

Pro- and antioxidant processes in schizophrenics with tardive dyskinesia

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Summary

Central nervous system diseases are connected with the production of an increased amount of reactive oxygen species. Decreased antioxidant activity is considered as one of the reasons of tardive dyskinesia (TD) in schizophrenic patients in a prolonged neuroleptic treatment course.

Aim. The evaluation of superoxide dismutase, catalase, glutathione peroxidase activity as well as lipid peroxidation by TBARS saturation in blood paleted in schizophrenic patients with or without tardive dyskinesia symptoms.

Methods. 84 paranoid schizophrenic patients took part in the study, 40 of them with TD symptoms. The groups were comparative in clinical and demographic terms. Indication of TBARS in blood paleted was performed by Placed and others' method. GSH-Px activity was indicated by Little and O'Brien method. CAT activity was indicated according to the Beers and others' method. CuZnSOD activity in blood paleted was indicated by Mirsa and Fridovich method.

Results. CuZnSOD activity in schizophrenic patients without TD is 820.23 and accordingly 710.75 U/g in TD symptoms patients and it is statistical essential. TBARS for patients with TD is 1.06 and accordingly without TD is 0.92 $\mu\text{mol}/109$, the difference is statistically essential. For groups with and without TD CAT activity is 19.87 and 17.93 U/g accordingly and GSH-Px is 32.30 and 30.48 U/g.

Conclusions. Schizophrenic patients with TD symptoms have lower CuZnSOD activity and higher concentration of TBARS in the paleted blood than patients without TD. CAT activity is higher in patients with TD symptoms. CuZnSOD activity and concentration of TBARS are in correlation with age in both groups under study.

Key words: schizophrenia, tardive dyskinesia, oxidative stress.

Introduction

Tardive dyskinesias (TD) are extrapyramidal symptoms occurring in the course of long-term neuroleptic treatment. TD is found in 20% of people taking neuroleptics on a chronic basis [1]. TD can co-occur together with other dysfunctions from the extrapyramidal range such as Parkinson's symptoms or dystonia [2, 3]. There are several hypotheses trying to explain TD origin. Many of them concentrate on the role of the neurotransmitter systems and oxidative stress function in extrapyramidal symptom

pathogenesis [4, 5, 6, 7]. “Free radical” hypothesis of TD genesis was defined due to observation of movements occurrence which are similar to TD in elderly people not treated with neuroleptics and suffering from schizophrenia [8]. In the context of this hypothesis it seems interesting to ask if schizophrenia patients with accompanying TD are different in terms of antioxidant enzymes activity from schizophrenia patients without symptoms of extrapyramidal range.

Cellular and extracellular compartments are equipped with a protective system against free radical damages. It consists of non-enzymatic ‘sweepers’ of reactive oxygen species (ROS) such as metal sequestering proteins, tocopherol, ascorbic acid, glutathione and antioxidant enzymes. Among important antioxidant enzymes superoxide dismutase – SOD, catalase – CAT and glutathione peroxidase – GSH-Px have an important significance. There are three SOD isoenzymes in humans: cytosole, containing copper and zinc in an active centre (CuZnSOD), mitochondrial with manganese in the centre of enzyme molecule (MnSOD) and extracellular SOD (ECSOD) also containing copper and zinc [9, 10]. Platelet blood cells were used excessively in scientific researches both to evaluate oxidative stress in the central nervous system and to know the molecular mechanism of drugs action used in psychiatric therapy in a better way [11, 12, 13, 14].

Aim of the study

In this study an attempt of evaluating the CuZnSOD, CAT, GSH-Px activity and lipid peroxidation level was made via the measurement of TBARS (thiobarbituro-reactive acid species) compounds concentration in blood platelets in schizophrenia patients with tardive dyskinesia presence and in patients without extrapyramidal symptoms. The following questions were formulated in the study:

- Is antioxidant enzyme activity in peripheral blood thrombocytes in TD patients different from activity measured in patients without extrapyramidal symptoms?
- Is the difference of TBARS concentrations in blood platelets isolated from both group patients stated?

Material and methods

84 paranoid schizophrenia patients took part in the study. They are treated in the Adult Psychiatry Department of the Medical University in Łódź. The diagnosis was made according to Psychiatric Disorders and Behaviour Disorders ICD-10 criteria, independently by two psychiatrists [15]. Tardive dyskinesia symptoms occurred in 40 patients. Socio-demographic and clinical characteristics of the groups under study are presented in Tab. I. Clinical evaluation was performed using the PANSS scale, whereas tardive dyskinesia exacerbation was evaluated with the AIMS scale [16, 17]. Presence of alcohol or other substance addiction as well as epilepsy were all excluded. Patients with clinical severe systemic disease symptoms, such as kidney, heart, severe hypertension, hyperthyroidism, auto-immunological diseases, bronchial asthma, chronic obstructive pulmonary disease, cirrhosis of the liver, tumour disease history

and gout were also excluded from the study. Patients taking part in the examination did not take such drugs as: aspirin, thymoleptic or antibiotics in the course of 7 days preceding the blood sample taking. They also did not fall ill with any respiratory system infection nor had fever.

Blood samples were taken from the elbow vein into a test tube with an anticoagulant (EDTA). TBARS marking in thrombocytes was made with Placer and others' method [18]. GSH-Px was marked with the Little and O'Brien method [19]. CAT activity in platelet blood cells was marked according to Beers and others method [20]. CuZnSOD activity in platelet blood cells was marked with the Misra and Fridovich method [21].

Statistical calculations were performed on an IBM PC computer with the application of automatic statistical analysis package-Statistica 5,1 PL (SN:SP8018052912G5). Statistical gravity was marked for $p < 0.5$. The study procedures were approved by the Bioethics Committee of Medical University in Łódź, decision No.RNN/76/03/KB.

Tab. 1. **Clinical and demographic features of schizophrenia patients with tardive dyskinesia and those without tardive dyskinesia**

Group under study	Patients with tardive dyskinesia symptoms N=40	Patients without tardive dyskinesia symptoms N=44
Sex (M/F)	5/35	7/37
Age (years)	52.3±9.2	51.8±8.5
Age of disease onset (years)	23.5±5.0	24.2±5.2
Length of disease duration (years)	32.2±11.3	31.1±10.2
Time of neuroleptic first dosage (years)	24.1±5.1	24.5±4.9
Current neuroleptic dosage(chlorpromazine equivalent)	320.1±156.3	335.3±147.7
Taking the patient's history in the direction of mental diseases (yes/no)	15/25	15/27
Results in AIMS	5.5±3.1	X
Results in PANSS: P/N/G	26.5±5.1/21.3±4.2/40.2±11.2	23.8±7.2/18.9±5.3/41.5±12.8

Results

Study results are presented in Tab. 2. Correlation of selected socio-demographic indicators, clinical scales results and results of examined parameters are shown in Tab.3.

Discussion

In many neurodegenerative diseases (dementia, Parkinson's disease, amyotrophic lateral sclerosis), as well as auto-immunological diseases (sclerosis multiplex), reactive oxygen species are a very important element of degenerative changes the pathogenesis of the central nervous system [22, 23]. Basal nuclei are especially prone to oxidative damage due to an intensive oxygen metabolism, high dopamine concentration and

Tab. 2. **Markers of oxidative stress for patients with schizophrenia who did or did not have tardive dyskinesia, SD – standard deviation**

	Patients with tardive dyskinesia average / SD	Patients without tardive dyskinesia average / SD	Statistical analysis
CuZnSOD (U/g)	710.75/142.68	820.23/146.38	t=2.979/p<0.05
CAT (UB/g)	19.87/2.46	17.93/3.93	t=2.302/p<0.05
GSH-Px (U/g)	32.30/7.01	30.48/8.15	t=1.491/p>0.05
TBARS (nmol/109)	1.06/0.20	0.92/0.17	t=3.401/p<0.01

Tab. 3. **Correlation between antioxidant enzyme activities, concentration of TBARS and clinical features of schizophrenic patients. *p<0.05**

Schizophrenia patients with tardive dyskinesia symptoms									
							PANSS		
							P	N	G
CuZn SOD	-0.36*	-0.03	-0.16	-0.10	-0.12	-0.25	0.04	-0.01	-0.11
CAT	0.17	0.01	0.07	0.02	0.07	0.21	0.04	0.0	0.03
GSH-Px	0.05	0.11	0.09	0.07	-0.03	0.09	0.02	-0.01	0.07
TBARS	0.37*	0.08	0.18	0.06	0.16	0.26	0.02	0.06	0.02
Schizophrenia patients without tardive dyskinesia symptoms									
CuZn SOD	-0.35*	-0.07	-0.14	-0.09	-0.11	X	0.07	0.03	0.02
CAT	0.19	0.03	0.06	-0.02	0.02	X	-0.02	0.05	0.01
GSH-Px	0.09	0.06	0.07	0.07	0.01	X	0.03	-0.02	0.0
TBARS	0.38*	0.09	0.21	0.10	0.13	X	0.06	0.06	0.04

significant contents of iron [24]. Neuroleptic medications usage induces dopamine production in corpus striatum and indirectly exacerbates oxidative stress [25]. Bivalent iron reacting with ROS (Fenton's reaction) catalyses coming into existence an especially toxic hydroxyl radical which initiates lipid peroxidation process [26].

In the 1980's some attention was paid to the fact that many mental diseases are accompanied by discreet anatomical changes of the encephalon structure connected with disordered central nervous system (the neuro-developmental hypothesis of schizophrenia) [27]. Accepting the research perspective of the neuro-developmental paradigm of schizophrenia attracted attention to the apoptosis role in schizophrenia pathogenesis and to a role of oxidative stress as a potential source of encephalon damage [28, 29]. It was indicated that thiobarbituro-dependent compounds concentration in schizophrenia patients is bigger in comparison to healthy people [30, 31, 32]. Arvindaksan and others found that a positive correlation exists between TBARS concentration in the serum

and seriousness of psychotic symptoms in paranoid schizophrenia patients [33]. The difference in TBARS concentration was observed between the groups under study. This indirectly proves a bigger exacerbation of tissue damage in tardive dyskinesia patients in comparison to patients without extrapyramidal symptoms. It should be also indicated that TBARS is a poorly sensitive and poorly specific marker of oxidative tissue damage. Thus, observation of the difference in their concentration is only of a certain heuristic value, not of a diagnostic or pathogenetic one. It should be stressed that marking of TBARS concentration is a common research procedure, but an interpretation of the obtained results allows for observing only a direction change and it should not lead to jumping into conclusions, which was emphasized by Janero [34].

The observed difference in the zinc-copper dismutase activity proves the diminished antioxidant potential in tardive dyskinesia patients. CuZnSOD is the “first” tissue defence enzyme against reactive oxygen species. This enzyme begins a reaction sequence where superoxide anion (O_2^-), and then hydrogen peroxide (H_2O_2) are used as substrates, and it leads to in-activation of the most toxic of the ROS group, namely hydroxyl radical (OH) [35]. In many independent research centres, a decrease in the CuZnSOD activity in schizophrenia patients was observed [36, 37]. It is worth mentioning that zinc-copper dismutase activity does not return to the activity level in the control group even after obtaining full clinical remission [38]. It can be assumed that decreased CuZnSOD activity is one of the risk factors of neuro-developmental changes directing to the clinical manifestation in the form of a schizophrenic process. The very same processes, which lead to neuro-developmental changes in the prenatal and adolescence periods, are the cause of neurodegenerative changes of the extrapyramidal system. It is manifested by the clinical presence of tardive dyskinesias. Parikh and others compared the influence of selected neuroleptic drugs on CuZnSOD activity. The biggest pro-oxidative potential was demonstrated for haloperidol, the smallest- for olanzapine [39]. Chronic treatment with typical neuroleptic drugs (Fluphenazine, Haloperidol, Perphenazine) resulted in the 10%-20% decrease of neuron number in the corpus striatum of rats, as compared the to starting value [40]. Even a single haloperidol administration to rats resulted in apoptosis, mainly in striatum and substantia nigra [41]. Qing et al. and Zhang and other researches have shown that in comparison to classical neuroleptic drugs, atypical neuroleptic drugs, mainly olanzapine, affect the balance oxidant/antioxidant to a lesser degree [42, 43]. According to Wei and others, Olanzapine has additional antioxidant action by increasing gene induction for manganese dismutase, which protects the mitochondria against negative ROS activity [44]. Classical neuroleptic drugs additionally inhibit I complex of respiratory chain (NADH –dehydrogenasis) by increasing the reactive oxygen species production in mitochondria, which secondarily induces apoptosis and brain damage [45]. Damaged mitochondria emit pro-apoptogenic factors - cytochrome C included - into the cytoplasm [46]. That is why extrapyramidal symptoms onset cannot be explained only due to dopamine receptors blockage. Brown et al. focused their attention on the antioxidant activity of schizophrenia patients depending on the presence or absence of TD symptoms. They reported the a significant CuZnSOD activity TD decrease in TD patients in comparison to schizophrenia patients without extrapyramidal symptoms

[47]. Similar observations confirm our earlier and current research [48]. Zhang and others have especially interesting observations, as they demonstrated a negative correlation between dismutase activity in the serum and a result in the AIMS scale [49]. Our results did not show such a dependence.

In these studies a slightly bigger catalase activity was shown in TD patients in comparison to those without extrapyramidal symptoms. CAT is a peroxysomal enzyme decomposing peroxides, hydrogen peroxide included. It is a result of the catalytic SOD function and also plays a part in reactions catalysed by numerous oxidases and oxygenases. Processes of catecholeamine autooxidation are a very important source of hydrogen superoxide in the central nervous system. The observed CAT activity increase in the group of patients with extrapyramidal symptoms is, probably, connected with adaptation changes on the level of gene expression coding antioxidative enzymes [50].

In our studies glutathione peroxidase was no different in patients from both groups under study. Studies describing both, decreasing this enzyme activity, and also lack of essential differences in its activity between schizophrenia patients with extrapyramidal symptoms and without TD presence were already published earlier. GSH-Px does not play such a key role in the protection against ROS as SOD, and it may be the cause of the lack of differences. Both, in TD patients, as in those without extrapyramidal symptoms, CuZnSOD activity correlated negatively with the age (Tab. I). TBARS concentration correlated positively in both groups together with the age. Sokal et al. stated that life longevity of many species depends on SOD activity [51]. It is possible that ageing is connected with SOD activity decrease with following lipid peroxidation exacerbation, which is depicted by TBARS concentration increase in elderly people.

Conclusions

- Schizophrenia patients with tardive dyskinesia symptoms have a lower zinc-copper dismutase activity and higher thiobarbiturate compounds concentration in blood platelets in comparison to people without extrapyramidal symptoms.
- Catalase activity in blood platelets is higher in patients with tardive dyskinesia symptoms.
- Along with the age of schizophrenia patients, zinc-copper dismutase activity decreases, whereas thiobarbituric-dependent compounds concentration increases.
- Glutathione peroxidase activity in the thrombocytes does not differ in patients with tardive dyskinesia symptoms and those without extrapyramidal symptoms in the course of schizophrenia.

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