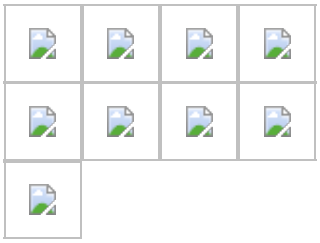

Decreased Superoxide Dismutase Activity after ECT and Correlation between Higher Oxidant Levels and Poor Response to ECT in Depression

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INTRODUCTION

Electroconvulsive therapy (ECT) has been used as a treatment for mental disorders since 1938. For refractory **depression** it is still the most effective treatment (1). A brief electrical stimulus is delivered to the brain through external electrodes and a cerebral seizure is induced under controlled conditions (2). Some cognitive adverse events may occur due to ECT (3). The hippocampus, frontal cortex, striatum, entorhinal cortex, temporal-parietal cortex are primary brain regions impacted by ECT (4).

The findings from a study of electroconvulsive shock (ECS), the animal equivalent of ECT, on animals have contributed to explain the therapeutic effect of ECT (5). In neurotransmitters and their receptors in the brain, ECS produces various changes, many of which can be linked to its therapeutic effect. Chronic ECS application influences 5-hydroxytryptamine (5-HT) 1A receptors, b-adrenoreceptors, a2-adrenoreceptors, adenosine A1 receptors, muscarinic receptors (6) and N-methyl-D-aspartic acid (NMDA) receptor complex (7). ECS also activates cell signaling and leads to release of second messengers (8). Protein and lipid changes in cell membrane and activations within the cell result in intracellular free fatty acid accumulation, which in turn causes formation of eicosanoids, lipid peroxides, and free radicals [9]. Antioxidant mechanisms try to minimize the cellular damage by removing hazardous oxidants (10).

In ECS studies on rats, conflicting values of oxidants and antioxidants were observed at various regions of the brain. While some researchers found an increase (4) in oxidants, others measured a decrease (11); meanwhile, Jornada et al. [12] reported no significant changes in oxidative metabolites **after** ECS. Regarding antioxidants, Erakovic et al. (10) and Feier et al. [4] measured a decrease, but Baricello et al. (11) found an increase. As we could not identify any literature about ECT and oxidative metabolism relationship in the human brain or blood, we conducted this study to investigate the changes in oxidants and antioxidants in the plasma of depressed patients **after** ECT. We studied plasma **levels** of nitric oxide (NO), xanthine oxidase (XO) and malondialdehyde (MDA) as oxidants and **activity** of **superoxide dismutase** (SOD) as antioxidant.

METHODS

Patients

A total of 16 consequent patients were included in the study. They were admitted for ECT **after** being assessed by one psychiatrist (HAS) at the Psychiatry Clinic of Gaziantep School of Medicine. Based on DSM-IV (13) criteria, all patients had been diagnosed with unipolar **depression**, except two, who met criteria for bipolar **depression**. All participating patients provided written informed consent separately for ECT and this study **after** standard education about ECT and the study protocol. The protocol had been approved by the Institutional Review Board of Gaziantep University, School of Medicine in accordance with the Declaration of Helsinki. ECT indication criteria were limited to presence of catatonic or psychotic features, significant suicide risk, or lack of **response** to at least two antidepressants at adequate doses **after** adequate duration of treatment.

Exclusion criteria included alcohol or substance abuse or dependence, presence of severe organic disorders, use of any antioxidants such as vitamin E or C, presence of epilepsy or other severe neurological disorders, presence of any infectious or viral disease, presence of diabetes mellitus or excessive obesity, and other severe medical conditions. Routine electrocardiogram, chest X-ray, complete blood count and blood chemistry panel were obtained from all patients prior to ECT. These routine tests were used to detect possible problems for exclusion criteria. All patients continued their outpatient medication regimen, which included antidepressants, antipsychotics, lithium, or benzodiazepines during ECT treatment. No medication or dose changes were made during the study.

The mean age of the patients was 39±17.98 years. Seven (43.8%) of them were men and 9 (56.2%) were women. Fourteen (87.5%) patients had previous history of ECT, 11 patients (68.8%) had previous inpatient psychiatric treatment and 4 (25%) patients had positive psychiatric family history. The mean illness duration was 3.44±3.18 (range 1-9) years. Two (12.5%) patients had comorbid medical disease, 14 (87.5%) did not. The comorbid medical diseases were migraine and gonarthrosis, which were not included in the exclusion criteria.

The severity of **depression** and **response** to the treatment were assessed by the 17-item Hamilton **Depression** Rating Scale (HDRS) [14]. The ratings were performed before the 1st and **after** the 7th ECT sessions. The HDRS was adapted for use in Turkish speaking patients with established validity and reliability (15).

ECT procedure

Each patient received at least 7 ECT treatments; there were no upper limit regarding the number of ECTs. In order to establish a standard among the subjects, blood samples for testing were obtained within 1 hour before the 1st ECT and within 1 hour **after** the 7th ECT sessions. ECT sessions were carried out under general anesthesia 3 times per week. The anesthesia procedure consisted of administration of propofol (1 mg/kg), an anesthetic agent considered to have no opioid or glucocorticoid properties, and succinylcholine (0.5 mg/kg) and oxygenation. ECT was delivered through bitemporally (one in each temporal fossa) placed classical electrodes between 09.00AM and 11.00 A.M. **after** overnight fasting. The current was sinusoidal, and the stimulus intensity was standardized at 700 mA for 5 seconds. Seizures were monitored by the cuff method. ECT was administered using a T-03 Basak Elektronik (Başak Elektronik, Istanbul, Turkey) machine. Seizure durations of 30-60 seconds were generated at each ECT session for each patient.

Blood collection and biochemical analyses

Blood samples were collected from the antecubital veins of each patient into 5 ml vacutainer tubes within one-hour period before the first ECT and within one hour **after** the 7th ECT sessions performed **after** an overnight fast. Blood samples were taken to normal laboratory tubes without anticoagulants. **After** immediate centrifugation (1000?g, 10 min), serum samples were stored frozen at -70 °C. The biochemical analyses were performed at Biochemistry Laboratories of Gaziantep University, School of

Medicine **after** the blood samples of all patients were collected.

Measurement of XO level

Plasma XO **activity** was measured spectrophotometrically by the formation of uric acid (UA) from xanthine through the increase in absorbance at 293 nm (Prajda and Weber, 1975). A calibration curve was constructed by using 10–50 milliunits/ml concentrations of standard XO solutions (Sigma X-1875). One unit of **activity** was defined as 1 mmol UA formed per minute at 37°C and pH 7.5. Results were expressed in units per liter plasma.

Measurement of NO level

The stable oxidation end products of NO, nitrite (NO2-) and nitrate (NO3-) were measured as indicators of NO production [16]. For nitrite plus nitrate (total nitrite) detection, an aliquot of the sample was treated with copperized cadmium (Cd) in glycine buffer at pH 9.7 (2.5 to 3g of Cd granules for a 4 mL reaction mixture) to reduce nitrate to nitrite and then mixed with fresh reagent, and the absorbance was measured in a spectrophotometer (UV-1601 Shimadzu, Japan). Linear regression was done using the peak areas from the nitrite standards. The resulting equation was then used to calculate the unknown sample concentrations. All chemicals used in this assay were obtained from Sigma, except cadmium granules. The results were expressed as micromoles per liter (mmol/L).

Measurement of MDA level

The plasma MDA **levels** were determined by the method of Draper and Hadley (1990) based on the reaction of MDA with thiobarbituric acid (TBA) at 95°C. In the TBA test reaction, MDA and TBA react to form a pink pigment with an absorption maximum at 532 nm. The reaction was performed at pH 2-3 at 95 °C for 15 minutes. The sample was mixed with 2.5 volumes of 10% (w/v) trichloroaceticacid to precipitate the protein. The precipitate was pelleted by centrifugation and an aliquot of the supernatant was allowed to react with an equal volume of 0.67% TBA in a boiling water bath for 15 minutes. **After** cooling, the absorbance was read at 532 nm. Arbitrary values obtained were compared with a series of standard solutions (1,1,3,3 tetramethoxypropane). The results were expressed as nmol/mL.

Measurement of SOD activity

The principle of the total SOD **activity** method is based, briefly, on the inhibition of nitroblue tetrazolium (NBT) reduction by O2- U generated by xanthine/xanthine oxidase system (17,18). One unit of SOD was defined as the enzyme amount causing

50% inhibition in the NBT reduction rate. **Activity** was expressed as Units per milliliter serum (U/mL).

Data analysis

SPSS 13.0 for Windows (SPSS, Chicago, IL) was used for the statistical analysis of data. The Wilcoxon signed-rank test was performed to compare the mean values obtained before and **after** the treatment. The differences were considered significant when p value was less than 0.05. Bivariate statistics were performed using Spearman's rho. Two-tailed significance values were used.

FINDINGS

We did not find any significant relationship **between** oxidants and antioxidant and following variables: gender, illness duration, number of inpatient episodes, history of previous **ECT** treatment, comorbid medical diseases and family psychiatric history ($p>0.05$).

Post-7th **ECT** values were significantly lower than pre-1st **ECT** SOD ones ($p<0.05$, $Z= -3.155$). No significant differences were detected **between** pre-1st **ECT** MDA, NO, XO and post-7th **ECT** MDA, NO and XO values ($p>0.05$). Pre-1st **ECT** and post-7th **ECT** MDA, NO, XO, SOD values are listed in Table 1. Pre-1st **ECT** and post-7th **ECT** HDRS scores were 32.63±9.54 (mean±SD) (range: 27-37), and 16.50±7.22 (mean±SD) (range: 12-20), (Z:-3.519, $p<0.0001$), respectively.

The **depression** in three patients showed psychotic features. There was no significant difference **between** patients with and without psychotic features with regard to pre- and post-**ECT** SOD, MDA, NO and XO values.

There was a significant positive **correlation between** pre-1st **ECT** SOD and pre-1st **ECT** HDRS scores (r=0.641, p=0.007). In other words, as the severity of **depression** increased, so did pre-1st **ECT** SOD values. In addition, we found a significant negative **correlation between** pre-1st **ECT** NO (r=-0.563, p=0.02) and pre-1st **ECT** XO (r=-0.584 p=0.018) **levels** and HDRS total score changes from pre-1st **ECT** to post-7th **ECT**. Otherwise, as pre-1st **ECT** NO and XO values got **higher**, the drop in HDRS scores **after** **ECT** treatment became smaller.

DISCUSSION

The first important finding of this study was that post-7th **ECT** SOD were significantly lower than pre-1st **ECT** SOD, while there were no significant differences **between** pre-1st **ECT** NO, XO, and MDA and post-7th **ECT** NO, XO, and MDA values, respectively, in the plasma of depressed patients.

We could not identify any study on this topic with **ECT** in humans in the literature, but there are animal studies about **oxidant** and antioxidant **levels** with different ECS applications to various areas of the brain in rats. Similar to our results Erakovic et al. (10) reported that single or repeated ECS induced a decrease in SOD and glutation peroxidase (GPX) activities at various brain regions in rats. Baricello et al. (11) demonstrated an increase in catalase (CAT) and SOD activities, a decrease in lipid peroxidation, and protein damage in the hippocampus immediately **after** and up to 30 days **after** a single or multiple ECS. Feier et al. (4) found a delayed increase in oxidative damage **after** ECS and a decrease in SOD and CAT activities in hippocampus and striatum in rats. Jornada et al. [12] reported no alteration in the lipid peroxidation and no protein damage in the

hippocampus, cortex, cerebellum, and striatum immediately **after**, 48 h and 7 days **after** the last maintenance ECS. Zupan et al. (19) found oxidative lipid damage only in the frontal cortex, but not in the hippocampus, cerebellum, and the pons/medulla region in their experimental animal conditions. Also they reported that the SOD and GPX activities significantly increased in the hippocampus and cerebellum, but did not change in the pons/medulla region.

The exact cause of decrease in SOD in our study is not known. As suggested in an animal study, oxidative stress and accumulation of hydrogen peroxide, which inactivates SOD irreversibly, possibly disturbs the SOD synthesis by damaging the mitochondrial function [20]. The mentioned oxidants were not increased in our study, but some oxidative products resulting from increased neuronal **activity** **after** **ECT** might have caused SOD inhibition. In addition, collecting blood samples before adequate normalization of SOD might have resulted in lower SOD **levels** **after** the 7th **ECT** (10).

Decrease in SOD **levels** may lead to deficiency in oxidative defense, and hence causes oxidative stress, which has hazardous effects on neurons. Interestingly, a study in rats reported an association **between** a decrease in SOD **activity** in frontal cortex and amnesia **after** **ECT** (21). In another significant report in the literature, it was observed that exogenously administered enzymatic and non-enzymatic antioxidants successfully protected the brain against seizure-induced damage (10).

It is important to note that these studies were conducted in rats, and the measurements were done at different regions of rat brains, while our results were obtained from plasma of depressed humans. The human studies investigating **oxidant** and antioxidant changes in antidepressant receiving depressed patients resulted in a variety of findings. Herken et al. (2007) found no change in NO **levels**, but XO and adenosine deaminase (ADA) **levels** were significantly **higher** in major depressive disorder (MDD) patients, and SOD **activity** was significantlylower than in the controls. **After** 8 weeks of antidepressant treatment, ADA and SOD activities increased, and NO and XO **levels** **decreased** significantly. They concluded that ADA, XO, and SOD **activity** might have a pathophysiological role in MDD and predict the prognosis of MDD. The **activity** of these enzymes might be used to monitor the **response** to treatment. Selek et al. (22) discovered that NO was **higher** and SOD **activity** was lower in bipolar **depression** patients prior to treatment than in the controls. **After** 30 days treatment with antidepressants, mood stabilizers, other drugs, or **ECT**, NO **levels** normalized, but SOD **levels** remained low. Bilici et al. (23) reported that MDD patients, especially patients with melancholia, had **higher** antioxidant enzyme **activity** and lipid peroxidation **levels** than healthy controls. **After** 3 months treatment with selective serotonin reuptake inhibitors (SSRIs), both measurements **decreased** to

normal **levels**.

The second important finding of this study was the discovery of some relationships between oxidant and antioxidant **levels** and **depression** severity, and also **response** to ECT. There was a significant positive relationship between pre1st ECT SOD **activity** and before 1st ECT HDRS scores. Basically, high HDRS scores were correlated with high SOD1 **activity**, which may indicate a reactive antioxidant **response** by the body to severe **depression** (24). In addition, there was a significant negative correlation between NO1 and XO1 **levels** and decreases in HDRS total scores obtained before the 1st ECT and **after** the 7th ECT sessions. In other words, the higher the NO1 and XO1 **levels** were, the smaller the decreases were between pre-ECT and post-ECT HDRS scores. High oxidant **levels** might have impacted the **response** to ECT negatively; hence, the **levels** of oxidants prior to ECT may be a factor in determining ECT responsiveness. This is an important finding and further research is needed. Some authors studied associations between oxidative metabolism (OM) and severity of illness and **response** to pharmacotherapy in depressed patients. Herken et al. (25) found no correlation between HDRS scores and SOD, ADA, XO and NO **levels**. Bilici et al. (23) reported a positive correlation between erythrocyte SOD **levels** and HDRS scores and suggested that antidepressant treatment **decreased** SOD **levels**. They also proposed that antioxidant enzyme **activity** might be used to monitor the **response** to SSRIs in MDD.Some important limitations of this study include relatively low number of patients, lack of control group, and missing data regarding smoking status of patients. Smoking status might have possibly interfered with the results as a confounding factor, only if any change occurred during the study (26). The patients likely did not change their smoking habits during short study period. All patients continued their medication regimen during ECT

course. Despite no medication and dosage changes, ECT outcome and oxidative parameters might possibly have been effected (23). Finally, propofol, which was used for anesthesia, was demonstrated to decrease oxidant enzyme activities. However, some reports mentioned that it had no effect on SOD (27,28). Theoretically, it is possible that the oxidant enzyme activities were **decreased** by propofol and increased back by ECT. The possible effect of propofol, given one hour before blood sampling, on oxidative status is also a limitation of this study.

In conclusion, there were two important results of this study: The first one was the decrease in antioxidant enzyme SOD **after** ECT, which may cause oxidative stress by weakening the oxidative defenses. Oxidative stress may hazard neurons through adverse events. Regarding this finding, effect(s) of antioxidant supplementation during ECT should be investigated separately. The second result is that high oxidant (NO1 & XO1) **levels** before the 1st ECT session were significantly correlated with a poorer **response** to ECT. In other words, oxidative status might determine or predict the **response** to ECT in depressed patients. Future studies with larger and controlled designs should explore the effects of ECT on OM in humans. Furthermore, OM may be investigated under single, multiple, and maintenance ECT courses at different time points.

Abbreviations

ECT, electroconvulsive therapy; ECS, electroconvulsive shock; OM, oxidative metabolism; MDA, malondialdehyde; NO, nitric oxide; XO, xanthine oxidase; SOD, **superoxide dismutase**; TBA, thiobarbituric acid; GPX, glutation peroxidase activities; CAT, catalase; ADA, adenosine deaminase; HDRS, Hamilton **Depression** Rating Scale; MDD, major depressive disorder; SSRI, selective serotonin reuptake inhibitor.

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