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ORIGINAL PAPER

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The role of the arginine-nitric oxide pathway in the pathogenesis of bipolar affective disorder

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Abstract There is a reciprocal regulation of arginase and nitric oxide synthase (NOS) in L-arginine-metabolizing pathways. Nitric oxide (NO) may be involved in some psychiatric disorders like schizophrenia, depression and bipolar affective disorder (BPAD). To our knowledge, there is no study in the literature in which the role of arginase, an important part of the arginine regulatory system affecting NOS activity, was investigated in BPAD. This study aims to investigate arginase, manganese (Mn) and total nitrite levels (a metabolite of NO) and their relationship to the arginine-NO pathway in patients with BPAD. Arginase activities, Mn and total nitrite levels were measured in plasma from forty-three patients with BPAD (Type one) and thirty-one healthy control subjects. Plasma arginase activities and Mn were found to be significantly lower and total nitrite level higher in patients with BPAD compared with controls. Our results suggest that the arginine-NO pathway is involved in the pathogenesis of BPAD.

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Introduction

Bipolar affective disorders (BPAD) are common, recurrent and disabling diseases. Although the etiopathogenesis of BPAD are still unclear, psychological, social or genetic factors may be involved in the pathogenesis.

Nitric oxide (NO) is a soluble gas produced by the activity of an enzyme, that is present in peripheric tissues and in neurons. NO is a free oxygen radical and neurotransmitter both in the central and peripheral nervous system. It is involved in numerous physiologic functions such as noradrenaline and dopamine release, memory and learning and regulation of the cerebrovascular system as well as some pathologies such as Alzheimer's and Huntington's disease, cerebral ischemia, and stroke (Das et al. 1995). Recently, the role of NO has been investigated in neuropsychiatric disorders such as, depression (Suzuki et al. 2001; Srivastava et al. 2002), bipolar affective disorders (Savas et al. 2002) schizophrenia (Akbarian et al. 1993; Das et al. 1995; Deutsch et al. 1997; Akyol et al. 2002; Zoroglu et al. 2002), and migraine (Shukla et al. 2001).

Arginase (EC 3.5.3.1), which exists primarily in the liver, catalyzes the hydrolysis of L-arginine to urea and ornithine. There are also modest levels of arginase activity in a variety of extrahepatic tissues like brain, kidney, small intestine and mammary gland (Jenkinson et al. 1996). Extra hepatic arginase functions in the production of ornithine which, in turn, is converted to glutamate and proline. Glutamate acts as an inhibitory neurotransmitter in the central nervous system. However, it can also be converted to another structure, γ-aminobutyric acid. Thus, arginase is important in the metabolic control of neurotransmitter synthesis (Jenkinson et al. 1996). Arginase needs manganese (Mn) for its catalytic activity and stability (Brock et al. 1994). There is a direct correlation between arginase activity and the Mn level in the body (Diez et al. 1992).

It is well known that arginase and NOS compete for L-arginine. The reciprocal regulation of arginase and NOS in L-arginine-metabolizing pathways has recently been demonstrated. This reciprocal relationship between NOS and arginase suggests a regulatory role for arginine in wound repair (Albina et al. 1990), chronic renal failure (Durak et al. 2001), rheumatoid arthritis (Huang et al. 2001) and nephritic glomeruli (Jansen et al. 1992). Also according to our previous results, schizophrenic patients had high NO and low arginase levels (Yanik et al. 2003).

Both NO and arginase have not been studied well in mood disorders. There is only one study in which the patients with depression had high arginase levels (Elgun and Kumbassar 2000). In another study, NO was found to be increased in bipolar disorder (Savas et al. 2002). However, despite the presence of these studies, there is no study that Mn and NO levels, and arginase activity were investigated. Therefore, we investigated the arginine-NO pathway by measuring plasma total nitrite, arginase and Mn in BPAD. To our knowledge, this is the first study in this topic.

Materials and methods

The study was conducted by the collaboration of the Departments of Psychiatry and Biochemistry, Medical Faculty of Harran University and the Department of Psychiatry, Medical Faculty of Gaziantep University, Turkey. A complete description of the study was given to each patient, the patient's relatives, the hospital authority and to control subjects. Written informed consent was obtained from all subjects and if the cooperation with patients was impossible, from the patients' relatives and hospital authority.

Patients

Forty-three patients with BPAD (Type I, manic episode) were included in this study. Control group consisted of thirty-one age- and sex-matched healthy subjects. The patients and control subjects were also matched according to smoking. All patients were being treated with stable doses of mood stabilizers, and typical and atypical antipsychotics. Patients with a history of drug abuse, chronic systemic diseases such as diabetes mellitus, hypertension, etc., severe head injury or seizure disorders and who were treated with electroconvulsive therapy, were excluded from the study. DSM IV (APA 1994) diagnosis of BPAD was established by two qualified psychiatrists independently, and the Turkish version of the Bech-Rafaelson Mania Scale (BRMS) was administered (Bech at al. 1979; Kantarci at al. 1993). Also a semistructured form was used to detect several sociodemographic and clinical variables of the patients. The medical records of the patients were also retrospectively reviewed.

Sample collection and preparation

Blood samples were drawn into heparin-containing tubes during routine blood sampling for biochemical analyses. After immediate centrifugation, plasma samples were stored and frozen at –70 °C until assayed, and all the measurements were performed at the same time.

Measurement of plasma levels of total nitrite

Since NO is a very labile molecule, its direct measurement in the biological samples is very difficult (Moncada et al. 1991). In an aqueous solution, NO reacts with molecular oxygen and accumulates in the plasma as nitrite and nitrate ions. Therefore, the stable oxidation end products of NO, nitrite (NO_2^-) and nitrate (NO_3^-), can be readily measured in biological fluids and have been used in vitro and in vivo as indicators of NO production (Koltuksuz et al. 2000) Plasma nitrite levels were measured with the Griess reaction (Cortas and Wakid 1990). Briefly, samples were initially deproteinized with Somogyi reagent (Somogyi 1930). Total nitrite (nitrite + nitrate) was measured after the conversion of nitrate to nitrite by copporized cadmium granules by a spectrophotometer at 545 nm (Ultraspect Plus, Pharmacia LKB Biochrom Ltd, UK). A standard curve was established with a set of serial dilutions (10^{-8} – 10^{-3} mol/l) of sodium nitrite. Linear regression was made by using the peak area from the nitrite standard. The resulting equation was then used to calculate the unknown sample concentrations. Results were expressed as micromoles per liter plasma. Therefore, plasma nitrite and nitrate (total nitrite) concentrations were accepted as an index of NO.

Measurement of plasma arginase levels

Plasma arginase activity was measured according to the method of Geyer and Dabich (1971) with some modification for plasma. Briefly, plasma was diluted 10 times with a solution of 5 mmol/l Mn^{2+} and incubated for 8 min at 55 °C. Then, 0.2 preincubated plasma, 0.4 ml 25 mmol/l L-arginine, and 0.4 ml 40 mmol/l carbonate tampon (pH: 9.7) were incubated for 60 min at 37 °C. After incubation, the reaction was stopped and the sample was deproteinized by adding 1 ml of 0.5 ml 1 N $HClO_4$. The urea level was measured spectrophotometrically through the method of thiosemicarbazide-diacetylmonoxime-urea in the supernatants obtained by the centrifugation of the tubes for 5 min at 5000 rpm. A zero time blank which was not incubated and of which the ingredient was the same as the experiment tube in which enzyme activity was measured was used. One unit plasma arginase was defined as the enzyme activity that produces 1 μmol of urea per minute. In addition, protein was determined using the Biuret method (Silvermen and Christenson 1994).

As enzyme activity was very low in plasma, the specific activity was expressed as units per gram protein by the measurement of the activity in 1 ml plasma per hour divided by the amount of protein in 1 ml plasma.

Measurement of plasma Mn levels

One volume of plasma (usually 0.2 ml) was added to an equal volume of aqueous 0.1% Triton X-100 solution, and thoroughly mixed in a vortex mixer. The diluted plasma was used directly for the analysis. Plasma Mn determination was performed by a SpectrAA 250 Plus Zeeman atomic absorption spectrometer with a graphite furnace GTA-96 (Varian, Australia), according to the method of Brodie and Routh (1984).

Statistical procedure

A two-tailed parametric *t*-test for independent samples was used to compare mean ages of patients and controls. The pearson chi-squared test was used to compare gender and smoking status between patients and controls. Group comparisons of NO and Mn levels and arginase activities were conducted using two-way analysis of variance (ANOVA) in which diagnosis and sex were designated as main effects. We performed supplementary a three-way ANOVA in which mean level of NO was the dependent variable and a competing explanatory variable (smoking status) was included with diagnosis and sex as an additional main effect. Where ANOVA revealed a significant effect, Tukey's post hoc pairwise comparison tests were performed. Pearson correlation tests were performed in order to analyze the relationship between NO and arginase activity. $p < 0.05$ was considered to indicate statistical significance in all analyses. SPSS® for Windows (10.0) computing program was used for the statistical analysis of data.

Results

As to the social and demographic data (e. g., age, or sex), patients and their controls showed homogeneity, and there were no significant differences between the groups ($p > 0.05$). There was no correlation between the plasma arginase and NO levels in either the patients or controls (*Pearson correlation test* $r = 1.13$; $p > 0.05$).

Table 1 shows plasma NO and Mn levels and arginase activities, expressed as group mean values and standard deviations in the sample of the study grouped by both diagnosis and sex. The sample included 43 patients with bipolar disorder (manic) (33 male, 10 female), and 31 normal controls (22 male and 9 female). For NO and Mn levels and arginase activities, two-way ANOVAs were performed separately to test effects of diagnosis and sex, and revealed similar results. For each one of the three, the diagnosis effect was statistically significant (*for NO* $F = 28.06$; $p < 0.001$, *for Mn* $F = 7.25$; $p < 0.001$ and *for arginase* $F = 4.32$; $p < 0.001$); but the sex effect and the interaction between diagnosis and sex were nonsignificant (see Table 1). Post hoc pairwise comparisons showed that the differences originated from the diagnosis effect (Tukey's $p < 0.05$); the mean plasma NO level in bipolar I (manic) group was significantly higher and the plasma Mn levels and arginase activities were significantly lower than those of controls.

A supplementary three way ANOVA was performed to assess the impact of smoking as a potential confounding factor on the relationship between plasma NO levels and diagnosis, and plasma NO levels and sex, as reported above. Analysis revealed no significant effect of smoking ($F = 0.78$, $p > 0.05$), but a significant effect of diagnosis ($F = 18.79$, $p < 0.001$); while the diagnosis x smoking interaction ($F = 0.34$; $p > 0.05$) and the sex x smoking interaction ($F = 1.6$, $p > 0.05$) were also not significant.

Table 1 The characteristics and levels of arginase, Mn and total nitrite in patients with BPAD and controls

	Total Nitrite (μmol/l)	Mn (μg/l)	Arginase (U/g protein)
Patients (n = 43)			
Male (n = 33)	46.43 ± 13.79	13.11 ± 6.13	9.61 ± 4.35
Female (n = 10)	46.72 ± 14.58	13.29 ± 6.49	10.04 ± 4.96
Controls (n = 31)			
Male (n = 22)	32.46 ± 9.09	16.01 ± 8.55	12.18 ± 6.05
Female (n = 9)	30.89 ± 6.36	21.29 ± 13.75	12.72 ± 6.08
Diagnosis effect			
F(df) and p	28.06, p < 0.001	7.25, p < 0.001	4.32, p < 0.001
Sex effect			
F(df) and p	0.052, p = 0.82	1.81, p = 0.18	0.14, p = 0.7
Diagnosis x sex interaction			
F(df) and p	0.11, p = 0.74	1.57, p = 0.21	0.001, p = 0.97

Discussion

In this study, the total nitrite levels were found to be elevated while arginase and Mn levels were lower in BPAD when compared to healthy controls. So far, there is only one study regarding the NO level in bipolar patients, in which NO level was found to be high (Savas et al. 2002). There are three studies regarding the NO levels in depression. Two of them found high NO levels (Papageorgiou et al. 2001; Suzuki et al. 2001), while the NO level was found to be low in the other study (Srivastava et al. 2002). Another study showed that arginase activity is elevated in depression (Elgun and Kumbassar 2000). There is no study regarding the arginase activity in BPAD.

A great number of studies in the literature suggest that there is a competition between arginase and NOS and that they control each other's level. Albina et al. (1990) found NOS to be dominant in the first 3 days and that of arginase after the 3rd day in wound repair. In another study, arginase was found to be the major pathway of L-arginine metabolism in nephritic glomeruli (Jansen et al. 1992). Likewise, it was found that while arginase increases, NOS decreases in the erythrocytes of the patients with chronic renal failure (Durak et al. 2001). Low arginase activity in our study supports the high level of NO because of a reciprocal relationship.

Decreased plasma Mn and arginase levels in bipolar disorders can be explained in two different ways. First, possible reason of low arginase and Mn levels may result from high NO levels. Second, it is reported that dietary Mn deficiency reduces the arginase level (Brock et al. 1994), which is also expected to be seen in bipolar patients, thus causing arginine metabolism to tend toward NO. Therefore, it is not clear whether low arginase activity due to reciprocal arrangement leads to a high NO level or whether a high NO level leads to low arginase activity.

It may be debated whether peripheral total nitrite level indicates NO activity in the brain of the patients with BPAD. Serum total nitrite was increased in a group of demyelinating diseases including multiple sclerosis, inflammatory neurological diseases and AIDS patients studied by Giovannoni et al. (1997). These studies suggest that peripheral NO metabolites can be used as a marker of central nervous system (CNS)-dependent NO changes. The relationship of plasma arginase to brain arginase activity remains unclear. However, this decrease in plasma arginase activity may be the reflection of reduced CNS arginase activity (Hampel et al. 1997). Since all the groups were medically healthy, it seems possible that the altered activity of arginine-NO pathway in our patients may reflect a disorder in the CNS.

The limitation of our study is that group of patients takes mood stabilizers and antipsychotic drugs. Behavioral and neurochemical studies implicate the action of mood stabilizers and antipsychotic drugs in nitric oxide biosynthesis. According to these studies mood stabiliz-

ers and antipsychotic drugs decreased nitric oxide levels (Kowalski et al. 2003; Tarazi et al. 2002; Harvey and Bester 2000). However, NO levels were found to be high in our study. Therefore, we think that drugs do not change the basic results of our study. In addition, in the two studies performed by Zoroglu et al. (2002) and Akyol et al. (2002), no difference in NO serum levels of the schizophrenic patients taking high and low antipsychotic treatment was found.

In conclusion, the significant decrease in arginase and Mn, and an increase in NO in the patients may suggest a shift in the arginine-NO pathway facilitating NO production in bipolar disorders. Our study is expected to contribute to the further studies concerning arginase activity and NO levels in neuropsychiatric diseases.

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