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OXIDATIVE STRESS AS MARKER OF POSITIVE SYMPTOMS IN SCHIZOPHRENIA

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Summary. *Schizophrenia, a serious hereditary disease, is a biological disorder of the brain resulting from abnormalities that arise early in life and disrupt a normal development of the brain. The chemical nature of schizophrenic brain is still not completely understood. The brain and nervous system are particularly prone to free radical damage since the membrane lipids are very rich in polyunsaturated fatty acid chains, and areas of human brain are very rich in iron, which plays an essential role in generating free radical species. Following the hypothesis that chronic schizophrenics are under oxidative stress which has exhausted the ability of their antioxidative capacity to adapt the elevated levels of circulating peroxides, we decided to examine the erythrocyte levels of lipid peroxidation products and reduced glutathione and the activities of antioxidative defence enzymes - superoxide dismutase (EC 1.15.1.1), glutathione peroxidase (EC 1.11.1.9) and catalase (EC 1.11.1.6) - as well as erythrocyte susceptibility to H_2O_2-induced oxidative stress in schizophrenic patients. The obtained results suggest a misbalance in pro/antioxidant status of chronic schizophrenics, which is more expressed in patients with positive symptoms of the disease.*

Key words: *Schizophrenia, oxidative stress, antioxidative defence*

Introduction

A major mental disorder that affects young people, schizophrenia is characterised by unknown etiology, complex pathology and long-lasting and not completely successful treatment. A growing body of evidence suggests that peripheral activities of antioxidant enzymes and lipid peroxidation are abnormal in schizophrenic subjects (1,2). Mahadik found increased lipid peroxidation products and altered defence system in both chronic and drug-naive first episode schizophrenics (3). The accumulated results indicate that oxidative stress is integral to this disease and not the result of neuroleptic treatment (4,5).

Oxidative damage inflected by reactive oxygen species is also referred to as oxidative stress. Oxidative stress is a result of increased formation of free radicals and/or reduced antioxidative system capacity. Neurons are particularly vulnerable to radical-mediated damage. High oxygen consumption, lipid content and transition metals are particular risk factors (6,7). Free radicals contribute to neuronal loss in cerebral ischemia and haemorrhage, and may be involved in degeneration of neurons in normal aging (8), epilepsy (9), Parkinson's disease (10), Alzheimer's disease (11), and possibly in schizophrenia (12). In addition to their pathological role, free radicals have critical physiological functions in neuronal development, differentiation and signal transduction (13,14), all of which may be altered in some cases of schizophrenia.

The effect of oxidative modification of neuronal phospholipids, DNA, and proteins on their function (i.e. membrane transport, loss of mitochondrial energy production, gene expression and, therefore, receptor-mediated phospholipid-dependent signal transduction (15,16)) may explain altered information processing in schizophrenia. Crow divided schizophrenia into two types (Crow's type I and II) according to clinical phenomenology, therapy response, and biochemical basis. Although it is obvious that these two syndromes frequently appear as two parts of a single process, it is possible to distinguish them according to their structural, biochemical and endocrinological specificities. Therefore, we wanted to explore possible changes in pro/antioxidant status in schizophrenics with positive and negative symptoms. Recently, it was shown that red blood cells superoxide dismutase increase in positive schizophrenia (Crow's type I), but not in Crow's type II (17). Due to a lack of literature data about this problem, we set out to explore possible markers of distinguishing and, perhaps, predicting the symptoms of this serious disease.

Since neuronal oxidative injury processes and underlying dynamic molecular regulatory mechanisms are reflected in peripheral blood cells, we could use red blood cells, platelets, lymphocytes and cultured skin fibroblasts in order to define these processes and find ways to prevent these kinds of injuries (18,19,20).

Patients and Methods

The study included 34 chronic schizophrenic patients: 20 (15 female and 5 male, aged 29 to 60, mean value = 42) in the phase of acute clinical impairment (patients with acute positive psychosis - Crow's type I) and 14 (11 female and 3 male, aged 32 to 61, mean value = 43) with negative schizophrenia (Crow's type II). The patients of Crow's type I showed hallucinatory and paranoid symptoms. The control group consisted of 15 voluntary blood donors of similar age and sex. Fasting blood samples obtained by venepuncture from patients and controls were drawn into heparinised tubes, which were then centrifuged at 2000 g for 15 min, plasma was carefully removed and the erythrocyte pellet was washed twice with equal volumes of saline and centrifuged at 2000 g for 15 min. Washed pellet was stored at -20^{o}C until analyses were carried out. The following parameters were determined in erythrocyte hemolyzate: Erythrocyte level of lipid peroxidation products was estimated by the spectrophotometric measuring of thiobarbituric acid (TBA) reactivity and expressed as malondialdehyde (MDA) content (21). The content of reduced glutathione (GSH) in red blood cells (RBC) was assayed using Elman's reagent (22). SOD activity in RBC was estimated using spectrophotometrical method based on auto-oxidation of pyrogallol (23). Selenium-dependent glutathione peroxidase activity in RBC was measured according to the method of Moin (24). Catalase activity in RBC was determined by the method of Beutler (25). For the estimation of erythrocyte susceptibility to H_2O_2-induced oxidative stress, MDA was detected in fresh untreated erythrocytes and after exposing to 3 mM H_2O_2 (dissolved in isotonic saline phosphate buffer pH 7.4, containing 2.0 mM of sodium azide to inhibit catalase activity) (26). The results were expressed as a difference between the two values. All the values were presented as a mean value ± SD and compared using Student's T-test.

Results

The obtained results are presented in Table 1. Lipid peroxides in erythrocytes (MDA) of the control group were 2.74 ± 0.41 nmol/g Hb. In Crow's type I, the mean value was 3.91 ± 0.82 nmol/g Hb, while in Crow's type II we also found an increase in this parameter (3.62 ±0.32 nmol/g Hb). The extent of lipid peroxidation in erythrocytes in both schizophrenic groups was similar ($p<0.001$) compared to the control. We found a significant decrease in GSH content in both groups of schizophrenic subjects (4.8 ± 0.91 μmol/g Hb; 5.8 ± 1.03 μmol/g Hb), but this decrease was statistically more significant in Crow's type I ($p<0.001$). There is also a statistically significant difference between the two schizophrenic groups ($p<0.05$). Erythrocyte Cu, Zn superoxide dismutase activity was significantly increased in Crow's type I (176 ± 39 U/g Hb, $p<0.01$) compared to control values (136 ± 28), while there were no significant differences in Crow's type II (145 ± 21). There is also statistically significant difference between Crow's types I and II ($p<0.05$). The estimation of glutathione peroxidase activity suggests a statistically significant decrease in both groups of schizophrenic patients, which was more expressed in Crow's type I (261 ± 62 U/g Hb, $p<0.001$; 301 ± 59, $p< 0.05$) in comparison to control values (364 ± 70). Erythrocyte catalase activity didn't show any significant difference between the groups studied. Baseline levels of erythrocyte peroxidation in both Crow's type I and Crow's type II were similar. In contrast, the susceptibility of erythrocyte lipids to peroxidation was significantly higher in Crow's type I after oxidative stress induced by H_2O_2 (40.06 ± 5.21, $p<0.001$), than in those with Crow's type II (35.62 ± 4.36, $p<0.01$). There is a statistically significant difference between Crow's type I and Crow's type II ($p<0.05$) as well.

Table 1. Parameters of oxidative stress in erythrocytes of schizophrenic patients

Variables	Control	Crow's type I	Crow's type II
Erythrocyte MDA (nmol/g Hb)	2.74 ± 0.41	3.91 ± 0.82 a***	3.62 ± 0.32 a***
Erythrocyte GSH (μmol/ g Hb)	7.07 ± 1.23	4.81 ± 0.91 a*** b*	5.8 ± 1.03 a*
Erythrocyte SOD (U/g Hb)	136 ± 28	176 ± 39 a** b*	145 ± 21
Erythrocyte GSH-Px (U/g Hb)	364 ± 70	261 ± 62 a** b*	301 ± 59 a*
Erythrocyte Catalase (U/g Hb)	12.36 ± 2.38	11.21 ± 2.06	12.87 ± 1.72
Erythrocyte MDA after H_2O_2-induced oxidative stress (nmol/g Hb)	29.05 ± 8.48	40.06 ± 5.21 a*** b*	35.62 ± 4.36 a**

a* **b*** $p < 0.05$
a** **b**** $p < 0.01$
a*** **b***** $p < 0.001$
a (statistical significance compared to control group)
b (statistical significance between Crow's type I and II)

Discussion

Contemporary knowledge in neuro-biochemistry increasingly emphasises the role of free radicals in the genesis of structural and functional changes of neuronal membrane that could be responsible for the beginning or aggravation of the basic disease (9,10,11,12). The brain and nervous system possess high potentials for the initiation of free radical reactions (6), which, relative to other tissues, can cause more damage in the brain and nervous system due to insufficient antioxidative protection and existing intensive aerobic metabolism accompanied with oxygen radical production. The brain contains both enzymatic and non-enzymatic antioxidants against free radical damage. The enzymatic antioxidants include superoxide dismutase (SOD), glutathione peroxidase (GSH-Px), catalase, glutathione reductase and

glucose-6-phosphate dehydrogenase (27). As the intensity of lipid peroxidation and antioxidative defence in erythrocytes to a certain extent reflects the state of the cell membranes of different tissues, including brain tissue (28), we investigated pro/antioxidant status in erythrocytes of schizophrenic patients with both positive and negative symptoms. The identified increase in lipid peroxidation products in erythrocytes in both schizophrenic groups supports the reports of other investigators (29,30). The changes in erythrocyte SOD activity in positive schizophrenia found in our study confirm the results of Vaiva et al. (17). Their hypothesis on the relationship between catecholaminergic hyper-metabolism and SOD increase in positive psychosis is supported by the fact that SOD activity could be an adaptive response of this enzyme to increased production of O_2 following oxidative decomposition of catecholamines.

Decreased erythrocyte GSH-Px activity in both types of schizophrenia could be explained by several factors. It is possible that it is a consequence of its oxidative inactivation (31). It could also be explained by its kinetic properties. The affinity of selenium GSH-Px for glutathione is low (32), so GSH-Px is not saturated with glutathione even at high concentrations of this substrate. Decreased glutathione content, found in our study, supports this hypothesis. Taking into account the fact that there were no significant changes in GSH-Px activity in drug-naive schizophrenic patients (33), the obtained decrease in this enzyme activity could be a consequence of exhausted adaptive response to a long-lasting oxidative stress during chronicity of this disease.

Erythrocytes have been extensively studied as a susceptible target for oxidative damage, since they are long-lived cells and very rich in Fe^{2+}-containing molecules, primarily Hb, that generate oxygen radicals (34, 35).

Taken together, the data suggest that changes in susceptibility of erythrocyte lipids to peroxidation observed in schizophrenics with positive and negative symptomatology may be explained in part by changes in levels of saturated and unsaturated fatty acids in RBC membranes in two types of schizophrenic subjects. Glen et al. suggest that negative symptoms are associated with high levels of saturated fatty acids and low levels of long-chain unsaturated ones in RBC membranes, while positive symptom patients show the opposite picture (36).

On the basis of these findings, it may be concluded that schizophrenic patients with positive symptoms are faced with increased oxidative stress. However, the underlying mechanisms have not yet been identified. It seems reasonable to assume that increased SOD activity and decreased GSH-Px activity, as well as decreased GSH content, might result in accumulation of H_2O_2 and other hydroperoxides in erythrocytes of schizophrenics with Crow's type I. We propose that it could be responsible for further production of free radicals in Fenton reaction (37) in brain tissue and amplification of oxidation of susceptible molecules, which could amplify the damage of neurons and lead to their death.

On the other hand, an increase in SOD activity results in reduction of quantity of $O_2^{\cdot}$, which is important for the process of nitric oxide (NO) degradation (38). Despite important physiological roles of NO, excessive formation or inadequate degradation of this compound has been suggested an important factor in the etiology of neurological disorders (38). In addition, there is an increasing body of evidence to support the concept that disruption of brain energy metabolism may be mediated by pro/antioxidant perturbation. Further more, NO may lead to mitochondrial permeability transition (39), which would compromise ATP synthesis and organelle's ability to sequester excess cellular Ca^{2+}(18), both of which could contribute to neuronal death and alter the information processing in schizophrenia.

Intensive oxidative stress in schizophrenia, increased level of intracellular Ca^{2+} and ROS can be potent activators of (MAP) kinases (40,41) and associated activation of transcription factor NF-κB (42). This factor regulates the expression of genes coding cell adhesion molecule proteins, nitric oxide synthase, proinflamatory cytokines, all of which play diverse roles in neuronal development, signal transduction, synaptic stabilization, neurogenesis, learning, and memory.

Conclusion

The obtained results support potential links between these observations. The fact that potentiated RBC susceptibility to oxidative stress, in Crow's type I, may reflect increased oxidative stress in brain tissue in this type of schizophrenia, gives an opportunity for using this parameter as one of markers for evaluation of the course and degree of this disease. These findings provide a theoretical basis for the development of novel therapeutic strategies, such as antioxidant supplementation.

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References

1. Reddy RD, Yao JK. Free radical pathology in schizophrenia. Prostaglandins Leukot Essent Fatty Acids 1996; 55(1-2):33-43.
2. Cadet JL, Kahler LA. Free radical mechanisms in schizophrenia and tardive diskynesia. NeurosciBiobehav Rev 1994;18(4):457-467.
3. Mahadik SP, Scheffer RE. Oxidative injury and potential use of antioxidants in schizophrenia. Prostaglandins Leukot Essent Fatty Acids 1996;55(1-2):45-54.
4. Yao JK, Reddy R, McElhinny LG, van Kammen DP. Reduced status of plasma total antioxidant capacity in schizophrenia. Schizohr Res 1998; 32(1): 1-8.

5. Yao JK, Reddy R, van Kammen DP. Reduced level of plasma antioxidant uric acid in schizophrenia. Psychiatry Res 1998; 80(1): 29-39.
6. Halliwell B. Oxidants and central nervous system. Some fundamental questions. Acta neurol Scand 1989;126:23-33.
7. Jesberger JA, Richardson JS. Oxygen free radicals and brain dysfunction. Int J Neurosci 1991;57:1-17.
8. Berlett SB, Stadtman ER. Protein oxidation in aging, disease and oxidative stress. J Clin Chem 1997;272(33):20313-20316.
9. Nikushkin EV, Kryzhanovski GN, Tupeev IR, Bordyukov MM, Yuzefova SM. Blood anitoxidant enzymes during epileptic activity. Biull Eksp Biol Med 1987;3:297-299.
10. Ceballos I, Lafon M, Javoy-Agid F, Hirsch E, Nicole A, Sinet PM, Agid Y. Superoxide dismutase in Parkinson's disease. Lancet 1990;335:1035-1036.
11. Smith CD, Carney JM, Starke-Reed PE, Oliver CN, Stadtman ER, Floyd RA, Markesbery WR. Excess brain protein oxidation and enzyme dysfunction in normal aging and in Alzheimer disease. Proc Natl Acad Sci USA 1991;88:10540-10543.
12. Abdalla DSP, Monteiro HP, Oliveira JAC, Bechara EJH. Activities of superoxide dismutase and glutathione peroxidase in schizophrenic and manic-depressive patients. Clin Chem 1986;32/5:805-807.
13. Garthwaite J, Charles SL, Chess-Williams R. Endothelium-derived relaxing factor release on activation of NMDA receptors suggests role as intercellular messenger in the brain. Nature 1988;336:385-387.
14. Matsumoto T, Pollock J, Nakane M, Forstermann U. Development changes of cytosolic and particulate nitric oxide synthase in rat brain. Dev Brain Res 1993;73:199-203.
15. Bennet CN, Horrobin DF. Gene targets related to phospholipid and fatty acid metabolism in schizophrenia and other psychiatric disorders: an update. Prostaglandins Leukot Essent Fatty Acids 2000; 63(1-2): 47-59.
16. Gispen WH. Nobel prize in physiology of medicine for year 2000 for research of signal trasduction in the nervous system. Ned Tijdschr Geneeskd 2000; 144(46): 2184-7.
17. Vaiva G, Thomas P, Leroux JM, Cottencin O, Dutoit D, Erb F, Goudemand M. Erythrocyte superoxide dismutase (eSOD) determination in positive moments of psychosis. Therapie 1994;49(4):343-348.
18. Strunecka A, Ripova D. What can the investigation of phosphoinositide signalling system in platelets of schizophrenic patients tell us? Prostaglandins Leukot Essent Fatty Acids 1999; 61(1): 1-5.
19. Yao JK, Reddy R, van Kammen DP. Abnormal age-related changes of plasma antioxidant proteins in schizophrenia. Psychiatry Res 2000; 97(2-3): 137-51.
20. Ramchand CN, Davies JI, Tresman RL, Grifiths IC, Peet M. Reduced susceptibility to oxidative damage of erythrocyte membranes from medicated schizophrenic patients. Prostaglandins Leukotr Essent Fatty Acids 1996; 55(1-2): 27-31.
21. Andreeva II, Kotemjakin AA, Kishkun AA. A modified thiobarbituric acid test for measuring lipid peroxidation products. Lab Delo 1988; 1: 41-43.
22. Beutler E, Duron O, Kelly BM. Improved methods for determination of blood glutathione. J Lab Clin Med 1968;61:882-888.
23. Marklund S, Marklund G. Involvement of the superoxide anion radical in the autooxidation of pyrogallol and a convenient assay for superoxide dismutase. Eur J Biochem 1974;47:469-474.
24. Moin VM. A simple specific method for assays of red cells glutathione peroxidase activity. Lab Delo 1986;12:724-727.
25. Beutler E. Catalase In: Beutler E (ed) Cell metabolism mannual and biochemical method. Grune and Stratton, New York 1982; 105-106.
26. Stocks J, Dormandy TL. The autooxidation of human red cell lipids induced by hydrogen peroxide. Br J Haemotol 1971; 20: 95-111.
27. Singh R, Pathak DN. Lipid peroxidation and glutathione peroxidase, glutathione reductase, superoxide dismutase, catalase and glucose-6-phosphate dehydrogenase activities in FeCl3-induced epileptogenic foci in rat brain. Epilepsia 1990;31:15-26.
28. Vilkov GA, Kiroi RI, Stepnina EG, Smirnova OB, Kovalenko VA, Trapezontseva RA. Lipid peroxidation and microviscosity of erythrocyte membranes in patients with schizophrenia. Zh Nevropatol Psikhiatr 1991;91(10):45-47.
29. Govorin NV, Govorin AV, Skazhutin SA. Significance of disorders of the process of lipid peroxidation in patients with persistant paranoid schizophrenia resistant to the treatment. Zh Nevropatol Psikhiatr 1991;91(7):121-124.
30. Arnao MB, Acosta M, Rio JA, Varom T, Garcia-Canovas FA. A kinetic study on the suicide inactivation of peroxidase by hydorgen peroxide. Biochim Biophys Acta 1990; (1):43-47.
31. Awasthi YC, Beutler E, Srivastava SK. Purification and properties of human erythrocyte glutathione peroxidase. J Biol Chem 1975;250(13):5144-5149.
32. Mukerjee S, Mahadik SP, Scheffer R, Correnti EE, Kelker H. Impaired antioxidative defense at the onset of psychosis. Schizophr Res 1996;19(1):19-26.
33. Davies KJA, Goldberg AL. Oxygen radicals stimulate intracellular proteolysis and lipid peroxidation by independent mechanisms in erythrocytes. J Biol Chem 1987;262(17):8220-8226.
34. Fung LWM, Zhang Y. A method to evaluate the antioxidant system for radicals in erythrocyte membranes. Free Radical Biol Med 1990;9:289-298.
35. Glen AI, Glen EM, Horrobin DF, Vaddadi KS, Spellman M, Morse-Fisher N, Ellis K, Skinner FS. A red cell membrane abnormality in a subgroup of schizophrenic patients: evidence for two diseases. Schizophr Res 1994;12(1),53-61.
36. Graf E, Mahoney JR, Bryant RG, Eaton JW. Iron-catalyzed hydroxyl radical formation. J Biol Chem 1984;259(6):3620-3624.
37. Murphy ME,Sies H. Reversible conversion of nitroxyl anion to nitric oxide by superoxide dismutase. Proc Natl Acad Sci USA 1991;88:10860-10864.
38. Heales SJR, Barker JE, Stewart VC, Brand MP, Hargreaves IP, Foppa P, Land JM, Clark JB, Bolanos JP. Nitric oxide, energy metabolism and neurological disease. Biochem Soc Trans 1997,25(3):939-943.
39. Packer MA, Scarlett JL, Martin SW, Murphy MP. Induction of the mitochondrial permeability transition by peroxynitrite. Biochem Soc Trans 1997,25(3):909-914.
40. Kyosseva SV, Elbein AD, Griffin WS, Mrak RE, Lyon M, Karson CN. Mitogen-activated protein kinases in schizophrenia. Biol Psychiatry 1999; 46(5): 689-96.
41. Kyosseva SV, Elbein AD, Hutton TL,Griffin ST, Mrak RE, Sturner WQ. Increased levels of trnscription factors Elk-1, cyclic adenosine monophosphate response element-binding protein, and activating transcription factor 2 in the cerebellar vermis of schizophrenic patients. Arch Gen Psychiatry 2000; 57(7): 685-91.
42. Mattson MP, Camandola S. NF-kB in neuronal plasticity and neurodegenerative disorders. J Clin Invest 2001; 107(3): 247-254.

OKSIDACIONI STRES KAO MARKER POZITIVNIH SIMPTOMA KOD SHIZOFRENIJE

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Kratak sažetak: *Shizofrenija, ozbiljno nasledno oboljenje, predstavlja biološki poremećaj mozga koji nastaje kao rezultat abnormalnosti u ranom životnom dobu i koji ometa normalan razvoj mozga. Hemijska priroda poremećaja u mozgu kod shizofrenije još uvek je nepoznata. Mozak i nervni sistem su naročito skloni oštećenju koje prouzrokuju slobodni radikali, zato što su membranski lipoproteidi bogati polinezasićenim masnim kiselinama, a centralni nervni sistem je bogat gvožđem koje ima suštinsku ulogu u stvaranju slobodnih radikala. U skladu sa hipotezom da su hronični shizofreni bolesnici pod oksidacionim stresom, koji iscrpljuje sposobnost antioksidacionog sistema za adaptaciju na povećanu produkciju peroksida, cilj ovog rada je ispitivanje nivoa lipidne peroksidacije u eritrocitima, određivanje sadržaja redukovanog glutationa, aktivnost antioksidacionih enzima – superoksid dismutaze (EC 1.15.1.1), glutation peroksidaze (EC 1.11.1.9) i katalaze (EC 1.11.1.6) - kao i osetljivost eritrocita na H2O2 indukovani oksidacioni stres kod shizofrenih pacijenata. Dobijeni rezultati pokazuju disbalans u pro/antioksidacionom statusu hroničnih shizofrenih bolesnika, koji je izraženiji kod pacijenata sa pozitivnim simptomima bolesti.*

Ključne reči: shizofrenija, oksidacioni stres, antioksidaciona zaštita