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 H2O2-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-naïve 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 inflicted 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°C until analyses were carried out. The following parameters were determined in erythrocyte hemolysate: 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 H₂O₂-induced oxidative stress, MDA was detected in fresh untreated erythrocytes and after exposing to 3 mM H₂O₂ (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 erythrocytes to peroxidation was significantly higher in Crow's type I after oxidative stress induced by H₂O₂ (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](image)

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...
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 increased level of intracellular Ca2+ and ROS can be a consequence of exhausted adaptive response to a long-lasting oxidative stress during chronicity of this disease.

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 Fe2+-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 H2O2 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 O2, 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 Ca2+(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 Ca2+ 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, proinflammatory 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.

**Acknowledgment:** This study is part of the MODULATORS OF REDOX CELL SIGNALING project (No 1711) that was financially supported by the Ministry of Science, Technology and Development of the Republic of Serbia.

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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 priroha 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 polinezasičenim 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 redukovanih glutathione, aktivnosti antioksidacionih enzima – superoksid dismutaze (EC 1.15.1.1), glutatian peroksidaze (EC 1.11.1.9) i katalaze (EC 1.11.1.6) – kao i osetljivost eritrocita na H2O2 indukovan 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 zaštit