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Effects of Duloxetine on Oxidant-Antioxidant System in Rat Brain Tissues

Duloksetinin Rat Beyin Dokularındaki Oksidan-Antioksidan Sistem Üzerine Etkisi

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ABSTRACT

Objective: *After the relationship between depression and oxidative stress (OS) was demonstrated, the effect of antidepressant drugs on OS has become important. In this study, we aimed to determine the effects of the antidepressant duloxetine on the activities of the superoxide dismutase (SOD), catalase (CAT), adenosine deaminase (ADA), xanthine oxidase (XO) and glutathione peroxidase (GSH-Px) enzymes as well as the lipid peroxidation (LP) product malondialdehyde (MDA) and nitric oxide (NO) levels in rat brains.*

Material and Method: *Twenty male Sprague-Dawley rats were used for the study. The first group was the control group (n=10) and the second group was the duloxetine group (n=10). Duloxetine was administered intragastrically once a day at a dose of 10 mg/kg for two weeks in the second group. Water was administered intragastrically once a day for two weeks in the first group. Rats were sacrificed at the end of the fourteenth day. The brain tissues were collected and then analyzes were performed.*

Results: *As a result of this study, we found that duloxetine increased the SOD (P=0.026) activity and decreased the ADA (P=0.041), XO (P=0.034) and CAT (P=0.006) activities significantly compared to the control group. We also found an increase in the GSH-Px enzyme activity and decrease in the NO and MDA levels at non-significant rates in the duloxetine group brain tissues.*

Conclusion: *The significant increase in the activity of the antioxidant enzyme SOD, the significant decrease in the activities of the XO and ADA enzymes, which can cause the formation of reactive oxygen products in the organism, and the insignificant decrease in the LP indicator MDA suggest that duloxetine can positively change the antioxidant status in rat brain tissues.*

ÖZET

Amaç: *Oksidatif stres (OS) ve depresyon arasındaki ilişki gösterildikten sonra antidepresanların OS üzerine etkisi önemli hale gelmiştir. Bu çalışmanın amacı duloksetinin rat beyin dokularındaki katalaz (CAT), süperoksit dismutaz (SOD), adenozin deaminaz (ADA), ksantin oksidaz (XO) ve glutatyon peroksidaz (GSH-Px) enzim aktiviteleri ile nitrik oksit (NO) ve malondialdehid (MDA) düzeylerine etkilerini araştırmaktır.*

Gereç ve Yöntem: *Çalışmaya birinci grup kontrol grubu (n=10) ve ikinci grup duloksetin grubu (n=10) olmak üzere toplam yirmi tane Sprague-Dawley cinsi erkek rat alındı. Duloksetin grubuna günde bir defa 10 mg/kg dozunda intragastrik yoldan iki hafta süreyle duloksetin verildi. Kontrol grubuna da günde bir defa iki hafta süreyle intragastrik olarak su verildi. On beşinci günde tüm ratlar sakrifiye edilerek beyin dokuları çıkarıldı ve incelemeler yapıldı.*

Bulgular: *Çalışmamızda duloksetinin rat beyin dokularında kontrol grubuna kıyasla SOD (P=0,026) enzim aktivitesini anlamlı düzeyde artırdığını ve XO (P=0,034), ADA (P=0,041) ve CAT (P=0,006) enzim aktivitelerini anlamlı düzeyde azalttığını saptadık. Ayrıca GSH-Px enzim aktivitesini anlamlı olmayan düzeyde artma ve MDA ile NO düzeylerinde ise anlamlı olmayan düzeyde azalma saptadık.*

Sonuç: *Çalışmamızda antioksidan bir enzim olan SOD enzim aktivitesinin anlamlı düzeyde artması, organizmada reaktif oksijen ürünleri oluşumuna neden olabilen XO ve ADA enzim aktivitelerinin anlamlı düzeyde azalması ve LP göstergesi olan MDA'nın artmayıp, anlamlı düzeyde olmasa da azalması duloksetinin antioksidan durumu olumlu yönde değiştirebileceğini düşündürmektedir.*

Keywords:
Duloxetine
Adenosine deaminase
Superoxide dismutase
Xanthine oxidase
Malondialdehyde
Catalase

Anahtar Kelimeler:
Duloksetin
Adenozin deaminaz
Süperoksit dismutaz
Ksantin oksidaz
Malondialdehit
Katalaz

INTRODUCTION

Reactive oxygen species (ROS) are continuously produced by all body tissues, especially during oxidative phosphorylation (1). Under physiological conditions, these ROS are eliminated by cellular antioxidant mechanisms. However, under pathological conditions, there is a shift towards an oxidative state due to an increase in oxidant markers, a decrease in antioxidant mechanisms, or both (2). Oxidative stress (OS), defined as the disruption of this balance in the oxidant-antioxidant system in favor of the

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oxidant system (3). A persistent increase in OS can lead to cell and tissue damage (2). In particular, the brain is more sensitive to ROS due to its high lipid content and high energy demand (4).

Evidence, especially in the last two decades, reveals that an imbalance between the oxidant system and antioxidant defenses is involved in the pathogenesis of depression (5). For example, studies in patients with depression have indicated that increased ROS production and decreased antioxidative defense systems are responsible for the altered brain structure of these patients. (6,7). The mechanisms of action of antidepressants are still not fully understood despite their use for many years. The hypothesis that these drugs regulate noradrenergic and serotonergic neurotransmitter systems has been dominant until today (8). However, recent studies have shown that, these drugs also have antioxidant effects, based on which a new concept of antidepressant mechanism of action has been proposed (9).

Duloxetine is a drug, that acts by inhibiting the reuptake of both serotonin and noradrenaline in the central nervous system (CNS) (10). The positive role of duloxetine in the activation of antioxidant defense and its anti-inflammatory properties have been demonstrated in some studies (11-14). In an animal study, duloksetin significantly increased the expression of antioxidant enzyme Cu-Zn-SOD in gerbil hippocampal pyramidal neurons (14), in a cell culture study conducted with PC 12 neuronal cells, duloxetine increased the GSH-Px enzyme levels (11), in a mice study, duloxetine reduced the stressor-induced increased brain MDA levels, and increased the SOD and CAT enzyme levels (15), in an another rat study, it was found that duloxetine reversed the hippocampal methamphetamine-induced increased MDA levels and decreased SOD and GSH-Px enzyme activities (16). No study has been found in the literature examining the effects of duloxetine on ADA and XO enzymes. The data obtained from these studies suggest that duloxetine may activate mitochondrial antioxidant systems and plays a role in neuroprotection against some neurotoxic agents. However, there are very few studies directly demonstrating this neuroprotective effect. We thought that, in addition to its effect on neurotransmitters such as serotonin and noradrenaline, duloxetine may have positive effects on the oxidant-antioxidant system balance by strengthening antioxidant defense systems. In this study, we aimed to investigate the effects of duloxetine on SOD, XO, GSH-Px, ADA and CAT activities, which are enzymes related to the oxidant-antioxidant system, MDA levels, which is an indicator of LP, and NO levels, an inorganic radical, in rat brain tissues.

MATERIAL AND METHOD

This study was carried out in Süleyman Demirel University Faculty of Medicine Experimental Animal Research Laboratory and Department of Medical Biology Laboratory. The study was approved by the Local Ethics Committee for Animal Experiments of Süleyman Demirel University Faculty of Medicine (decision no. 04 dated 27.05.2010) and supported by Süleyman Demirel University Scientific Research Projects Management Unit under project no. 2183-TU-10.

Experimental Animals

The total number of animals included the study (sample size) was calculated by using the resource equation method (17). In this study, a total of 20 male Sprague-Dawley rats aged 8-12 weeks and weighing 200-250 g were used. They were obtained from Süleyman Demirel University Faculty of Medicine Experimental Animal Research Laboratory Production Unit. During the experiment, the rats were kept under standard light, humidity, and temperature (25º C) conditions. Feed and water were not restricted throughout the experiment. They were divided into 2 groups as control group and duloxetine experimental group. In the duloxetine experimental group, duloxetine was dissolved in water and administered intragastrically once daily at a dose of 10 mg/kg for two weeks. The control group was given intragastric water at a single dose of 10 ml/kg for two weeks (18). The weight of the animals was monitored every three days and the drug dose was adjusted accordingly.

Anesthesia and Tissue Samples

Feeding of all rats was discontinued overnight except water, and anesthesia was induced by i.p. administration of a ketamine hydrochloride (90 mg/kg) and xylazine (10 mg/kg) mixture approximately 24 hours after the last treatment, i.e., on the 15th day of the experiment.

All rats were sacrificed after anesthesia and brain tissues were removed. The brain tissue samples were stored at -20°C.

Preservation, Homogenization and Preparation of Samples for Experiment

Brain tissue samples were homogenized in 50 mM Tris-HCl buffer (pH 7.4) and transferred to glass tubes while maintaining their cold temperatures. After that, the samples were centrifuged at 16 000 rpm for 3 minutes. The homogenates were placed in Eppendorf tubes without increasing the temperature, and the NO, MDA and protein levels were determined. The homogenates were centrifuged again at 5000 rpm at +4°C for 30 minutes, and the supernatant was obtained. ADA, XO, and CAT detection assays were performed. The supernatants were diluted and vortexed with chloroform/ethanol to 1/1 (v/v) and then centrifuged at 3200 rpm at +4°C for 40 minutes. Protein levels and GSH-Px, SOD activities were determined on the supernatant.

Detection of Catalase Activity

The rate of degradation of hydrogen peroxide (H2O2) by CAT was measured spectrophotometrically by Aebi's method using the light absorption of H2O2 at a wavelength of 240 nm (19). The results obtained were calculated as k/gr protein.

Detection of Glutathione Peroxidase Activity

GSH-Px catalyzes the oxidation of reduced glutathione to oxidized glutathione. Oxidized glutathione is converted to reduced glutathione with the help of glutathione reductase and NADPH. The GSH-Px activity was calculated by measuring the change in absorbance at 340 nm due to the decrease in NADPH. The activity was recorded as units per gram of protein (U/gr) (20).

Detection of Superoxide Dismutase Activity

Detection of SOD activity is based on the inhibition by SOD of the reduction of nitroblue tetrazolium by superoxide anions in the medium. The resulting superoxide radicals

Table 1: SOD, CAT, XO, ADA and GSH-Px activities and MDA and NO levels in rat brain tissues.

Groups	SOD (U/mg protein)	CAT (k/g protein)	GSH-Px (U/g protein)	MDA (nmol/g protein)	NO (µmol/g protein)	XO (U/g protein)	ADA (U/g protein)
I-Control (n=10)	0.736 ± 0.120	0.083 ± 0.013	68.52 ± 17.40	15.78 ± 0.617	0.244 ± 0.089	0.454 ± 0.093	0.320 ± 0.040
II-Duloxetine (n=10)	0.858 ± 0.153	0.069 ± 0.005	86.24 ± 22.19	15.05 ± 1.123	0.168 ± 0.083	0.342 ± 0.106	0.276 ± 0.038
P values	0.026	0.006	0.089	0.112	0.140	0.034	0.041

The results are shown in arithmetical values mean ± standard deviation. SOD: Superoxide dismutase, CAT: Catalase, ADA: Adenosine deaminase, XO: Xanthine oxidase, GSH-Px: Glutathione peroxidase, MDA: Malondialdehyde, NO: Nitric oxide

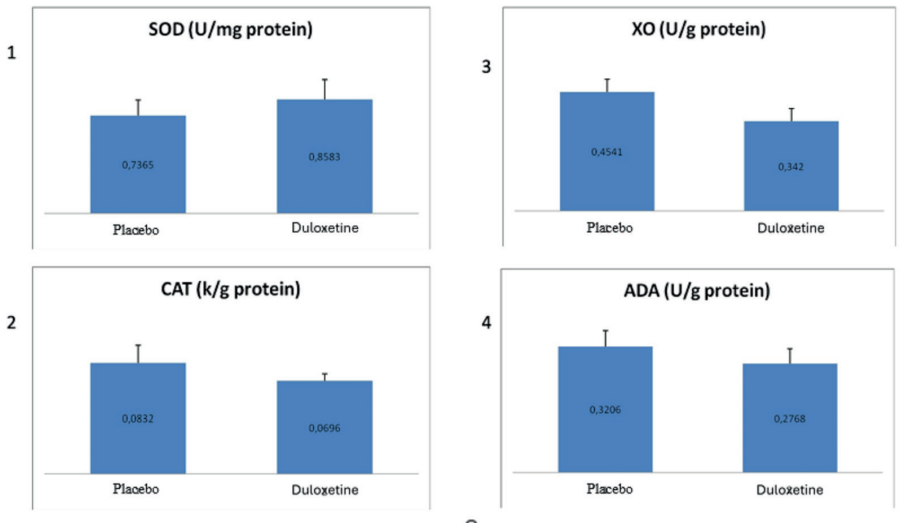

Figure 1: The duloxetine group was compared with the control (placebo) group. 1. The SOD activity was higher in duloxetine group (p= 0.026). 2. The CAT activity was lower in duloxetine group (p= 0.006). 3. The XO activity was lower in duloxetine group (p= 0.034). 4. The ADA activity was lower in duloxetine group (p= 0.041).

reduce nitroblue tetrazolium in the medium and form a colored complex. This complex formation was measured in a spectrophotometer at a wavelength of 560 nm. When SOD is present in the medium, there is no reduction, and a light color is observed in relation to the activity of the enzyme. The results obtained were calculated as U/mg protein (21).

Detection of Adenosine Deaminase Activity

The absorbance of ammonia by the reaction of ADA with adenosine was measured with a spectrophotometer at a wavelength of 628 nm. The results obtained were calculated as U/gr protein (22).

Detection of Xanthine Oxidase Activity

The absorbance of uric acid formed from xanthine was determined spectrophotometrically at a wavelength of 293 nm. The results were calculated as U/gr protein (23).

Detection of Nitric Oxide Levels: The NO levels in the tissues were measured by the Griess method (24). The results obtained were calculated as µmol/g protein.

Detection of Malondialdehyde Levels

Increased free radical formation at the end of LP was measured using the method of Draper and Hadley (25). MDA reacts with thiobarbituric acid to form a colored complex with maximum absorbance at 532 nm. The MDA concentration was calculated as nmol/g protein.

Protein Detection in Samples: The protein content was measured using bovine serum with albumin as the standard. This method is a combination of the biuret reaction and the Folin-Ciocalteau reaction under alkaline conditions. Formation of a dark blue color is characteristic. The darkness of the color is directly proportional to the protein concentration in the medium (26).

Statistical analysis

A Windows-compatible computer program SPSS 9.0 (SPSS, Inc., Chicago, IL, USA) was used for statistical analysis. Since the distributions of the groups were not normal, the Mann-Whitney U test, one of the non-parametric tests, was used to compare the groups. Values are given as arithmetic mean ± standard deviation and for statistical significance; $p<0.05$ values were considered significant.

RESULTS

Table 1 shows the SOD, CAT, GSH-Px, XO and ADA activities as well as the NO and MDA levels in the rat brain tissues of the two groups in our study. Duloxetine group revealed a statistically significant decrease in the CAT (p=0.006), XO (p=0.034) and ADA (p=0.041) activities, whereas a statistically significant increase was shown in the SOD activity (p=0.026), compared to the control group. There was a statistically non-significant decrease in the MDA and NO levels and a statistically non-significant increase in the GSH-Px activity in the duloxetine group compared to the control group. The graphical representation of the SOD, CAT, XO and ADA activities measured in the brain tissues are given in Figure 1, respectively.

DISCUSSION

With regard to the etiology of depression, evidence from the last two decades reveals an imbalance between the oxidant system and antioxidant defenses (5). Therefore, another potential target of antidepressant drug regulation should be intracellular antioxidant enzymes because antioxidant enzymes act to lower the OS levels of cells by scavenging free radicals, thereby working to prevent cell damage and neuronal death (27). Antioxidant enzymes are important for the brain as the brain is more vulnerable to OS (5). This study is the first in the literature to examine certain parameters of both oxidant and antioxidant systems of duloxetine in the same environment. The most important finding in our study was that we found a significant increase in the SOD activity and a significant decrease in the CAT, XO, and ADA activities in the duloxetine group. We also found a non-significant increase in the GSH-Px activity and a non-significant decrease in the MDA and NO levels in the duloxetine group.

The literature involves several studies showing that duloxetine has a protective effect against OS (11,12,16,28,29). In an in vitro experimental study, it was found that TRPM2 and TRPV1 channel activities involved in Ca2+ entry-induced oxidative neuronal death in rat hippocampus and DRGs decreased with duloxetine treatment, and it was suggested that this was the mechanism for apoptosis and the neuroprotective effect (12). In an in vitro experimental study in human neuroblastoma SH-SY5Y cells, it was shown that duloxetine had the potential to reduce ROS damage through the Akt/Nrf2/HO-1 protective signaling pathway and exhibited neuroprotective effects (28). In an ischemia-reperfusion animal experiment, pretreatment with duloxetine protected gerbil hippocampal pyramidal neurons from ischemia associated delayed neuronal damage. Pretreatment with duloxetine did not increase LP markers and significantly increased the SOD, an antioxidant enzyme, after ischemia-reperfusion, in neurons. As a result, it has been suggested that duloxetine has a neuroprotective effect against transient global cerebral ischemia, which may be due to the reduction of OS (14). In our study, in support of these findings in the literature, the duloxetine group demonstrated a significant increase in the activity of SOD, an antioxidant enzyme, and a decrease, although non-significant, in the levels of MDA, a LP product that is an OS indicator. These findings suggest that duloxetine may change the antioxidant status positively, hence may contribute positively to the imbalance between the oxidant system and antioxidant defenses, which is suggested to be involved in the etiology of depression. In a cell culture study in PC 12 neuronal cells, duloxetine was shown to be beneficial against apoptotic cell death and OS, which appear to be due to increased intracellular Ca+2 levels through activation of voltage-gated Ca+2 and TRPM2 channels, and the antioxidant GSH-Px and GSH levels were determined significantly higher in the duloxetine group (11). Although there was no statistically significant increase in the GSH-Px activity in our study, in the duloxetine group, the fact that it tended to increase supports this finding. That is because, SOD enzymes are involved in the catalytic dismutation of the toxic superoxide radical, and H2O2 is produced in this process. H2O2, the resulting reactive oxygen product, is eliminated by peroxidases such as GSH-Px (30).

An animal study with mice, evaluating the effect of duloxetine on chronic immobilization stress (CIS)-induced cognitive impairment and neurodegeneration, also assessed its effect on OS, as a result of which it was found that duloxetine pretreatment at doses of 10 and 20 mg/kg provided a dose-dependent decrease in elevated brain MDA levels and an increase in reduced brain GSH, SOD and catalase enzyme activities. Based on these findings, the authors concluded that the reduction of OS may be one of the mechanisms of the protective effect of duloxetine against neuropsychiatric symptoms caused by the CIS (15). Another study with rats showed that duloxetine treatment may have a protective effect against OS by reversing the increase in the level of MDA, which is a marker of methamphetamine-induced increased LP in animal brains, and the decrease in GSH-Px, SOD, and Glutathione reductase enzyme activities, which are the enzymes of the antioxidant defense system in hippocampal tissues (16). In a study analyzing the possible protective role of single-dose duloxetine against pentylenetetrazol (PTZ)-induced convulsive seizures in mice, brain OS parameters were also evaluated. There was a statistically significant decrease in both SOD and CAT activities and a statistically significant increase in LP in the cerebral cortex of PTZ-administered mice. However, in the group receiving a single dose of 20 mg/kg duloxetine, this effect of PTZ was not observed and SOD and CAT activities were preserved, and it was suggested that this modulation of SOD and CAT enzymes may have a role in antioxidant protection (31). We also found results that support these data in the literature. In our study, we found a significant increase in the brain's total SOD activity, an antioxidant enzyme. Although the significant decrease in CAT activity suggests that duloxetine may have a negative effect on the antioxidant system, an increase in the level of GSH-Px, another antioxidant enzyme that removes H2O2 in the environment like CAT, although not at a significant level, and not an increase but conversely a decrease in the level of MDA, a LP product, which is one of the important markers of OS in tissues, although not at a significant level, suggest that duloxetine may have positive effects on the antioxidant system.

XO forms reactive oxygen products in living organisms. It converts hypoxanthine into xanthine and xanthine into uric acid. In these reactions, molecular oxygen is converted to superoxide. In the brain tissue, which is rich in oxygen and requires a lot of energy, this enzyme is activated for the destruction of the ATP used and produces free radicals that damage the tissue as a result of their reactions (32). ADA is also an aminohydrolase in purine metabolism (33). In a study conducted in patients with major depression, ADA and XO levels were found to be high before treatment. Significant decrease in XO levels and increase in ADA levels were observed after eight weeks of antidepressant treatment (34). In another study conducted by the same researchers in panic patients, ADA and XO levels were

found to be significantly higher in patients, and after eight weeks of antidepressant treatment, ADA activity increased and XO activity significantly decreased (35). As a result, it has been stated that increased purine metabolism in depression and panic patients can be controlled with antidepressant treatment. Also in our study, the significant decreases in the XO and ADA activities of the duloxetine group support that purine catabolism is decreased in the organism, thereby reducing radical formation. These results can be considered as a supportive parameter that duloxetine may help increase the resistance of the brain against oxidative damage.

NO is an inorganic free radical that the form of a colorless gas and has an odd number of electrons. In vivo studies have shown that NO regulates the levels of serotonin, dopamine, GABA and glutamate in the CNS. However, excessive NO synthesis has been found to damage neurons (36). Studies evaluating the effect of duloxetine on NO are available in the literature (37). For example, in a study using the comet assay on mouse liver and brain cells, duloxetine caused significant DNA damage and increased DNA, lipid, protein and NO oxidation in both organs mainly after 9 hours. Even at a dose of 2 mg/kg, duloxetine has been reported to have the capacity to damage DNA, and it has been suggested that this effect may be due to its oxidative potential (37). In our study, we found a non-significant decrease in rat brain NO levels after duloxetine treatment for 14 days. This non-significant decrease caused by duloxetine on the NO levels suggested that it would not have a negative effect on the oxidant-antioxidant system at least through NO, which is an inorganic free radical. Perhaps duloxetine may have an oxidative potential by increasing NO oxidation in the acute period in short-term applications (37), however, in long-term treatments such as the one in our study, this oxidative effect it has through NO may be eliminated.

The most important limitation of our study is that the results are preliminary for clinical use because it was performed on rats. The superiority of our study over similar studies in the literature is that it is the only study that evaluated the direct effect of duloxetine on the oxidant-antioxidant system in such a wide range. The studies on this subject are predominantly laboratory studies similar to ours, thus there is a need for further clinical studies.

CONCLUSION

The importance of the antioxidant system is indisputable, especially in the brain tissue, which is weak against oxidant radicals formed due to high oxygen utilization. Increase in SOD activity by duloxetine, the antidepressant drug we use, may strengthen the protective system thereby rendering the brain more resistant and stronger against stress. Decreases in XO and ADA activity, which are involved in purine catabolism, are also supportive parameters related to the increase of this resistance. In addition, a decrease in the level of MDA, a LP product, and NO, an inorganic free radical, although not at a significant level, suggests that duloxetine may have a protective effect on the brain against OS.

Conflict of Interest: No conflict of interest was declared by the authors.

Ethics: The study was approved by the Local Ethics Committee for Animal Experiments of Süleyman Demirel University Faculty of Medicine (Decision no: 04 Date: 27.05.2010)

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