

OXIDATIVE STRESS STATUS IN PATIENTS WITH SCHIZOPHRENIA

Irina DOBRIN¹, Alin CIOBÎCĂ², Manuela PĂDURARIU³,
Cristinel ȘTEFĂNESCU⁴, Roxana CHIRIȚĂ⁵

Abstract:

We have 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, compared with a normal age-matched control group. The results showed an increased oxidative stress status in schizophrenic patients. These findings could raise some important issues concerning schizophrenia treatment, suggesting that one possible solution would be to associate antioxidants with the classical therapy.

Key words: schizophrenia, oxidative stress, antioxidant enzymes, lipid peroxidation

INTRODUCTION

Oxidative stress is the condition arising from the imbalance between toxic reactive oxygen species (ROS) and antioxidant defenses. Various tissues have different susceptibilities to oxidative stress. Brain is particularly more vulnerable to oxidative damage due to the relatively low levels of antioxidants, high levels of polyunsaturated fatty acids and the increase rate of oxygen utilization (1, 2).

It is believed that increased oxidative stress may be relevant to the pathophysiology of schizophrenia (3,4). Some authors suggested that the damage caused by the occurrence of oxidative stress might be the biochemical basis of neurodevelopmental abnormalities that are manifested in schizophrenia. Also, it is believed that oxidative stress may contribute to specific aspects of schizophrenic symptomatology and complications of its treatment (5).

The production of oxygen reactive species is a core part of mitochondrial energy generation, and these toxic compounds are dealt with by the body in multiple ways. These defenses include SOD, that catalyses the conversion of superoxide radicals to hydrogen peroxide, which is then converted into water by GPX and catalase. There is accumulating evidence of altered antioxidant enzyme activities and increased levels of lipid peroxidation in schizophrenia (6). Also, the concept of oxidative stress is intimately linked with the notion that, in many psychiatric disorders, there is an abnormality in mitochondrial energy generation. Molecular and genetic studies indicate that

¹ M.D., Drd, University of Medicine and Pharmacy "Gr. T. Popa" Iași; Resident in psychiatry, Clinical Psychiatric Hospital "Socola" Iași

² Lecturer, Ph.D., Researcher Assistant, University "Al. I. Cuza" Iași

³ Resident in psychiatry, Clinical Psychiatric Hospital "Socola" Iași

⁴ M.D., Ph.D., Assistant Professor University of Medicine and Pharmacy "Gr. T. Popa", Dept. of Psychiatry, Senior Psychiatrist, Clinical Psychiatric Hospital "Socola" Iași

⁵ M.D., Ph.D., Professor, University of Medicine and Pharmacy "Gr. T. Popa" Iași, Department of Psychiatry, Senior Psychiatrist, Clinical Psychiatric Hospital „Socola” Iași

disturbances in redox reactions are part of the pathophysiology of schizophrenia, including evidences of changes in proteins and metabolites that are involved in mitochondrial function, energy metabolism and oxidative stress responses. These were recently recognized as parts of the mitochondrial disorders, which are believed to contribute in some extent to various brain disorders, through the generation of ROS (7,8). Excessive formation of ROS can damage mitochondria, and at the same time, mitochondrial dysfunction increases the production of ROS (9). In this way, it is generally believed that an impairment of antioxidant defense system, mitochondrial dysfunctions and increased lipid peroxidation are very important parts in the schizophrenic pathology (10). However, despite the increasing information regarding oxidative stress mechanisms from the psychiatric literature of the last decade, the different biochemical pathways that might mediate oxidative stress in schizophrenia are not yet fully explained (11).

In this context, our objective was to determine some oxidative stress parameters (SOD, GPX and MDA) in patients with schizophrenia, 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, Iași, Romania and met DSM-IV criteria for schizophrenia (12). Patients were of paranoid subtype, with the duration of illness for at least 5 years. 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 °C 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 (13).

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 (14).

RESULTS

We report an increase oxidative stress status in patients with schizophrenia, as shown by a significant decrease of SOD specific activity ($*p<0,05$), compared to control subjects (Figure 1). Also, the specific activity of the other antioxidant enzyme determined, GPX, was reduced in comparison with the control group (Figure 2).

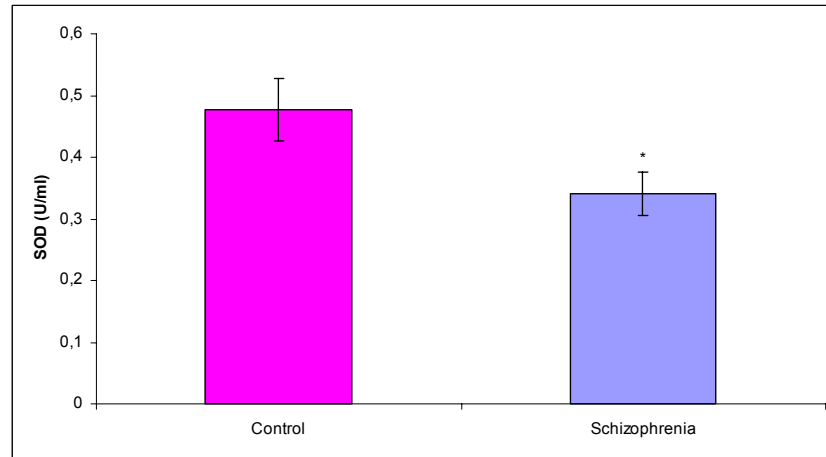


Figure 1. *Superoxide dismutase specific activity in the serum of control subjects and schizophrenic patients. The values are mean $\pm$ SEM (n=6 for control group, n=39 for schizophrenics group). $*p<0,05$ vs. control group*

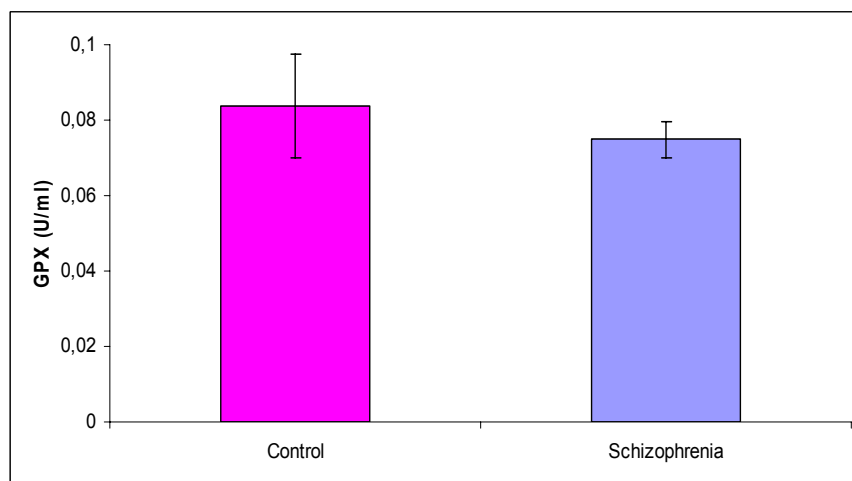


Fig. 2. *Glutathione peroxidase specific activity in the serum of control subjects and schizophrenic patients. The values are mean $\pm$ SEM (n=6 for control group, n=39 for schizophrenics group)*

Additionally, the concentration of serum MDA was significantly increased ($p<0,02$) in patients with schizophrenia, compared to the age-matched control group (figure 3).

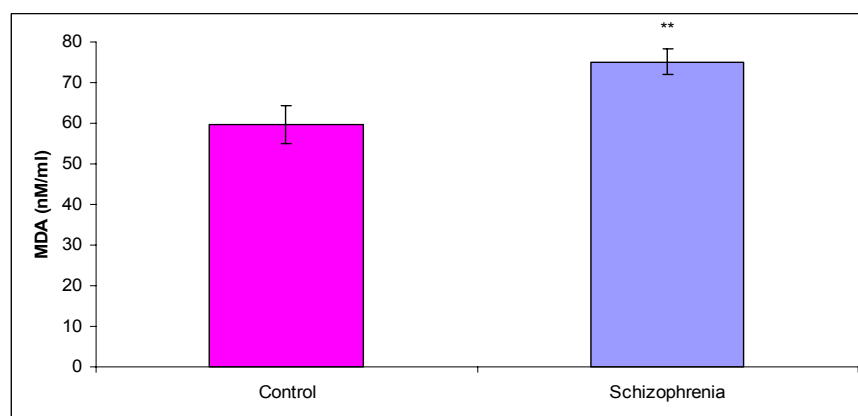


Fig. 3. Levels of malondialdehyde in the serum of control subjects and schizophrenic patients. The values are mean $\pm$ SEM ($n=6$ for control group, $n=39$ for schizophrenics group). ** $p<0,02$ vs. control group.

DISCUSSION

Generally, reduced levels of enzymatic oxidative stress markers are reported in patients with schizophrenia (15-18). However, few studies reported an increase of antioxidant status in schizophrenia, which could represent a preceding cellular oxidative stress or serve as a compensatory mechanism (19,20).

The equivocal results cited above may be due to differences in species used for each study (rats and humans), different tissues (brain and serum), therapeutic features or duration of the illness (21).

Our study clearly demonstrates that oxidative stress damage occurs in patients with schizophrenia. This could suggest that one possible solution for schizophrenia treatment would be the use of antioxidants. In this way, Zhang and colleagues demonstrated in two different studies from 2001 that adding a Ginkgo biloba extract to classical haloperidol treatment, results in enhancing the effectiveness of the antipsychotic and reduces some extrapyramidal side effects (22). In addition, adding Ginkgo biloba extract also resulted in better scores in the Scales for the Assessment of Positive and Negative Symptoms and decreased SOD levels (23).

The use of essential polyunsaturated fatty acids has also been suggested, considering that reduced levels of membrane essential polyunsaturated fatty acids and their association with psychopathology have been consistently reported in both chronic-medicated schizophrenic patients as well as in never-medicated patients soon after the first episode of psychosis (24,25).

In addition, a combination of eicosapentaenoic / docosahexaenoic acid and vitamin C/E resulted in a significant reduction of schizophrenia psychopathology, while the use of alfa-tocopherol alone has also been proved to be effective (26).

CONCLUSIONS

The present study provides additional evidences that oxidative stress occurs in patients with schizophrenia. This was demonstrated by a decrease of the main antioxidant enzymes (SOD and GPX) and an increase of MDA, as a lipid peroxidation marker, in schizophrenic patients, in comparison with the control subjects. In this way, oxidative stress seems to be a key component in the schizophrenia pathophysiology.

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REFERENCES:

1. Sultana, R., Piroddi, M., Galli, F., Butterfield, D.A. Protein levels and activity of some antioxidant enzymes in hippocampus of subjects with amnesic mild cognitive impairment. *Neurochem Res* 2008; 33: 2540-6.
2. Smith, M.A. Oxidative stress and iron imbalance in Alzheimer disease: how rust became the fuss! *J Alzheimers Dis* 2006; 9: 305-8.
3. Parikh, V., Khan, M.M., Mahadik, S.P. Differential effects of antipsychotics on expression of antioxidant enzymes and membrane lipid peroxidation in rat brain. *J Psychiatr Res* 2003; 37: 43-51.
4. Polydoro, M., Schroder, N., Lima, M.N. et al. Haloperidol and clozapine-induced oxidative stress in the rat brain. *Pharmacol Biochem Behav* 2004; 78: 751-6.
5. Zhang, X.Y., Tan, Y.L., Cao, L.Y. et al. Antioxidant enzymes and lipid peroxidation in different forms of schizophrenia treated with typical and atypical antipsychotics. *Schizophr Res* 2006; 81: 291-300.
6. Wood, S.J., Yücel, M., Pantelis, C., Berk, M. Neurobiology of schizophrenia spectrum disorders: the role of oxidative stress. *Ann Acad Med Singapore* 2009; 38: 396-6.
7. Rezin, G.T., Amboni, G., Zugno, A.I., Quevedo, J., Streck, E.L. Mitochondrial dysfunction and psychiatric disorders. *Neurochem Res* 2009; 34: 1021-9.
8. Jou, S.H., Chiu, N.Y., Liu, C.S. Mitochondrial dysfunction and psychiatric disorders. *Chang Gung Med J* 2009; 32: 370-9.
9. Serrano, F., Klann, E. Reactive oxygen species and synaptic plasticity in the aging hippocampus. *Ageing Res Rev* 2004; 3: 431-43.
10. Yao, J.K., Reddy, R.D., van Kammen, D.P. Oxidative damage and schizophrenia: an overview of the evidence and its therapeutic implications. *CNS Drugs* 2001; 15: 287-310.
11. Kunz, M., Gama, C.S., Andreazza, A.C. et al. Elevated serum superoxide dismutase and thiobarbituric acid reactive substances in different phases of bipolar disorder and in schizophrenia. *Prog Neuropsychopharmacol Biol Psychiatry* 2008; 32: 1677-81.
12. Azevedo, M.H., Soares, M.J., Coelho, I., Dourado, A., Valente, J., Macedo, A. Using consensus OPCRIT diagnoses. An efficient procedure for best estimate lifetime diagnoses. *Br J Psychiatry* 1999; 175: 154-7.
13. Artenie, V., Ungureanu, E., Negura, A., 2008, Metode de investigare a metabolismului glucidic și lipidic. Editura PIM, Iasi, 149-153.
14. Georgescu, G., Dascălu, C., 2003, Informatică aplicată și biostatistică, Editura Stef, Iași, 379-380.
15. Dadheech, G., Mishra, S., Gautam, S., Sharma, P. Evaluation of antioxidant deficit in schizophrenia. *Indian J Psychiatry* 2008; 50: 16-20.
16. Singh, O.P., Chakraborty, I., Dasgupta, A., Datta, S. A comparative study of oxidative stress and interrelationship of important antioxidants in haloperidol and olanzapine treated patients suffering from schizophrenia. *Indian J Psychiatry* 2008; 50: 171-6.
17. Raffa, M. A., Mechri, L.B., Othman et al. Decreased glutathione levels and antioxidant enzyme activities in untreated and treated schizophrenic patients. *Prog Neuropsychopharmacol Biol Psychiatry* 2009; 33: 1178-83.
18. Pavlović, D., Tamburić, I. Stojanović. Oxidative stress as marker of positive symptoms in schizophrenia, *Fact. Uni. Med. Biol* 2002; 9: 157-161.
19. Rukmini, M.S., B. D'Souza, V. D'Souza. Superoxide dismutase and catalase activities and their correlation with malondialdehyde in schizophrenic patients, *Indian J Clin Biochem* 2004; 19: 114-8.
20. Kuloglu, M., Ustundag, B., Atmaca, M., Canatan, H., Tezcan, A.E., Cinkilinc, N. Lipid peroxidation and antioxidant enzyme levels in patients with schizophrenia and bipolar disorder. *Cell Biochem Funct* 2002; 20: 171-5.
21. Pădurariu, M., Ciobîcă, A., Dobrin, I., Ștefănescu, C. Evaluation of antioxidant enzymes activities and lipid peroxidation in schizophrenic patients treated with typical and atypical antipsychotics, *Neurosci Lett* 2010. doi 10.1016 / j.neulet. 2010.05.088.
22. Zhang, X.Y., Zhou, D.F., Zhang, P.Y. et al. A double-blind, placebo-controlled trial of extract of Ginkgo biloba added to haloperidol in treatment-resistant patients with schizophrenia. *J Clin Psychiatry* 2001; 62: 878-83.
23. Zhang, X.Y., Zhou, D.F., Su, J.M., Zhang, P.Y. The effect of extract of Ginkgo biloba added to haloperidol on superoxide dismutase in inpatients with chronic schizophrenia. *J Clin Psychopharmacol* 2001; 21: 85-8.
24. Mahadik, S.P., Evans, D.R. Is schizophrenia a metabolic brain disorder? Membrane phospholipid dysregulation and its therapeutic implications. *Psychiatr Clin North Am* 2003; 26: 85-102.
25. Arvindakshan, M., Ghate, M., Ranjekar, P.K., Evans, D.R., Mahadik, S.P. Supplementation with a combination of omega-3 fatty acids and antioxidants (vitamins E and C) improves the outcome of schizophrenia. *Schizophr Res* 2003; 62: 195-204.
26. Yao, J.K., Reddy, R.D., van Kammen, D.P. Oxidative damage and schizophrenia: an overview of the evidence and its therapeutic implications. *CNS Drugs* 2001; 15: 287-310.