COMPARISON BETWEEN THE EFFECTS OF TYPICAL AND ATYPICAL ANTIPSYCHOTICS ON OXIDATIVE STRESS STATUS IN SCHIZOPHRENIC PATIENTS

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Abstract: We determined the specific activities of some enzymatic antioxidant defenses like superoxide dismutase (SOD) and glutathione peroxidase (GPX), as well as a lipid peroxidation marker (MDA-malondialdehyde) from the serum of patients with schizophrenia treated with typical and atypical antipsychotics, in comparison with a normal age-matched control group. We observed an increased oxidative stress in schizophrenic patients treated with typical antipsychotics, compared to controls. Moreover, we demonstrated an antioxidant effect of atypical antipsychotics, since these patients showed an increased activity of SOD, compared to control subjects and a slightly decrease of MDA.

INTRODUCTION

Currently there are growing evidences that oxidative stress is implicated in several neuropsychiatric disorders such as dementia, schizophrenia or Parkinson’s disease (Hritcu et al., 2008, Padurariu et al., 2010).

There is accumulating evidences of altered antioxidant enzyme activities and increased levels of lipid peroxidation in schizophrenia (Wood et al., 2009). Studies performed in schizophrenia patients have generally suggested a compromised antioxidant system, but this is not always consistent with specific observed parameters, which on the whole, showed evidences of dysregulation. Reduced levels of the antioxidant enzymes specific activity are generally mentioned in patients with schizophrenia (Rafa et al., 2009, Pavlovic et al., 2002), but also reports about an increase antioxidant status in schizophrenic patients are presented (Rukimi et al., 2004). In addition, elevated levels of MDA have been shown in plasma, erythrocytes, leucocytes and platelets of schizophrenia patients (Kunz et al., 2008). It is believed that a high level of TBARS is a sign of peroxidative injury to membrane phospholipids. Neuronal functioning is affected by this injury either by changes in membrane fluidity or by alterations in membrane receptors (Mahadik et al., 2001), which can cause neurotransmitter uptake, release impairment and even cell death (Gama et al., 2006).

In this context, our objective was to determine some oxidative stress parameters (SOD, GPX and MDA) in schizophrenic patients treated with both typical and atypical antipsychotics, compared to a normal age-matched control group.

MATERIALS AND METHODS

The subjects of this study (45 patients) consisted of 39 patients with schizophrenia and 6 healthy age-matched controls. Patients were recruited from the Psychiatry University Hospital, Iasi, Romania and met DSM-IV criteria for schizophrenia (Azevedo et al., 1999). Patients were of paranoid subtype, with duration of illness for at least 5 years. They had all been receiving stable doses of oral neuroleptic medications for at least two years prior this study. In this way, the typical antipsychotics group (n=11) received haloperidol (1-2 mg daily dose) and fluanxol (20 mg/2 weeks) treatment, while the atypical antipsychotics (n=28) used were quetiapine (300 mg daily dose), olanzapine (20 mg daily dose), risperidone (2-4 mg daily dose), clozapine (30 mg daily dose), amisulpride (400- 600 mg daily dose) and ziprasidone (40 mg daily dose).

Healthy control subjects had major psychiatric disorders excluded based on history taking and psychiatric examination, according to the DSM-IV check list. Demographic data of the controls were chosen in order to match with the schizophrenic patients. Additionally, none of the subjects studied was taking antioxidant supplements.

The study was conducted according to provisions of the Helsinki Declaration and the local ethics committee approved the study. All the patients signed the consent for the participation in this study.

Biochemical estimations

Blood samples were collected in the morning, before breakfast, allowed to clot and centrifuged immediately. Sera were aliquoted into Eppendorf tubes and stored at -35°C until measurement.

Determination of SOD

Superoxide dismutase (SOD) activity was measured by the percentage reaction inhibition rate of enzyme with WST-1 substrate (a water soluble tetrazolium dye) and xanthine oxidase using a SOD Assay Kit (Fluka, 19160) according to the manufacturer’s instructions. Each endpoint assay was monitored by absorbance at 450 nm (the absorbance

wavelength for the colored product of WST-1 reaction with superoxide) after 20 min of reaction time at 37°C. The percent inhibition was normalized by mg protein and presented as SOD activity units.

Determination of GPX

The glutathione peroxidase (GPX) activity was measured using the GPX cellular activity assay kit CGP-1 (Sigma Chemicals). This kit uses an indirect method, based on the oxidation of glutathione (GSH) to oxidized glutathione (GSSG) catalyzed by GPX, which is then coupled with recycling GSSG back to GSH utilizing glutathione reductase (GR) and NADPH. The decrease in NADPH at 340 nm during oxidation of NADPH to $NADP^+$ is indicative of GPX activity.

Determination of MDA

MDA levels were determined by thiobarbituric acid reactive substances (TBARs) assay. 200 µL serum was added and briefly mixed with 1 mL of trichloroacetic acid at 50%, 0.9 mL of TRIS-HCl (pH 7.4) and 1 mL of thiobarbituric acid 0.73%. After vortex mixing, samples were maintained at 100 ºC for 20 minutes. Afterwards, samples were centrifuged at 3000 rpm for 10 min and supernatant read at 532 nm. The signal was read against an MDA standard curve and the results were expressed as nmol (Artenie et al., 2008, Ciobica et al., 2009).

Statistical analysis

Results were expressed as mean ± S.E.M. The results were analyzed statistically by means of the Student's "t" test (T- test: Paired Two Sample for Means). $p<0.05$ was taken as the criterion for significance (Georgescu and Dascalu, 2003).

RESULTS AND DISSCUSIONS

We observed an increased oxidative stress status in schizophrenic patients treated with typical antipsychotics, as shown by decreased SOD and GPX ($p<0,05$) specific activities (figure 1 and 2) and increased MDA concentration (figure 3), compared to control subjects. Also, we demonstrated an antioxidant effect of atypical antipsychotics, since these patients showed an increased activity of SOD, compared to control subjects (figure 1) and a slightly decrease of MDA (figure 3).

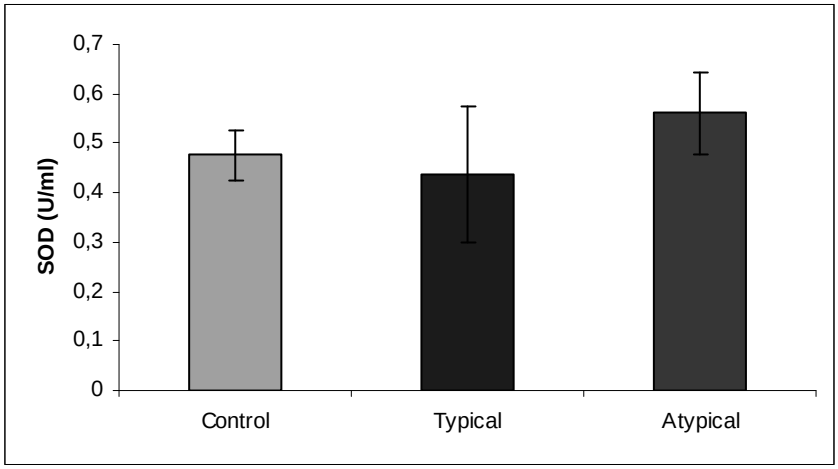

Figure 1. Superoxide dismutase specific activity in the serum of control subjects and schizophrenic patients treated with typical and atypical antipsychotics. The values are mean ± SEM (n=6 for control group, n=11 for typical antipsychotics group and n=28 for atypical antipsychotics).

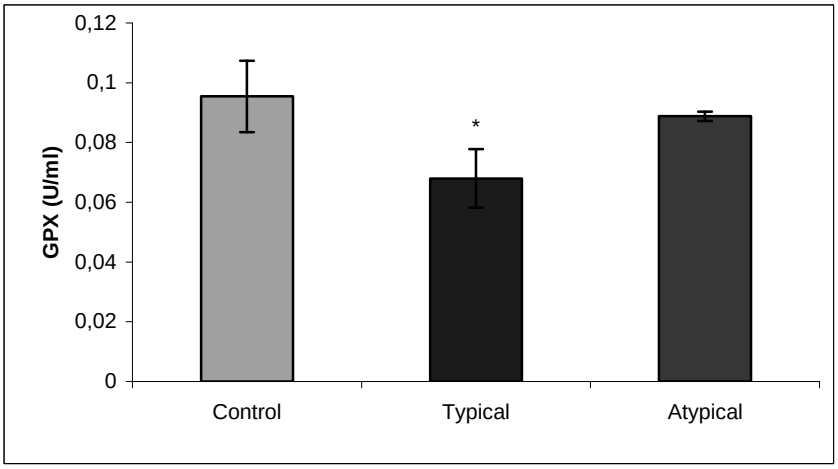

Figure 2. Glutathione peroxidase specific activity in the serum of control subjects and schizophrenic patients treated with typical and atypical antipsychotics. The values are mean ± SEM (n=6 for control group, n=11 for typical antipsychotics group and n=28 for atypical antipsychotics). *p<0,05 vs. control group.

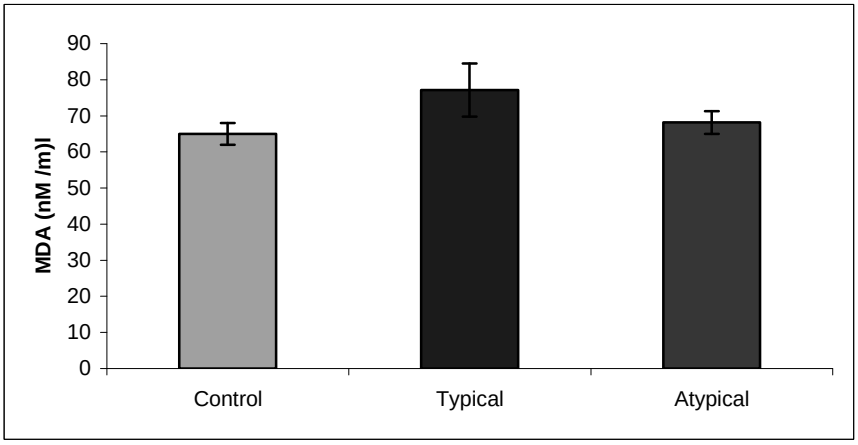

Figure 3. Levels of malondialdehyde in the serum of control subjects and schizophrenic patients treated with typical and atypical antipsychotics. The values are mean ± SEM (n=6 for control group, n=11 for typical antipsychotics group and n=28 for atypical antipsychotics).

As we previously mentioned, reduced levels of the antioxidant enzymes are generally reported in patients with schizophrenia compared with controls (Dadheech et al., 2008, Singh et al., 2008). However, few studies reported an increase of antioxidant status in schizophrenia. In this way, increased antioxidant activity may reflect a preceding cellular oxidative stress or serve as a compensatory mechanism (Kunz et al., 2008, Kuloglu et al., 2002). This difference in

observations may probably be attributed to varied clinical symptoms, therapeutic features or duration of the illness (Padurariu et al., 2010).

Also, there are controversies regarding the oxidative stress status in patients treated with typical vs. atypical antipsychotics. Some of the authors reported that chronic administration of typical antipsychotic haloperidol, but none of atypical antipsychotics like risperidone, clozapine or olanzapine induces oxidative stress by decreasing the activity of antioxidant enzymes causing membrane lipid peroxidation (Parikh et al., 2003). However, there are studies demonstrating a decreased level of lipid peroxidation in the cerebral cortex, as a result of haloperidol chronic administration (Martins et al., 2008), as well as significant reduction of GPX specific activity in the blood of long-term clozapine-treated schizophrenic patients (Miljevic et al., 2010, Polydoro et al., 2004).

In this paper we report an increased oxidative stress in schizophrenic patients treated with typical antipsychotics and an antioxidant effects of the aforementioned atypical antipsychotics.

CONCLUSIONS

Our results provide additional evidences that oxidative stress occurs in patients treated with typical antipsychotics. This was demonstrated by a decrease of the main antioxidant enzymes (SOD and GPX) and an increase of MDA, as a lipid peroxidation marker. Additionally, our results could raise some important issues for therapeutics in schizophrenia, considering the antioxidant action of the atypical antipsychotics treatment.

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