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Serum Paraoxonase and Arylesterase Activities and Oxidative Stress Levels in Patients with SSRI Intoxication

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Abstract Oxidative stress is a critical route of damage in various psychological stress-induced disorders, such as depression. Paraoxonase-1 (PON1) plays an important role as an endogenous free-radical scavenging molecule. The aim of this study was to evaluate the influence of serum PON1 activity and oxidative stress in patients with selective serotonin reuptake inhibitor (SSRI) intoxication. A total of 11 patients with SSRI intoxication and 20 healthy controls were enrolled. The serum total antioxidant capacity (TAC) and malondialdehyde (MDA) levels, as well as the paraoxonase and arylesterase activities, were measured spectrophotometrically. The serum TAC levels and the paraoxonase and arylesterase activities were significantly lower (for all, $p < 0.001$), whereas the serum MDA levels were significantly higher in the patients with SSRI intoxication than in the controls ($p < 0.001$). These results indicated that decreased PON1 activity and increased oxidative stress represent alternative mechanisms in SSRI toxicity. More studies are needed to elucidate the role of PON1 activity in the etiology of SSRI intoxication.

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Introduction

Antidepressant drugs are widely employed to treat stress and stress-related depression and anxiety. Selective serotonin reuptake inhibitors (SSRIs) are commonly prescribed rather than tricyclic antidepressants because of their diminished side-effect profile and reduced toxicity after overdose. SSRIs are a class of drugs used to treat depression. SSRIs can also be used to treat other conditions, such as anxiety disorder. This drug has a highly selective, potent and dose-dependent inhibitory effect on the human serotonin transporter (Kirino 2012).

It is well known that reactive oxygen species (ROS) can affect human physiological and pathophysiological processes. All ROS types, including superoxide anions and hydrogen peroxide, have unpaired valence electrons or unstable bonds (Valko et al. 2007). However, when the ROS concentration exceeds the antioxidative capacity of an organism, animal cells enter a state of oxidative stress and the excess ROS begin to induce oxidative damage on cellular components (Delattre et al. 2005). Oxidative stress has been implicated in the pathogenesis of a variety of diseases, including cancer, diabetes, autoimmune diseases, atherosclerosis, cardiovascular disorders (Valko et al. 2007; Delattre et al. 2005; Aitken and Roman 2008), neurodegenerative diseases and neuropsychiatric diseases, such as schizophrenia and major depressive disorder (Valko et al. 2007; Delattre et al. 2005; Bilici et al. 2001).

Paraoxonase-1 (PON1) is a calcium-dependent glycoprotein that is associated with high density lipoprotein (HDL). PON1 also acts as an enzyme that hydrolyzes

organophosphorus (Sams and Mason 1999). PON1 was shown to hydrolyze specific oxidized lipids (Aviram et al. 2000) and to protect HDL particles from oxidation and increase their ability to induce macrophage cholesterol efflux (Shamir et al. 2005). It has been suggested that decreased serum PON1 activity might be associated with increased oxidative stress (Rozenberg et al. 2003). Furthermore, oxidative stress has been shown to decrease PON1 activity and down-regulate the serum expression of PON1 (Aviram et al. 1999; Kotur-Stevuljevic et al. 2008). Several studies have shown that the level of PON1 serves as an independent risk factor for coronary disease (Kabaroglu et al. 2004; Mackness et al. 2004).

Information regarding the effect of tricyclic antidepressant intoxication on serum PON1 activity in vivo is very limited (Alagoz et al. 2007). Therefore, the aim of this study was to investigate the influence of SSRI toxicity on PON1 activity and oxidative stress levels.

Materials and Methods

Subjects

In this prospective study, eleven patients (8 females and 3 males) with SSRI intoxication and 20 healthy controls (12 females and 8 males) were enrolled. The serum SSRI levels could not be measured because of technical difficulties.

All intentional drug overdose patients were included in the study. Patients were asked for both generic and specific brand names with emphasis on the commonest products. Family members or attendants were questioned and pill packages were sought, whenever possible.

None of the study subjects (neither the patients nor the controls) had been taking antioxidant vitamin supplements, including vitamins E or C. All of the study subjects were nonsmokers.

The control group was selected from 20 healthy volunteers. All of the control subjects were asymptomatic and presented unremarkable medical histories and normal physical examinations.

The study protocol was conducted in accordance with the 2,000 revision of the Helsinki Declaration and was approved by the local ethics committee. All of the subjects were informed about the study, and written consent was obtained from each subject.

Exclusion Criteria

The exclusion criteria included a history of alcohol abuse, habitual smoking, intravenous drug abuse, pregnancy, antioxidant supplements, hypertension, diabetes mellitus, liver or renal disease, rheumatoid arthritis, pulmonary disease and coronary artery disease.

Blood Samples

Blood samples were collected from the study groups, placed into empty tubes and immediately stored at 4 °C. The serum samples were then separated from the cells by centrifugation at 3,000 rpm for 10 min. The serum samples were stored in plastic tubes at −80 °C and used to analyze the TAC levels, PON1 activity and MDA levels.

Measuring of Paraoxonase and Arylesterase Activities

The paraoxonase and arylesterase activities were measured using a spectrophotometer to measure the absorbances (Genesys 10 UV Scanning UV/Vis spectrophotometer, Shimadzu, Japan) with kits (Rel Assay Diagnostics kit, Mega Tıp, Gaziantep, Turkey). The PON1 activity was assayed using two different substrates (Eckerson et al. 1983). The paraoxonase activity was expressed as U/L. Phenylacetate was used as a substrate to measure the arylesterase activity. The arylesterase activity was expressed as kU/L and defined as 1 μmol of phenol generated per minute under the reaction conditions (Haagen and Brock 1992).

Measuring of Serum Total Antioxidant Capacity

The serum TAC levels were determined spectrophotometrically (Genesys 10 UV Scanning UV/Vis Spectrophotometer, Shimadzu, Japan) at 660 nm using kits (Rel Assay Diagnostics kit, Mega Tıp, Gaziantep, Turkey) that were previously developed by Erel (2004). Hydroxyl radicals (the most potent biological radicals) are produced using this method. In the assay, ferrous ion solution, which is part of Reagent 1, is mixed with hydrogen peroxide, which part of Reagent 2. Potent radicals are produced, such as the brown-colored dianisidinyl radical cation that is sequentially produced by the hydroxyl radical. Using this method, the antioxidative effect of the sample is measured against the potent free radical reactions, which is initiated by the generated hydroxyl radical. The results are expressed as mmol Trolox Equiv./L.

Measurement of Serum Lipid Peroxidation

To determine the amount of lipid peroxidation in serum, levels of MDA were analyzed spectrophotometrically, using the modified thiobarbituric acid-reactive substance (TBARS) method by Yoshioka et al. (1979). The results were expressed as nmol/mL.

Other Parameters

The serum aspartate aminotransferase (AST) and alanine aminotransferase (ALT) levels were determined using commercially available assay kits (Roche®, Mannheim, Germany) with an autoanalyzer (Roche®/Hitachi Modular P-800, Mannheim, Germany).

Statistical Analysis

The results were expressed as mean ± standard deviation. The parametric variables were compared using Student's *t* test. The correlation analyses were performed using Pearson's correlation analysis. The results were considered to be statistically significant when $p < 0.05$. The data were analyzed using the SPSS® for Windows computing program (Version 11.0).

Results

The demographic characteristics of the cases with SSRIs intoxication and the controls are shown in Table 1. There were no significant differences between the groups with respect to age and gender ($p > 0.05$) (Table 1).

The serum TAC levels and the paraoxonase and arylesterase activities were significantly lower (for all, $p < 0.001$) and the serum MDA levels were significantly higher in the patients with SSRI intoxication than those in the controls ($p < 0.001$) (Table 2).

Table 1 Demographic characteristics of the patients with SSRI intoxication and the control subjects

Parameters	Controls ($n = 20$)	Patients ($n = 11$)	p
Age (years)	34 ± 10	29 ± 8	0.162
Sex (female/male)	12/8	8/3	0.479
AST (U/L)	23.15 ± 6.13	23.18 ± 5.38	0.989
ALT (U/L)	25.45 ± 5.76	23.27 ± 5.96	0.328

Values are mean ± SD

ALT alanine aminotransferase, *AST* aspartate aminotransferase

Table 2 Serum oxidant and antioxidant levels, as well as paraoxonase and arylesterase activities, in the SSRI intoxication and control subjects

Parameters	Controls ($n = 20$)	Patients ($n = 11$)	p
MDA (nmol/mL)	12.61 ± 1.43	27.74 ± 4.02	0.001
TAC (mmol Trolox Equiv./L)	2.93 ± 0.57	0.95 ± 0.72	0.001
Paraoxonase (U/L)	75.51 ± 4.30	44.47 ± 3.57	0.001
Arylesterase (kU/L)	40.44 ± 8.89	16.01 ± 4.12	0.001

Values are mean ± SD

MDA malondialdehyde, *TAC* total antioxidant capacity

No significant correlation was found between the serum MDA levels and the TAC, paraoxonase and arylesterase activities ($p > 0.05$) in the patient group.

Discussion

In the present study, we investigated the influence of SSRI toxicity on PON1 activity and oxidative stress levels. In addition, we measured MDA levels as one of the end products of lipid peroxidation. We observed that the serum PON1 and arylesterase activities were significantly lower in the patients with SSRI intoxication. In the present study, we have observed the increased TBARS in patients with SSRI intoxication. In patients with SSRI intoxication, there is an increased production of free radicals which promotes lipid peroxidation. To the best of our knowledge, serum PON1 activity has not previously been evaluated in patients with SSRI intoxication. This report is the first to investigate the serum PON1 activity in patients with SSRI intoxication. Reduced serum PON1 enzyme activity may play a role in the etiopathogenesis of SSRI intoxication and in the increased susceptibility to oxidative stresses observed during SSRI intoxication.

SSRIs are widely prescribed to treat stress and stress-related depression and anxiety (Kirino 2012). SSRIs are potent inhibitors of the hepatic isoenzyme P450-2D6 and likely affect the clearance of drugs metabolized by this enzyme (Gury and Cousin 1999).

MDA is a highly toxic by-product formed in part by lipid oxidation derived free radicals. Serum MDA levels are widely accepted as markers of oxidative stress and lipid peroxidation. Oxidative stress is one of the main causes of damage to cell membranes in result of exacerbated lipid peroxidation process. End products of lipid peroxidation (aldehydes, organic peroxides) react with important biological macromolecules such as DNA and proteins (Gutteridge 1995).

Oxidative stress results from an imbalance between oxidants and antioxidants; this imbalance is theorized to play a key role in certain physiological conditions, such as aging, and in the pathogenesis of numerous diseases, such as liver, renal and multiple organ failure (Zafarullah et al. 2003). Oxidative stress has also been implicated in many pathophysiological conditions that result from either an overproduction of reactive oxygen species or from a failure of the antioxidant defense systems (Torun et al. 2009; Yildiz et al. 2008). ROS, including superoxide anion, hydrogen peroxide, and singlet oxygen, act as subcellular messengers in many complex processes (e.g., mitogenic signal transduction, gene expression, and the regulation of cell proliferation) when they are generated excessively or when enzymatic and nonenzymatic defense systems are impaired (Fujii et al. 2003). Several studies have observed

an increased production of ROS under conditions of psychological stress (Wittchen and Jacobi 2005; Tuthill et al. 2006), whereas others have shown a decrease in ROS production (Krömer et al. 2005; Ditzen et al. 2006). There is evidence that ROS play an important role in the pathogenesis of many neurodegenerative and neuropsychiatric diseases, such as schizophrenia and major depressive disorder (Valko et al. 2007; Delattre et al. 2005; Bilici et al. 2001).

PON1 is an HDL-associated antioxidant enzyme (Leviev et al. 1997). Increasing evidence has demonstrated that other environmental factors, in addition to genetics, also influence PON1 activity. Multiple lines of evidence have indicated that exposure to environmental chemicals can inhibit PON1 activity (Blatter et al. 1993). The enzymatic activity levels of PON-HDL vary greatly among healthy people, and individuals with low PON activity are at a greater risk for developing diseases that involve oxidative stress and lipid peroxidation (Chandra et al. 1994). PON1 is one of the antioxidants that acts as an enzymatic defense against lipid hydroperoxides and lipid peroxides in low density lipoprotein (LDL) under in vitro oxidizing conditions (Mackness et al. 1996). In certain diseases, the serum PON1 activity was found to be inversely correlated with the level of oxidative stress (Ali et al. 2009; Ates et al. 2009). In addition, oxidative stress has been observed to increase the expression and activity of PON1 (Aviram et al. 1999; Kotur-Stevuljevic et al. 2008). Furthermore, low serum PON1 activity represents a risk factor for atherosclerosis (Kabaroglu et al. 2004; Mackness et al. 2004). It has also been noted that serum paraoxonase and arylesterase activities are significantly reduced in patients with chronic liver disease (Ferré et al. 2002; Kilic et al. 2005).

Information on the effect of tricyclic antidepressant intoxication on serum PON1 activity in vivo is very limited (Alagoz et al. 2007). Alagoz et al. (2007) investigated serum PON1 activity and oxidative stress levels after tricyclic antidepressant intoxication. The results of that study suggested that tricyclic antidepressant intoxication does not appear to induce serum PON1 activity and oxidative stress levels (Alagoz et al. 2007). Furthermore, they investigated the relationships of serum PON1 activity and oxidative stress levels with serum liver function tests, such AST and ALT, in patients with tricyclic antidepressant intoxication and reported that no significant differences were found between serum PON1 activity and oxidative stress levels and AST and ALT levels in those with tricyclic antidepressant intoxication (Alagoz et al. 2007).

Saadaoui et al. (2012) investigated the in vitro inhibitory effects of three antidepressants (imipramine, amitriptyline and fluoxetine) on PON1 activity. The results of that study suggested that tricyclic antidepressants significantly inhibit PON1 activity in a concentration-dependent manner (Saadaoui et al. 2012). Furthermore, they suggested that amitriptyline exhibited a more potent inhibitory effect than did imipramine. In another study, Abdel-Salam et al. (2013) investigated the in vitro effect of antidepressants, such as sertraline, on PON1 activity. The results of that study suggested that sertraline significantly inhibited PON1 activity, and the authors therefore concluded that sertraline decreased the PON1 activity and might have exposed the brain to further oxidative insults (Abdel-Salam et al. 2013).

AST and ALT are a transaminase enzymes. ALT is found in serum and in various bodily tissues, but is most commonly associated with the liver. It is also called serum glutamic pyruvic transaminase (SGPT) or ALT. AST is normally found in red blood cells, liver, heart, muscle tissue, pancreas, and kidneys. AST formerly was also called serum glutamic oxaloacetic transaminase (SGOT). The serum levels of ALT and AST are the main indices that reflect liver cell injury (B). An important limitation for using AST as an indicator of liver toxicity is the fact that this enzyme is also present in the heart, skeletal muscle, kidney and brain. In contrast, serum ALT levels are considered the most frequently relied upon laboratory indicator of hepatotoxic effects (C). In current study, there was no significant differences in the serum aspartate and alanine transferases levels in patients with SSRI intoxication compared to the healthy controls.

There were several limitations in the present study. First, the number of patients with SSRI intoxication who were enrolled in the study was relatively small. However, a large sample would have increased the power to detect serum PON1 activity in patients with SSRI intoxication. Second, serum ROS and some ROS scavenging enzymes due to technical limitations were not measured in the study population.

These results indicated that lower levels of PON1 activity were associated with an oxidant–antioxidant imbalance. Decreased PON1 activity may be part of the mechanism of SSRI toxicity. For this reason, measuring serum PON1 activity may be beneficial for assessing the risk during SSRI toxicity. Consuming antioxidant vitamins, such as vitamins C and E, might be useful for these patients. Further studies are necessary to confirm this observed relationship.

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