figure 2). Increasing SRT concentrations did not cause a significant increase in the percentage of the binuclear cells with micronuclei (%MNBN) for 24- and 48-h treatment periods. On the other hand, similar to the concentration-related decrease in the %MN, SRT also induced a significant concentration-dependent decrease in the %MNBN ($r=-0.930$, $p<0.05$) when it was present for the last 48 hr (48-h treatment) of the culture period (Figure 3).

DNA cleavage activity of SRT was assessed by relaxation of supercoiled circular form of pBR322 plasmid DNA (SC DNA) into the nicked open circular (OC DNA) and linear (LIN DNA) form. If one strand of SC DNA is cleaved, the supercoil form will relax and produce slower moving OC DNA. If both strands of SC DNA are cleaved, a linear form (LIN DNA) will occur and migrate between SC DNA and OC DNA. Figure 3 depicts agarose gel electrophoresis patterns of pBR322 DNA after incubation with SRT for 5 min. Compared with control (DNA control, lane 1), the banding patterns of Lanes 3–6, demonstrate that no observable DNA cleavage occurs when incubated with four different concentrations of SRT, which is indicative of the inability of SRT to damage pBR322 DNA. Figure 5 demonstrates the DNA damage protection potential of four different concentrations of SRT. Hydrogen peroxide treatment of plasmid DNA (lane 2) resulted in the cleavage of linear DNA (LIN DNA) to faint and low molecular weight bands between OC and SC DNA. However, SRT could not show a protection potential against the DNA breaking effect of H_2O_2 on LIN DNA (lanes 3–6).

Plasma total oxidant status (TOS), total antioxidant status (TAS) and oxidative stress index (OSI) values of control and SRT-treated human peripheral blood lymphocytes are shown in Table 2. TOS were found to increase significantly in both 24- (3.75 and 5 µg/mL) and 48-h (1.25, 2.5, 3.75 and 5 µg/mL) treatment periods when compared to control. Moreover, the increase in TOS observed in 48-h treatment period showed no significant difference when compared to positive contol (Mitomycin C), indicating that SRT itself was as potent as MMC in inducing the formation of various oxidant species in human peripheral blood lymphocytes (Table 2). However, addition of SRT in human peripheral blood culture did not increase TAS in either 24- or 48-h treatment periods when compared to control. As a result of the increase in TOS, addition of SRT into human peripheral blood cultures significantly increased the OSI in 24- (3.75 and 5 µg/mL) and 48-h (2.5, 3.75 and 5 µg/mL) treatment periods.

The effects of various concentrations of SRT on cell cycling kinetics in human peripheral lymphocytes have been assessed by using the nuclear division index (NDI). The NDI shows the effect of SRT on nuclear division and hence cytotoxicity. NDI was significantly reduced at the first three concentrations (1.25, 2.50 and 3.75 µg/mL) in 24-h treatment period and at the highest concentration (5 µg/mL) in 48-h treatment period compared with control (Table 1).

Discussion

Antidepressant drugs are long-term medications used by millions of patients with or without prescription. When determining benefit/risk ratio for treatment with these drugs, it should be considered that the genotoxic and carcinogenic side effects caused by these drugs can not be ignored. Brambilla *et al.* (2009), reviewing data from several genotoxicity assays, reported that in the long-term carcinogenicity tests of 33 antidepressant molecules, 17 showed positive results in terms of genotoxicity or carcinogenicity.

SRT, the test substance of this study, is among the most widely prescribed SSRIs today (Sanches *et al.*., 2014). SRT is a type of SSRI antidepressant used mainly in major depression, obsessive-compulsive disorder, widespread and social anxiety disorder. SSRIs are preferred more

Table 1. The percentage of micronucleus (MN), micronucleated binuclear (MNBN) cells and nuclear division index (NDI) in cultured human peripheral lymphocytes treated with SRT for 24- and 48-h.

Test substance	Treatment time (hr)	Treatment concentration (µg/mL)	MN±SE (%)	MNBN±SE (%)	NDI
Control	–	–	0.10±0.02	0.07±0.01	1.32±0.05
MM	24	0.25	0.42±0.11*	0.37±0.10*	1.13±0.02*
SRT	24	1.25	0.11±0.02b_2	0.10±0.02 b_2	1.21±0.03 a_1
SRT	24	2.50	0.18±0.07 b_1	0.16±0.07 b_1	1.18±0.02 a_2
SRT	24	3.75	0.17±0.03 b_1	0.17±0.03 b_1	1.19±0.03 a_1
SRT	24	5	0.20±0.07 b_1	0.17±0.07 b_1	1.24±0.01 b_1
MMC	48	0.25	0.42±0.20*	0.39±0.18*	1.06±0.03*
SRT	48	1.25	0.22±0.08	0.22±0.08	1.27±0.03 b_3
SRT	48	2.50	0.15±0.02	0.13±0.02	1.24±0.03 b_2
SRT	48	3.75	0.13±0.03 b_1	0.13±0.03	1.25±0.01 b_2
SRT	48	5	0.08±0.02 b1	0.08±0.02 b_1	1.18±0.02 a_2b_1

Data are expressed as the mean ± SE (n=4). * Statistically significant increase in positive control vs. control for 24 and/or 48 h (*: $p<0.05$). a Statistically significant *vs.* control, b Statistically significant *vs.* positive control. a_1b_1: $p<0.05$; a_2b_2: $p<0.01$; a_3b_3: $p<0.001$

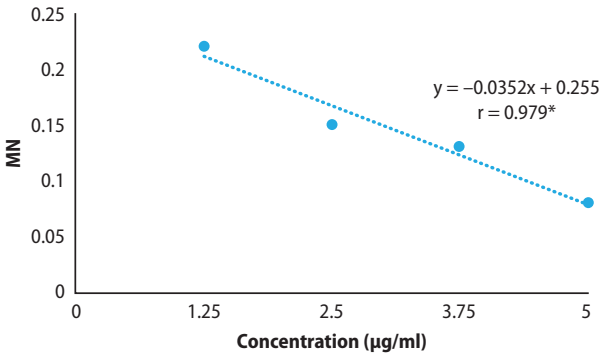

Figure 2. Regression line and correlation coefficient depicting concentration-dependent reduction of MN in human peripheral blood lymphocytes treated with sertraline for 48 h at different concentrations. *$p<0.01$

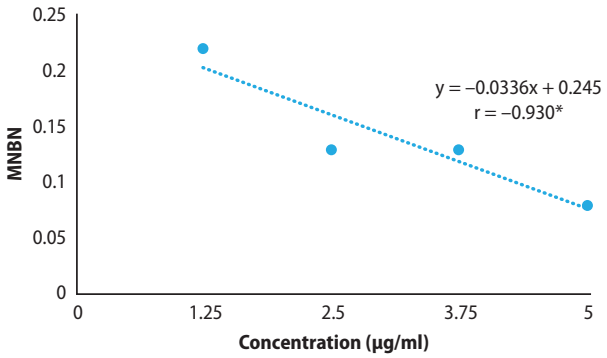

Figure 3. Regression line and correlation coefficient depicting concentration-dependent reduction of MNBN in human peripheral blood lymphocytes treated with sertraline for 48 h at different concentrations. *$p<0.05$

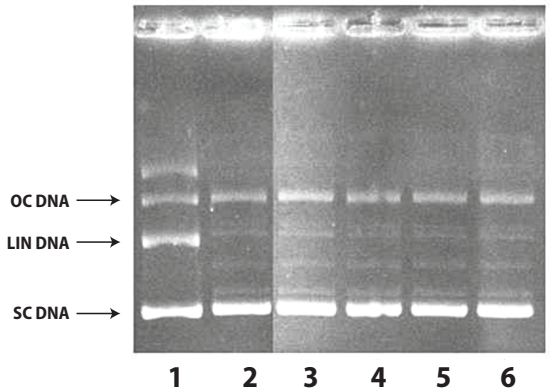

Figure 4. Agarose gel electrophoresis cleavage pattern of pBR322 plasmid DNA after treatment with SRT in presence of H_2O_2 (lanes: 3, 4, 5, 6) for 5 min. Lane 1: DNA control (H_2O); Lane 3: 1.25 µg/mL SRT+H_2O+DNA; Lane 4: 2.50 µg/mL SRT+H_2O+DNA; Lane 5: 3.75 µg/mL SRT+H_2O+DNA; Lane 6: 5 µg/mL SRT+H_2O+DNA

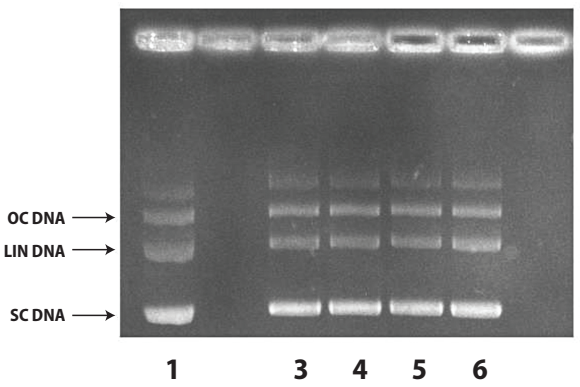

Figure 5. Agarose gel electrophoresis cleavage pattern of pBR322 plasmid DNA after treatment with SRT alone (lanes: 3, 4, 5, 6) for 5 min. Lane 1: DNA control (H_2O); Lane 2: DNA+H_2O_2+dH2O; Lane 3: 1.25 µg/mL SRT+H_2O_2+DNA; Lane 4: 2.50 µg/mL SRT+H_2O_2+DNA; Lane 5: 3.75 µg/inL SRT+H_2O_2+DNA; Lane 6: 5 µg/mL SRT+H_2O_2+DNA.

Table 2. The levels of total oxidant status (TOS), total antioxidant status (TAS) and oxidative stress index (OSI) in cultured human blood lymphocytes treated with SRT for 24- and 48-h.

Test substance	Treatment time (hr)	Treatment concentration (µg/mL)	TOS (µmol H_2O_2 equiv./L)	TAS (mmol Trolox equiv./L)	OSI (Oxidative Stress Index)
Control	–	–	3.34±1.04	0.26±0.01	13.09±4.38
MMC	24	0.25	4.31±0.62	0.27±0.004	15.75±2.40
SRT	24	1.25	4.69±0.65	0.25±0.01	18.14±2.40
SRT	24	2.50	4.29±0.68	0.26±0.01	16.59±2.83
SRT	24	3.75	5.55±0.24 a_2	0.27±0.01	20.43±0.96 a_1
SRT	24	5	5.25±0.65 a_1	0.26±0.01	19.94±2.27 a_1
MMC	48	0.25	5.55±0.04**	0.25±0.01	22.16±1.67**
SRT	48	1.25	4.94±0.42 a_1	0.25±0.01	18.97±0.77
SRT	48	2.50	5.63±0.37 a_2	0.29±0.02	20.14±2.74 a_1
SRT	48	3.75	5.40±0.09 a_1	0.26±0.01	20.60±1.75 a_1
SRT	48	5	5.58±0.14 a_2	0.26±0.004	19.88±0.96 a_1

Data are expressed as the mean ± SE (n=4). ** Statistically significant increase in positive control vs. control for 24 and/or 48 h (**: $p<0.01$), a Statistically significant vs. control, b Statistically significant vs. positive control. a_1b_1: $p<0.05$; a_2b_2: $p<0.01$; a_3b_3: $p<0.001$.

often than older generation antidepressants because of the low side effects. However, some studies in the literature indicate that members of this class of antidepressants exert genotoxic/carcinogenic effects.

In this study, we evaluated the genotoxic potential of SRT using cytokinesis-blocked MN cytome assay. To the best of our knowledge, this is the first study to evaluate the *in vitro* genotoxic effect of SRT in cultured human peripheral lymphocytes. We used the NDI as indicator of cytotoxicity to determine the effects of SRT on the nuclear division of lymphocytes. Moreover, a quantitative approach using a novel spectrophotometric method was undertaken to quantify the amount of total antioxidants and oxidants in human peripheral lymphocytes exposed to SRT. The data obtained separately as the total antioxidant and oxidant status were further evaluated to calculate the oxidative stress index (OSI) in human lymphocytes.

In this study, SRT was unable to induce the formation of MN in human peripheral lymphocytes for 24- and 48-h treatment periods. Neither was SRT demonstrated a cleavage activity on plasmid DNA nor conferred DNA protection against H_2O_2. In accordance with our findings, SRT was shown to have no indication of clastogenic hazard in patients with generalized anxiety disorder and major depression (Bozkurt *et al.*, 2004). Bozkurt *et al.* concluded that the increased SCE frequency in patients was based on psychogenic stress rather than SRT treatment. Andreazza *et al.* (2007) reported that the DNA damage observed in peripheral leukocytes of patients in the comet assay was correlated with the symptoms of depression and mania, but not with SRT treatment. Furthermore, SRT, though not citalopram, did not show any genotoxic effect in balancer heterozygous wings of D. melanogaster (Gürbüzel *et al.*, 2012). However, Battal *et al.* (2013) reported that SRT induced a significant increase in the frequency of MN (in chronic treatments) in peripheral blood of Wistar albino rats in the CBMN assay.

The reason for the apparent discordance that SRT could not induce DNA damage in our study and in the comet assay (Bozkurt *et al.*, 2004; Andreazza *et al.*, 2007; Gürbüzel *et al.*, 2012), but apparently induced DNA damage in the *in vivo* CBMN assay (Battal *et al.*, 2013) is not clear but negative comet assay results versus positive MN results should be interpreted carefully. Comet assay measures primary DNA damage, which is usually completely repaired within a few hours by the DNA repair mechanisms of the cell. If the comet assay is performed a few hours after the induction of DNA damage, comet data could be negative because of the effective repair of DNA damage. Improperly repaired damage can cause MN in cells undergoing second mitotic division (M2). The data can be interpreted as the absence of induction of primary DNA damage and as a negative comet assay result (Wiedemann & Schutz, 2008). On the other hand, high-dose SRT administration has been shown to enhance oxidative stress, lipid peroxidation (Battal *et al.*, 2014) and the oxidative stress caused by SRT leads to mitochondrial dysfunction in eukaryotic cells (Li *et al.*, 2012). Furthermore, it was shown that ROS inducing chemicals could induce secondary DNA damage (Jeong and Swenberg, 2004). Thus, we are of the opinion that high MN frequency observed in the study of Battal *et al.* (2013) may be secondary DNA damage caused by oxidative cell injury resulting from long-term chronic administration of SRT in rats.

The present study clearly demonstrates a SRT-induced cytotoxicity in human peripheral lymphocytes as revealed by significant decreases in the NDI. Interestingly, at a 48-h culture period, SRT induced a significant concentration-dependent decrease in the percentage of MN and MNBN. We are of the opinion that this effect of SRT could be explained by the suppression of nuclear division due to oxidative stress. For the formation of micronucleus, binuclear cells that have undergone only one nuclear division must be present. The decrease in the number of binuclear

cells (due to decrease in nuclear division) was determined as a decrease of MN in our study. At the molecular level, this effect on lymphocyte cell division was shown to be related to the suppressive effect of SRT on expression of genes (Cdc6) and proteins (STAT3 and COX2) involved in cell proliferation (Taler *et al.*, 2007). SRT was also shown to inhibit translation by altering the localization of eIF4E and increasing eIF2α phosphorylation. In addition, these effects were followed by the downregulation of mTOR signaling pathway in a REDD1-dependent manner (Lin *et al.*, 2010).

The results obtained with SRT on the cellular antioxidant and oxidant status in our study showed that SRT is a potent inducer of cellular oxidant species as well as oxidative stress. Although a measurement of the total oxidant status (but not different oxidant molecules separately) performed in this study showed that our results on the cytotoxicity of SRT are in apparent concordance with the study of Chen *et al.* (2014). In that study, SRT decreased the cell viability and induced apoptosis in human hepatoma cell line HepG2 through the TNFα-MAP4K4-JNK pathway. These data clearly suggest that SRT can pose cytotoxic risk even at very low concentrations *in vitro*. All SSRI antidepressants carry apolar aromatic ring systems in their molecular structures. Although we did not perform a study in this context, we propose that the oxidative stress induced by SRT in our study may be correlated to its ability to easily cross and damage biological membranes (both cellular and mitochondrial). Consistent with our argument, SRT has been also shown to diffuse passively through lysosomal membranes and accumulate in the organelle (lysosomotropy) *via* a proton-trap mechanism (Daniel *et al.*, 2001). This feature of SRT leads to damage to the lysosomal membrane through increased ROS, lipid peroxidation, and activation of cathepsins (Dielschneider *et al.* 2017). In conclusion, SRT and other cationic amphiphilic drugs demonstrate antiproliferative effect by ion trapping, vacuolar alterations and these effects are inversely correlated with their lipophilicity (Parks & Marceau, 2016).

Conclusion

In this study, oxidant, antioxidant, genotoxic and DNA cleavage-damage protection potential of SRT were determined. In general, SRT was unable to induce micronuclei and did not demonstrate DNA cleavage activity on pBR322 DNA. Neither was SRT potent enough to reverse the DNA cleavage activity of H_2O_2. Our results are in accordance with the study of Synder *et al.* (2006) who reported that unlike genotoxic citalopram, SRT does not have the N-dialkyl group that enables the molecule for possible DNA intercalation. However, SRT exerted cytotoxic effect by decreasing the NDI and increased the oxidative stress index in human peripheral lymphocytes. Previous studies have also confirmed that the use of SRT is associated with increased oxidative stress, lipid peroxidation (Chung *et al.*, 2013; Battal *et al.*, 2014), mitochondrial and lysosomal dysfunction (Li *et al.*, 2012; Dielschneider *et al.* 2017). Therefore, it is essential that antidepressant drugs should be tested not only for their genotoxic potential but also for their ability to disturb mitochondrial and lysosomal processes which lead to cytotoxicity and thus to provide a deeper understanding of the potential risks related to treatment in patients with depression.

Acknowledgements

This study was sponsored by Cukurova University Research Fund; Grant Number FBA-2016-6877. Also, the authors thank Prof. Dr. Mehmet Topaktaş for his valuable intellectual assistance through the critical evaluation of the article.

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