

Studies regarding the protective effects exhibited by antidepressants on cell models

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Abstract

The study aimed to assess *in vitro* the short-term effects exerted by fluoxetine, sertraline and venlafaxine on certain physiological properties in two different study models: U937 monocytes and erythrocytes isolated from patients treated with the above-mentioned molecules. Results on U937 cell suspensions revealed the depolarization of the cell membrane induced by the three antidepressants. The maximal depolarization effect was registered after 15 minutes of cell exposure and was concentration-dependent, in a non-monotonic manner. The effect was also dependent on the tested compound, fluoxetine presenting the strongest depolarizing effect compared to sertraline and venlafaxine. The erythrocyte susceptibility to lipid peroxidation and glucose-6-phosphate dehydrogenase activity were assessed on red blood cells isolated from patients with depressive disorder. Our results revealed that antidepressant treatment induced the antioxidant defense, by decreasing erythrocyte susceptibility to lipid peroxidation and increasing glucose-6-phosphate dehydrogenase activity. The effect is more intense in the case of severe pathology and less evident in the case of moderate or minor disorder, as expressed by MADRS (Montgomery-Åsberg Depression Rating Scale) score. Our results could indicate that selected antidepressants at therapeutic concentrations, besides their known pharmacological effects, exhibit a protective effect against oxidative stress and also influence cells with immune properties.

Keywords: fluoxetine, sertraline, venlafaxine, transmembrane potential, antioxidant potential.

Introduction

The imbalance between the production of reactive oxygen species (ROS) and the activity of the endogenous antioxidants (*e.g.*, tocopherol) is involved in numerous pathologies such as atherosclerosis, diabetes mellitus, neurodegenerative diseases, but also in the pathogenesis of neuropsychiatric disorders [1]. It is known that many of the cellular disturbances in these pathological conditions are caused by the formation of lipid peroxidation products, such as 4-hydroxy-2-nonenal and malondialdehyde (MDA), which are highly reactive to proteins and are prone to generate a wide variety of inter- and intra-molecular adducts. It has been shown that in the case of major depressive disorders, there is a certain overproduction of ROS and/or a decrease in antioxidant status, which is correlated with increased susceptibility to lipid peroxidation [2, 3].

A statistically significant correlation between the severity of the depressive disorder and the alteration of superoxide dismutase (SOD) activity, especially on red blood cells, has been demonstrated in clinical trials [1], suggesting that SOD activity might represent a biomarker of major depressive disorder. At central level, the cellular changes due to overproduction of ROS and correlated with altered membrane composition [4] are manifested by neuronal degeneration. The neuronal degeneration is associated with neurodegenerative diseases and apoptosis.

Peripherally, an over-production of ROS is associated with the presence of significant changes in the composition of cell membranes (particularly the ratio cholesterol/phospholipids) and causes loss of cell membrane fluidity [5] but also modifies the membrane potential, leading to a possible membrane rupture [6–8]. Also, changing the levels of lipids in membranes might influence different neurotransmissions, particularly the one regarding the serotonergic system [9, 10].

The severity of the depressive disorder was also correlated with the change of ratio between arachidonic acid (AA) and eicosapentaenoic acid (EPA) in the composition of the red cell membrane of patients [11]. A higher ratio was observed in patients with severe depressive disorder, thus confirming that the pathology is correlated with an abnormal metabolism and/or reuptake of essential fatty acids.

The protective effects of selective serotonin reuptake inhibitors (SSRIs) against redox imbalance were widely investigated *in vivo*, especially in the case of fluoxetine. In an animal model with experimentally induced depression, fluoxetine significantly stimulated the plasmatic SOD and glutathione activity and significantly decreased the plasmatic MDA levels [12]. These results are consistent with those observed *in vitro*, where fluoxetine demonstrated cytoprotective effect on PC12 cells (cultured rat pheochromocytoma) against the neurotoxicity induced by hydrogen peroxide [13].

There are also studies that investigated the clinical relevance of protective antioxidant effect exhibited by SSRI antidepressants in patients with depressive disorders, but the results are inconclusive [2, 3, 14].

It was demonstrated that depressive disorder implies also a significant increase of immunoinflammatory markers, such as increased level of peripheral blood cells (leukocytes, monocytes, CD4+ T-cells) but also increased serum concentration of soluble interleukin-2 receptor [9, 15, 16]. There is also some evidence that the activation of the immune cells, especially polymorphonuclear cells, is related, at least partially, with the overproduction of ROS [17].

It was reported that antidepressants exert an inhibitory effect in the immune system [18]. Increased level of leukocytes and neutrophils are reduced by selective serotonin reuptake inhibitors (SSRIs) [19], while in other studies, SSRIs treatment inhibited the function of polymorphonuclear cells [20].

Some authors hypothesized that immune activation and lipid peroxidation are interrelated phenomena in major depression [1].

The aim of the present study was to assess the short-term effects exerted by three molecules used for their antidepressant properties (fluoxetine, sertraline and venlafaxine) on physiologic properties in two different study models: erythrocytes isolated from patients treated with the above-mentioned molecules and U937 cell line (monocytes). The selection of the cell models had in view that red blood cells are involved in the regulation of redox balance due to their specific metabolism. The second type of cells was selected because literature data proves the activation of immune cells in depressive disorders [9].

Fluoxetine and sertraline are SSRIs while venlafaxine is an antidepressant of serotonin-norepinephrine reuptake inhibitor (SNRI) class, the effect being dose-dependent. At low doses (<150 mg/day), it acts only on serotonergic transmission, while at moderate doses (>150 mg/day), it acts on both systems, whereas at high doses (>300 mg/day), it also affects dopaminergic neurotransmission [21].

Materials and Methods

Materials

HPLC (high performance liquid chromatography) grade sertraline hydrochloride (purity $\geq 98\%$, from Sigma, code S6319), venlafaxine hydrochloride (purity $\geq 98\%$, from Sigma, code V7264) and fluoxetine hydrochloride [USP (*United States Pharmacopeia*) reference standard from Fluka] were used for the *in vitro* stimulation of the cells.

The stock solutions (1 mg/mL of each antidepressant in methanol) were stable at least two weeks. RPMI 1640 (supplemented with L-glutamate) from Merck was used as the main medium for the cells suspension during all tests performed.

Bis-(1,3-dibutylbarbituric acid)trimethine oxonol [DiBAC₄(3)] was bought from Molecular Probes and was used as a fluorescent probe for the evaluation of transmembrane potential changes under the influence of the three antidepressants. DiBAC₄(3) stock solution (2 mM in DMSO – dimethyl sulfoxide) was kept at -20°C between different day experiments.

Cell culture

Experiments were performed using U937 cell line, from *European Collection of Cell Cultures*, at the 3th–4th passage. The U937 human macrophage cells were grown in RPMI 1640 supplemented with 10% heat-inactivated fetal calf serum (from Promega) at 37°C in 5% CO_2 atmosphere.

Prior to the experiments, cells were washed three times with phosphate buffer saline (pH 7) at 1200 rpm, for two minutes. After the last centrifugation, they were counted and standardized at 5×10^5 cells/mL to be used in aliquots of 2 mL in the fluorimeter cuvette. Results are the mean of three replicate determinations.

Study design

We recruited 21 female patients, 50 to 60-year-old, diagnosed with mental disorder (depressive disease), according to *Diagnostic and Statistical Manual of Mental Disorders Fourth Edition* (DSM-IV). Montgomery-Åsberg Depression Rating Scale (MADRS) was used in order to measure the severity of depression and to classify the patients in three subgroups:

- major depressive disorder ($n=4$, MADRS>34),
- moderate depressive disorder ($n=12$, MADRS 20–34) and
- mild depressive disorder ($n=5$, MADRS 12–19).

Results were matched against a group made up of 18 healthy subjects, 45 to 60-year-old. Patients with severe renal, diabetes, hepatic or hematological disease, overt cardiovascular disease or malignancy were excluded. All the patients in the study had illness history of more than six years, and were under treatment with sertraline 50 mg/day (38.09%), fluoxetine 20 mg/day (14.29%), venlafaxine 75 mg/day (47.62%) throughout the study (12 weeks). The treatment continued after the conclusion of the study.

Fasting venous blood samples drawn on Na₂EDTA (ethylenediaminetetraacetic acid disodium salt) as anticoagulant were used for the isolation of red blood cells. Separated erythrocytes were standardized at 1 g Hb (hemoglobin)/100 mL suspension and further used for the spectrophotometric assay of G-6-PDH (glucoso-6-phosphate dehydrogenase) and the erythrocyte susceptibility to lipid peroxidation (ESP) assay. Blood samples were collected at the beginning of the study as well as after 12 weeks of treatment; at the same timepoint, MADRS evaluation was performed.

The study was approved by the local Ethics Committee (from the “Prof. Dr. Alexandru Obregia” Clinical Hospital of Psychiatry, Bucharest, Romania) and informed consent was obtained from the patients and healthy subjects.

Methods

Transmembrane potential evaluation on U937 line

DiBAC₄(3) is a slow-response potential-sensitive probe that can enter depolarized cells, binds to intracellular proteins or membrane thus exhibiting enhanced fluorescence [22]. An increase in depolarization generates an additional influx of the anionic dye, resulting in the increase of fluorescence. Conversely, hyperpolarization

(increase in the absolute value of the membrane potential) is indicated by a decrease in fluorescence [23].

In a normalized suspension (2 μM probe concentration for 5×10^5 cells/mL), DiBAC₄(3)-labeled cells were excited at 493 nm and fluorescence emission spectra were collected within the range 500 to 600 nm. Fluorescence intensity was measured at 516 nm before and after 2, 15, 30, 45 and 60 minutes incubation of the cells with fluoxetine hydrochloride, sertraline hydrochloride and venlafaxine hydrochloride. A control sample of untreated cells was also evaluated similar to the samples, using methanol in the same amount as used to obtain a specific concentration of antidepressant and phosphate buffer saline.

The evaluation of cell transmembrane potential was performed using a LS 50B Perkin Elmer spectrofluorimeter equipped with thermostated cell holder and magnetic stirring.

The membrane potential was evaluated as absolute value intensity of the fluorescence signal at 516 nm.

Erythrocyte susceptibility to lipid peroxidation (ESP) on biological samples

ESP was determined by malondialdehyde (MDA) formation, using a modified technique proposed by Stocks, using the complexation with the thiobarbituric acid (TBA) [24, 25]. Briefly, red blood cells standardized (at 1 g Hb/100 mL) were incubated with hydrogen peroxide for one hour at 40°C in the presence of sodium azide (as inhibitor of catalase). After protein precipitation with trichloroacetic acid, the supernatant obtained after centrifugation was treated with TBA 1% solution. The optical density of each sample was read at 535 nm against TBA solution. TBA-reactive species (TBARS) were expressed in mM MDA/g Hb.

Determination of glucose-6-phosphate dehydrogenase (G-6-PDH) on biological samples

The activity of G-6-PDH was investigated using the method proposed Lohr & Waller [26], in which the NADPH formation is measured spectrophotometrically at 340 nm. Briefly, hemolyzed erythrocytes are incubated with triethanolamine buffer 50 mM (pH 7.5) and 30 mM NADP⁺ solution, for 5 minutes at 37°C. Then it was treated with 40 mM glucose-6-phosphate solution, and the optical density of each sample was read at 340 nm against the control (triethanolamine buffer and NADP⁺).

The activity of G-6-PDH was expressed as IU/g Hb.

Statistical analysis

Results are expressed as means \pm standard deviation (SD). Statistical analysis was performed using the Student's *t*-test. Differences were considered significant for $p < 0.05$.

Results

The aim of the present study was to assess the short-term effects exerted *in vitro* by the three antidepressants on transmembrane potential of U937 cell line, to investigate the parameters that influences the transmembrane potential, to establish a possible dose-effect correlation and to compare with the antioxidant potential exerted *in vitro* on erythrocytes isolated from treated patients.

In vitro experiments on U937 monocytes

Preliminary experiments on cell line

The preliminary experiments were focused on: (1) selection of the relevant cell line, (2) selection of the optimal sensitive probe and (3) setup the experimental parameters.

We selected human constitutively proliferating cell line (U937 monocytes) taking into account published studies that assessed on this cell line potential effects of different antipsychotic drugs [27]. The line is mimicking biochemical changes observed in schizophrenic disease, including increased activity of phospholipase A2 [28] or high levels of polyunsaturated fatty acids [29], both changes being correlated with an overproduction of ROS. Furthermore, the U937 cell line also corresponds to human peripheral leukocytes, therefore *in vitro* studies on this line could further be correlated with *in vivo* human studies.

Probe selection was made taking into account different factors such as the incorporation of the probe into the cell, the type of probe response and the cell toxicity induced by the probe.

The fluorochrome upload was checked microscopically in order to investigate the cellular distribution of a slow-response potential-sensitive probe as DiBAC₄(3). Figures of unstained cells (Figure 1) and stained cells (Figure 2) shown that DiBAC₄(3) had a cellular uniform distribution, without exclusively binding to the plasma membrane or the membranes of organelles, therefore is suitable to investigate transmembrane potential for this type of cells. The final concentration of the dye used [2 μL , corresponding to 100 nM DiBAC₄(3)] is similar to the procedures mentioned in the literature for assessment of changes in the transmembrane potential [30, 31].

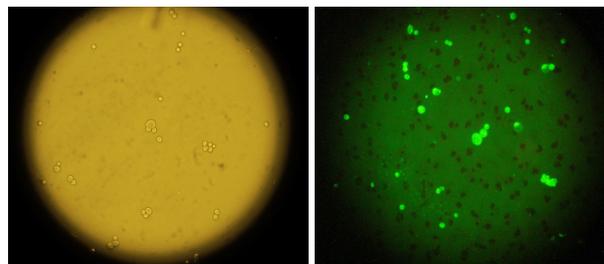


Figure 1 – Unstained cells.

Figure 2 – Cells after DiBAC₄(3) staining, showing the uniform distribution of the fluorescent marker.

Setup of the experimental model

For setting up the *in vitro* protocol, normalized suspensions were incubated for 2, 15, 30, 45 and 60 minutes with the 1 $\mu\text{g}/\text{mL}$ fluoxetine and fluorescence emission spectra of DiBAC₄(3)-labeled cells excited at 493 nm were collected within the range 500 to 600 nm. In order to exclude the effect induced by methanol (as fluoxetine was dissolved in this solvent); the effect induced by 1 μL of solvent was also tested. After stimulation with fluoxetine or methanol, an increase in the fluorescence intensity was observed, which in the case of DiBAC₄(3) corresponds to depolarization of the cell

membrane. The depolarization effect of fluoxetine on the membrane of U937 cells was much stronger than that induced by methanol (Figure 3). We noticed that the control fluorescence emission signals had constant values, thus indicating the fact that the depolarization is induced by the stimuli, and is not an effect of time lag.

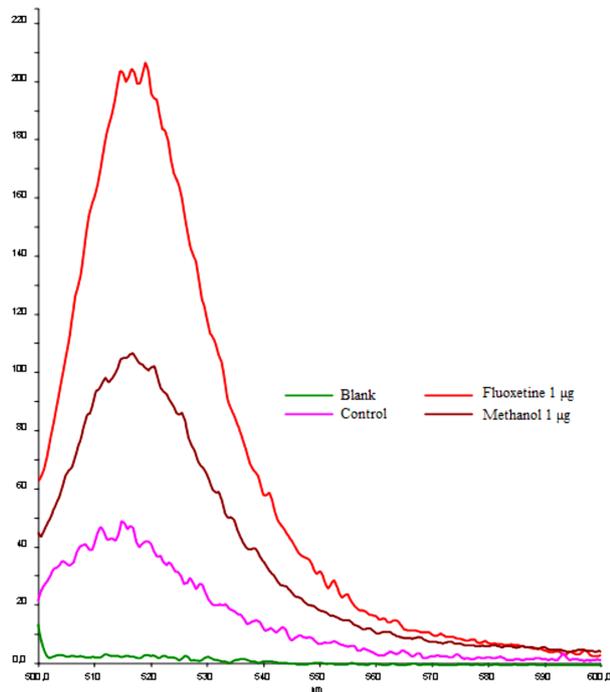


Figure 3 – Fluorescence uncorrected emission spectra of U937 cells labeled with DiBAC₄(3) (excitation at 493 nm).

When investigating the influence of incubation time (cell exposure) on the transmembrane potential changes (Figure 4), we noticed that the maximum depolarizing effect is obtained after incubation with the stimulus for 15 minutes, at room temperature; increased exposure to 30 minutes leads to a significant decrease of the effect on the transmembrane potential, while prolongation of the incubation up to 60 minutes does not induce any other significant change. The depolarizing effect of methanol remains constant throughout the period investigated. Based on these experimental results, further tests were performed using the 15 minutes incubation time.

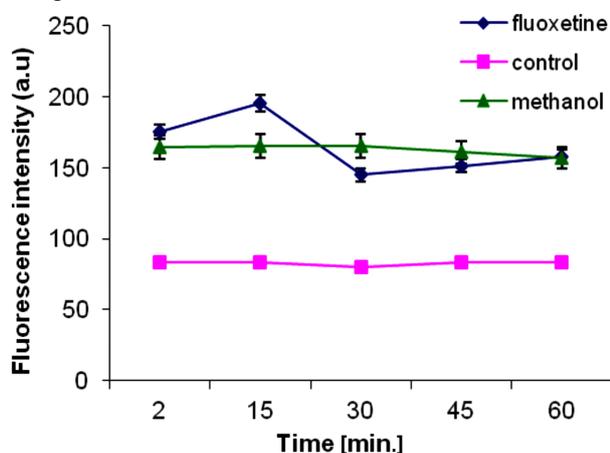


Figure 4 – Influence of the incubation time on the fluorescence emission signal intensity (excitation at 493 nm, emission at 516 nm).

Next, we studied the influence of fluoxetine concentration on the transmembrane potential of U937 cells, compared with methanol. Different volumes of fluoxetine hydrochloride, in a range of 0.01 to 10 µL stimuli/1 mL cell suspension (corresponding to 0.01–10 µg stimuli/5×10⁵ cells), were added on cells, while the incubation time used corresponds to 15 minutes.

The experimental results (Figure 5) suggest a similar behavior of the transmembrane potential under the influence of both stimuli (fluoxetine and methanol). At doses up to 3 µg/mL fluoxetine there is a marked depolarization of the cell membrane, while increasing the concentration over 3 µg/mL, we observed a marked decrease of depolarizing effect. At the highest concentration tested (10 µg/mL), depolarizing effect is still evident, but small. The range of 0.01–1 µg/mL fluoxetine is close to therapeutic levels; therefore, fluoxetine may exhibit a depolarizing effect also *in vivo*.

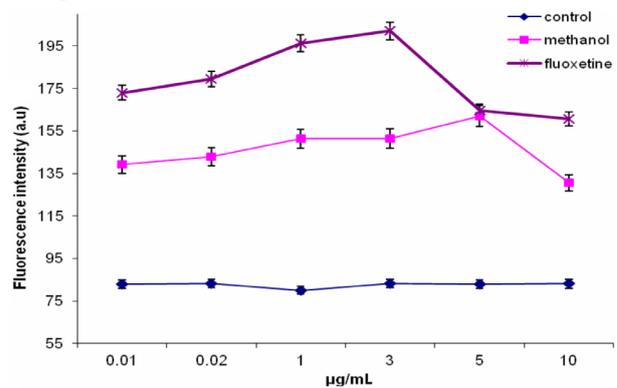


Figure 5 – Dose-dependent relative depolarizing effect of fluoxetine on U937 cells in the range of 0.01 to 10 µg stimulus/mL cell suspension.

In the case of methanol, we noted the relatively constant depolarizing effect at low to medium concentrations (up to 5 µg/mL), while increasing the dose to 10 µg/mL, a marked decrease of depolarizing effect was observed, similar to fluoxetine.

Comparative effects between antidepressants

The same preliminary studies, aimed at optimizing the parameters, which might influence the transmembrane potential changes, especially stimulus concentration and time of incubation, were conducted also on sertraline and venlafaxine. Similar to fluoxetine, sertraline and venlafaxine increased the fluorescence emission intensity of DiBAC₄(3), after membrane permeation, so they also depolarized the membrane of U937 cells. Comparing the intensity of effect (Figure 6), the depolarizing effect of fluoxetine is the strongest, while for venlafaxine it is the weakest.

Studying the influence of incubation time on the fluorescence signal intensity (Figure 6), for sertraline and venlafaxine we observed a behavior similar to fluoxetine. An incubation time of 15 minutes leads to a maximum fluorescence intensity, while the prolongation of incubation up to 60 minutes resulted in a decrease in signal intensity. These results confirm that the maximum depolarizing effect is obtained after 15 minutes incubation with stimuli.

Also, for these two antidepressants, we studied the influence of concentrations, in the range of 0.01–10 µg/mL

on the transmembrane potential. The experimental results (Figure 7) suggest a different behavior of transmembrane potential under the influence of two stimuli.

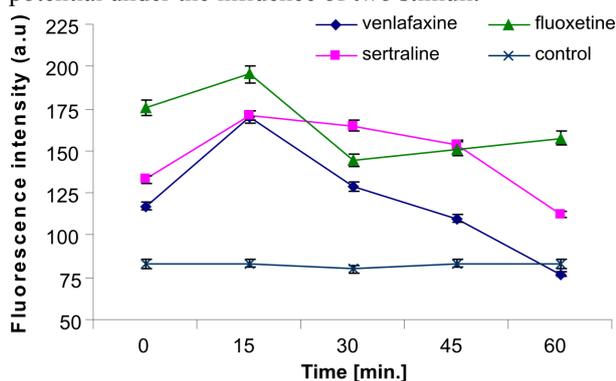


Figure 6 – Influence of the incubation time on the fluorescence emission signal intensity (excitation at 493 nm, emission at 516 nm).

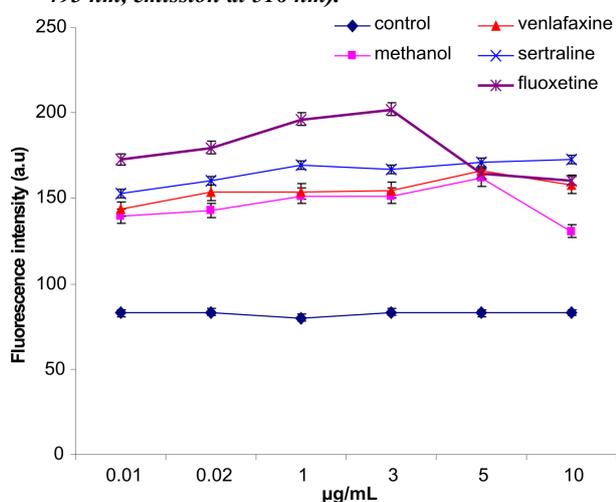


Figure 7 – Dose-dependent relative depolarizing effect of antidepressants on U937 cells.

For sertraline, while increasing the concentration up to 1 µg/mL, the depolarizing effect increased. Higher concentration leads to a decrease of the fluorescence intensity, up to 10 µg/mL, where the emission intensity of the dye is similar to that one of the unstimulated cells, thus suggesting the restoration of the membrane potential towards the initial value.

In the range investigated, venlafaxine presents the weakest depolarizing effect compared to sertraline and fluoxetine, respectively. The strongest effect was obtained at a concentration corresponding to 5 µg/mL, while for concentrations up to 10 µg/mL the intensity of the fluorescence emission signal of U937 cells labeled with DiBAC₄(3) was almost similar to the effect exhibited by methanol.

The constant responses obtained for control samples (unstimulated cells) in all measurements performed confirmed the absence of changes related to cell age, their variability (proliferating cells from a single source in the same environmental conditions) or the difference in cell concentrations.

In vitro experiments on erythrocytes isolated from treated patients

The selection of the cell models was made because

red blood cells are involved in the regulation of redox balance due to their specific metabolism. Moreover, they can be easily obtained, compared with the brain tissue or cerebrospinal fluid, from patients with depressive disorder.

The experimental results obtained at baseline and after 12 weeks of treatment for the subgroups of patients are presented in Table 1.

Table 1 – Experimental results on patients divided into subgroups depending on the initial MADRS score

Subgroups	Parameters	Baseline (mean value ± SD)	After 12 weeks (mean value ± SD)
Major depressive disorder (n=4)	G-6-PDH [IU]	11.43 ± 1.91 ^{a,b,c,d}	35.07 ± 0.48 ^a
	ESP	637.89	310.51
	[mM MDA/g Hb]	± 11.93 ^{a,d}	± 14.36 ^a
	MADRS score	35.25 ± 0.50	14.5 ± 0.70
Moderate depressive disorder (n=12)	G-6-PDH [IU]	21.44 ± 7.76 ^d	31.84 ± 3.9 ^a
	ESP	486.30	320.11
	[mM MDA/g Hb]	± 72.47 ^{a,d}	± 20.71 ^a
	MADRS score	25.25 ± 3.08	9.33 ± 1.21
Mild depressive disorder (n=5)	G-6-PDH [IU]	30.50 ± 7.43	34.05 ± 1.79
	ESP	322.93	310.34
	[mM MDA/g Hb]	± 7.41	± 4.69
	MADRS score	17.80 ± 0.45	9.5 ± 0.71
Control (n=18)	G-6-PDH [IU]	32.92 ± 8.99	
	ESP	316.23	
	[mM MDA/g Hb]	± 54.38	

G-6-PDH: Glucose-6-phosphate dehydrogenase; ESP: Erythrocyte susceptibility to lipid peroxidation; MDA: Malondialdehyde; Hb: Hemoglobin; MADRS: Montgomery–Åsberg Depression Rating Scale; SD: Standard deviation; ^a $p < 0.01$; ^bvs. baseline; ^cvs. moderate group; ^dvs. mild group; ^evs. control group.

On erythrocytes from patients with depressive disorder and from the control group, two parameters as ESP and G-6-PDH activity were assessed in a short-term study.

The analysis indicated that only for the subgroups of patients with major and moderate depressive disorder, the initial ESP values are significant higher compared with the control group, while the G-6-PDH activity is statistically significant lower compared with the control group ($p < 0.01$). Between subgroups, the initial ESP value of the subgroup with severe depressive disorder is statistically significant compared with moderate or mild groups ($p < 0.01$).

After 12 weeks, in the subgroup of patients with severe depressive disorder, ESP decreased significantly, while the G-6-PDH activity increased significantly, compared with the initial values of these two parameters ($p < 0.01$). The inverse correlation of the two parameters is obvious. In the subgroup of patients diagnosed with moderate depressive disorder the same trend was observed, slightly attenuated, but still statistically significant ($p < 0.01$), while in the subgroup of patients diagnosed with mild depressive disorder, both parameters (ESP and G-6-PDH) varied, but the differences were not statistically significant ($p > 0.05$).

The results suggest that the depression severity (as assessed by MADRS score) correlates with the increased value of ESP and inversely correlates with the G-6-PDH activity. This correlation between ESP and G-6-PDH is less obvious at the end of the study.

The initial G-6-PDH activity of the subgroup with severe depressive disorder is statistically significant lower compared with the patients with moderate or mild depressive disorder ($p < 0.01$). After 12 weeks, the highest increase of G-6-PDH activity was observed in patients with major depressive disorder, statistically significant compared with the baseline, and much less for other subgroups.

Discussion

Starting from the hypothesis that immune activation and lipid peroxidation are interrelated phenomena in major depression, the aim of our study was to investigate if three molecules used for their antidepressant properties (fluoxetine, sertraline and venlafaxine) have effects on both systems.

We used an *in vitro* "surrogate" for human peripheral leukocytes (the U937 monocytes cell line) to investigate the transmembrane potential, while the antioxidant potential was observed *in vitro* on red blood cells isolated from treated patients, on a short-term study.

Our experiments carried out on U937 cell suspensions using as stimuli solutions of fluoxetine, sertraline and venlafaxine in methanol revealed the depolarization of the cell membrane induced by antidepressants. The depolarization effect induced by antidepressants on U937 cells is maximal after 15 minutes incubation at room temperature and decreases when prolonging the incubation time.

The depolarizing effects of fluoxetine, sertraline or venlafaxine are also described in literature, but on other type of cells, like neurons [32] or ventricular myocytes [33], implying other mechanisms as inhibition of the sodium voltage-dependent channels [34], potassium or calcium channels [35].

In treated patients, therapeutic serum concentrations of fluoxetine correspond to 0.15–0.5 $\mu\text{g/mL}$, for sertraline to 0.05–0.25 $\mu\text{g/mL}$ while for venlafaxine are up to 0.163 $\mu\text{g/mL}$ [36]. The range investigated (0.01–1 $\mu\text{g/mL}$) is close to therapeutic levels; therefore, the compounds may exhibit a depolarizing effect also *in vivo*.

The depolarizing effect is concentration-dependent, but not in a linear manner and is also dependent on the compound. The concentration-effect profile of fluoxetine shows a marked depolarization effect exercised over U937 promyelocytic cells at 3 $\mu\text{g/mL}$, while 5 $\mu\text{g/mL}$ induced a marked decrease of depolarizing effect. At the highest concentration tested (10 $\mu\text{g/mL}$), depolarizing effect is still evident, but small.

For sertraline, increasing the concentration up to 1 $\mu\text{g/mL}$ results in an increased depolarizing effect exercised over U937 promyelocytic. Higher concentration (such as 3 $\mu\text{g/mL}$) leads to a decrease of the depolarizing effect, while increasing the stimulus up to 10 $\mu\text{g/mL}$ leads to a restoration of the membrane potential.

The strongest depolarizing effect of venlafaxine on U937 cells labeled with DiBAC₄(3) was obtained for 5 $\mu\text{g/mL}$, while concentrations up to 10 $\mu\text{g/mL}$ had an effect almost similar with the one exhibited by methanol (the solvent used for all antidepressants).

In the range investigated, fluoxetine induced the strongest depolarizing effect compared to sertraline and venlafaxine, respectively.

Previously published data correlate the response of

some cells (Jurkat, U937) to natural compounds or physiopathological conditions to the changes of transmembrane potential [37, 38].

Depolarizing effect exhibited by the antidepressants on U937 cells, which corresponds to human peripheral leukocytes, at concentrations close with those achieved therapeutically indicate a potential direct impact on the immune function, otherwise stimulated in depressive disorder. The effect is also exhibited even at concentrations corresponding to overdosage. Results are also in line with published *in vitro* studies that suggested a potent apoptotic activity of SSRI on different cell cultures [39, 40].

Pellegrino & Bayer also demonstrated *in vivo* that fluoxetine suppressed in rats the lymphocyte responses, not *via* direct immunomodulation but through its impact on the serotonergic axis [41]. It looks like antidepressants exhibit multiple modes of action on the immune system, at least at the leukocytes level, taking into account also the influence of SSRI on the 5-HT (5-hydroxytryptamine) transporter in human lymphocytes [42]. Further biophysical studies on U937 cells, like cell fluidity assay or measurement of membrane peroxides could fully elucidate the mechanisms of action.

Our *in vitro* experiments on erythrocytes isolated from treated patients investigated the antioxidant potential of the three antidepressants. The study did not investigate the clinical efficiency of the antidepressants, because the duration of treatment is not relevant for this kind of pathology.

The study was conducted on erythrocytes, because, similar to neurons, these are susceptible to oxidative challenge and, moreover, can be easily obtained, compared with the brain tissue or cerebrospinal fluid from patients with depressive disorder. It was suggested [3] that changes in the antioxidant defense of erythrocytes might reflect similar changes in other cells and tissues.

Our data indicate that only for the subgroups of patients with major and moderate depressive disorder the initial ESP values are statistically significant higher, while the G-6-PDH activity is statistically significant lower, both parameters being compared with the control group. Between subgroups, the initial ESP value of the subgroup with severe depressive disorder is statistically significant compared with moderate or mild groups ($p < 0.01$). Increased ESP values observed in the present study, especially for subgroups with severe and moderate disorder, is in line with other published investigation [3, 43], which reported high MDA levels in plasma of severe depressive patients or increased 4-hydroxy-2-nonenal.

After 12 weeks of treatment, in the subgroup of patients with severe depressive disorder, ESP decreased statistically significant, while the G-6-PDH activity increased significantly, compared with the initial values of these two parameters ($p < 0.01$). In the subgroup of patients diagnosed with moderate depressive disorder the same trend was observed, slightly attenuated, but still statistically significant ($p < 0.01$), while in the subgroup of patients diagnosed with mild depressive disorder, both parameters (ESP and G-6-PDH) varied, but the differences were not statistically significant ($p > 0.05$).

After analyzing the dynamics of both parameters, we

suggest that in patients with depressive disorder, erythrocyte membrane is more rigid, with less deformability and a short lifetime. These changes are caused to the fact that the erythrocytes are subjected to a chronic oxidative stress, so that deposits of lipophilic antioxidants that protect the membrane are exhausted. The *in vitro* results can be interpreted as the lipid-soluble antioxidant defense induced by treatment with the antidepressants (SSRI or SNRI) is more evident in the case of severe pathology and less evident in the case of moderate or minor disorder. There are several limitations of the study particularly on the number of the patients and the heterogeneity of subgroups (as age and size).

☒ Conclusions

All selected antidepressants at therapeutic concentrations exhibit a depolarization effect on U937 cells. The effect is concentration-dependent, in a non-monotonic manner. Fluoxetine exhibits the strongest effect compared to sertraline and venlafaxine, respectively. The results can be extrapolated to *in vivo*, antidepressants probably stimulating the leukocytes apoptosis. The lipid-soluble antioxidant defense, assessed on erythrocytes, is induced by the antidepressants even on short-term treatment and is most evident in the case of severe pathology. Our results could indicate that selected antidepressants at therapeutic concentrations, besides their known pharmacological effects, exhibit a protective effect against oxidative stress and also influence cells with the immune potential.

Conflict of interests

The authors declare that they have no conflict of interests.

References

- [1] Bilici M, Efe H, Koroğlu MA, Uydu HA, Bekaroğlu M, Değer O. Antioxidative enzyme activities and lipid peroxidation in major depression: alterations by antidepressant treatments. *J Affect Disord*, 2001, 64(1):43–51.
- [2] Khanzode SD, Dakhale GN, Khanzode SS, Saoji A, Palasodkar R. Oxidative damage and major depression: the potential antioxidant action of selective serotonin re-uptake inhibitors. *Redox Rep*, 2003, 8(6):365–370.
- [3] Sarandol A, Sarandol E, Eker SS, Erdinc S, Vatansever E, Kiri S. Major depressive disorder is accompanied with oxidative stress: short-term antidepressant treatment does not alter oxidative-antioxidative systems. *Hum Psychopharmacol*, 2007, 22(2):67–73.
- [4] Baghai TC, Eser D, Möller HJ. Effects of different antidepressant treatments on the core of depression. *Dialogues Clin Neurosci*, 2008, 10(3):309–320.
- [5] Papas AM. Determinants of antioxidant status in humans. *Lipids*, 1996, 31(Suppl):S77–S82.
- [6] Esterbauer H, Schaur RJ, Zollner H. Chemistry and biochemistry of 4-hydroxynonenal, malonaldehyde and related aldehydes. *Free Radic Biol Med*, 1991, 11(1):81–128.
- [7] Androutsopoulos VP, Papakyriakou A, Vourloumis D, Tsatsakis AM, Spandidos DA. Dietary flavonoids in cancer therapy and prevention: substrates and inhibitors of cytochrome P450 CYP1 enzymes. *Pharmacol Ther*, 2010, 126(1):9–20.
- [8] Veskokouk AS, Tsatsakis AM, Kouretas D. Dietary oxidative stress and antioxidant defense with an emphasis on plant extract administration. *Cell Stress Chaperones*, 2012, 17(1):11–21.
- [9] Maes M, Smith R, Christophe A, Cosyns P, Desnyde R, Meltzer H. Fatty acid composition in major depression: decreased ω 3 fractions in cholesteryl esters and increased C20:4 ω 6/C20:5 ω 3 ratio in cholesteryl esters and phospholipids. *J Affect Disord*, 1996, 38(1):35–46.
- [10] Engelberg H. Low serum cholesterol and suicide. *Lancet*, 1992, 339(8795):727–729.
- [11] Adams PB, Lawson S, Sanigorski A, Sinclair AJ. Arachidonic acid to eicosapentaenoic acid ratio in blood correlates positively with clinical symptoms of depression. *Lipids*, 1996, 31(Suppl):S157–S161.
- [12] Zafir A, Banu N. Antioxidant potential of fluoxetine in comparison to *Curcuma longa* in restraint-stressed rats. *Eur J Pharmacol*, 2007, 572(1):23–31.
- [13] Kolla N, Wei Z, Richardson JS, Li XM. Amitriptyline and fluoxetine protect PC12 cells from death induced by hydrogen peroxide. *J Psychiatry Neurosci*, 2005, 30(3):196–201.
- [14] Yanik M, Erel O, Kati M. The relationship between potency of oxidative stress and severity of depression. *Acta Neuropsychiatrica*, 2004, 16(4):200–203.
- [15] Değer O, Bekaroğlu M, Orem A, Orem S, Uluutku N, Soylu C. Polymorphonuclear (PMN) elastase levels in depressive disorders. *Biol Psychiatry*, 1996, 39(5):357–363.
- [16] Seidel A, Arolt V, Hunstiger M, Rink L, Behnisch A, Kirchner H. Increased CD56+ natural killer cells and related cytokines in major depression. *Clin Immunol Immunopathol*, 1996, 78(1):83–85.
- [17] Whittemore ER, Loo DT, Watt JA, Cotman CW. A detailed analysis of hydrogen peroxide-induced cell death in primary neuronal culture. *Neuroscience*, 1995, 67(4):921–932.
- [18] Müller N. Psychoneuroimmunology: implications for the drug treatment of psychiatric disorders. *CNS Drugs*, 1995, 4(2):125–140.
- [19] Maes M, Vandoolaeghe E, Van Hunsel F, Bril T, Demedts P, Wauters A, Neels H. Immune disturbances in treatment-resistant depression: modulation by antidepressant treatments. *Hum Psychopharmacol Clin Exp*, 1997, 12(2):153–162.
- [20] Bekaroğlu M, Bilici M, Değer O, Karahan SC, Örem A, Soylu C. Effect of antidepressant treatment on acute phase protein levels in patients with depression. *Turk J Psychiatry*, 1997, 8(4):260–265.
- [21] Redrobe JP, Bourin M, Colombel MC, Baker GB. Dose-dependent noradrenergic and serotonergic properties of venlafaxine in animal models indicative of antidepressant activity. *Psychopharmacology (Berl)*, 1998, 138(1):1–8.
- [22] Epps DE, Wolfe ML, Groppi V. Characterization of the steady-state and dynamic fluorescence properties of the potential-sensitive dye bis-(1,3-dibutylbarbituric acid)trimethine oxonol (Dibac4(3)) in model systems and cells. *Chem Phys Lipids*, 1994, 69(2):137–150.
- [23] Klapperstück T, Glanz D, Klapperstück M, Wohlrab J. Methodological aspects of measuring absolute values of membrane potential in human cells by flow cytometry. *Cytometry A*, 2009, 75(7):593–608.
- [24] Stocks J, Offerman EL, Modell CB, Dormandy TL. The susceptibility to autoxidation of human red cell lipids in health and disease. *Br J Haematol*, 1972, 23(6):713–724.
- [25] Ilie M, Margină D. Chapter 5: Trends in the evaluation of lipid peroxidation processes. In: Catala A (ed). *Lipid peroxidation*, InTechOpen, Rijeka, Croatia, 2012, 111–130.
- [26] Lohr GW, Waller HD. Glucose-6-phosphate dehydrogenase. In: Bergmeyer HU (ed). *Methods of enzymatic analysis*. 2nd edition, Academic Press, New York, 1974, 635–636.
- [27] Heiser P, Enning F, Krieg JC, Vedder H. Effects of haloperidol, clozapine and olanzapine on the survival of human neuronal and immune cells *in vitro*. *J Psychopharmacol*, 2007, 21(8):851–856.
- [28] Gattaz WF, Hübner CV, Nevalainen TJ, Thuren T, Kinnunen PK. Increased serum phospholipase A2 activity in schizophrenia: a replication study. *Biol Psychiatry*, 1990, 28(6):495–501.
- [29] Obermeier H, Hrboticky N, Sellmayer A. Differential effects of polyunsaturated fatty acids on cell growth and differentiation of premonocytic U937 cells. *Biochim Biophys Acta*, 1995, 1266(2):179–185.
- [30] Eisinger J. Membrane fluidity and diffusive transport. In: Jameson DM, Reinhart GD (eds). *Fluorescent biomolecules: methodologies and applications*. Plenum Press, New York, 1989, 151–171.
- [31] Hovius R, Vallotton P, Wohland T, Vogel H. Fluorescence techniques: shedding light on ligand-receptor interactions. *Topics Pharmacol Sci*, 2000, 21(7):266–273.

- [32] Pancrazio JJ, Kamatchi GL, Roscoe AK, Lynch C 3rd. Inhibition of neuronal Na⁺ channels by antidepressant drugs. *J Pharmacol Exp Ther*, 1998, 284(1):208–214.
- [33] Khalifa M, Daleau P, Turgeon J. Mechanism of sodium channel block by venlafaxine in guinea pig ventricular myocytes. *J Pharmacol Exp Ther*, 1999, 291(1):280–284.
- [34] Lenkey N, Karoly R, Kiss JP, Szasz BK, Vizi ES, Mike A. The mechanism of activity-dependent sodium channel inhibition by the antidepressants fluoxetine and desipramine. *Mol Pharmacol*, 2006, 70(6):2052–2063.
- [35] Deák F, Lasztóczy B, Pacher P, Petheö GL, Kecskeméti V, Spät A. Inhibition of voltage-gated calcium channels by fluoxetine in rat hippocampal pyramidal cells. *Neuropharmacology*, 2000, 39(6):1029–1036.
- [36] Moffat AC, Osselton MD, Widdop B. Clarke's analysis of drugs and poisons. 4th edition, Pharmaceutical Press, 2011, 1423–2223.
- [37] Margina D, Gradinaru D, Manda G, Neagoe I, Ilie M. Membranar effects exerted *in vitro* by polyphenols – quercetin, epigallocatechin gallate and curcumin – on HUVEC and Jurkat cells, relevant for diabetes mellitus. *Food Chem Toxicol*, 2013, 61:86–93.
- [38] Margina D, Ilie M, Manda G, Neagoe I, Mocanu M, Ionescu D, Gradinaru D, Ganea C. Quercetin and epigallocatechin gallate effects on the cell membranes biophysical properties correlate with their antioxidant potential. *Gen Physiol Biophys*, 2012, 31(1):47–55.
- [39] Levkovitz Y, Gil-Ad I, Zeldich E, Dayag M, Weizman A. Differential induction of apoptosis by antidepressants in glioma and neuroblastoma cell lines: evidence for p-c-Jun, cytochrome c, and caspase-3 involvement. *J Mol Neurosci*, 2005, 27(1):29–42.
- [40] Chen S, Xuan J, Wan L, Lin H, Couch L, Mei N, Dobrovolsky VN, Guo L. Sertraline, an antidepressant, induces apoptosis in hepatic cells through the mitogen-activated protein kinase pathway. *Toxicol Sci*, 2014, 137(2):404–415.
- [41] Pellegrino TC, Bayer BM. Specific serotonin reuptake inhibitor-induced decreases in lymphocyte activity require endogenous serotonin release. *Neuroimmunomodulation*, 2000, 8(4):179–187.
- [42] Gordon J, Barnes NM. Lymphocytes transport serotonin and dopamine: agony or ecstasy? *Trends Immunol*, 2003, 24(8):438–443.
- [43] Selly ML. Increased (*E*)-4-hydroxy-2-nonenal and asymmetric dimethylarginine concentrations and decreased nitric oxide concentrations in the plasma of patients with major depression. *J Affect Disord*, 2004, 80(2–3):249–256.

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Received: September 30, 2014

Accepted: September 16, 2015