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CLINICAL NEUROCHEMISTRY

The State of Albumin Thiol Groups in Patients with the First Episode of Schizophrenia

V. V. Brilliantova[a], N. V. Smolina[a], T. I. Syreishchikova[b], M. G. Uzbekov[a, 1], and G. E. Dobretsov[c]

[a] *Moscow Research Institute of Psychiatry, Ministry of Health of Russian Federation, Moscow, Russia*
[b] *Lebedev Physical Institute, Russian Academy of Sciences, Moscow, Russia*
[c] *Research and Clinical Center of Physicochemical Medicine, Federal Medical-Biological Agency of the Russian Federation, Moscow, Russia*

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Abstract—In this paper, the state of the body's antioxidant defense was studied using the state of thiol groups in the albumin in patients with their first episode of schizophrenia (FES). We examined 21 patients with the first psychotic attack of schizophrenia; the average severity of the disorders was 75 ± 2 points on the PANSS scale. All patients were examined prior to initiation of the drug therapy. The control group consisted of ten healthy volunteers. The concentration and reactivity of the albumin SH groups was determined in the reaction with dithionitrobenzoic acid. As a result of the study, we found a 24% decrease in the average reactivity of albumin SH groups in the FES group compared with the control group ($p = 0.02$). Using two parameters, that is, the concentration and reactivity of albumin thiols, it was possible to separate the patient group and the control group, with the probability of a relationship of the patient to the respective group of 86%. Thus, patients with the first episode of schizophrenia before the start of treatment are characterized by significant disturbances in the concentration and reactivity of the albumin SH-groups involved in redox processes in the body.

Keywords: first episode of schizophrenia, thiol group of albumin, concentration of reduced thiols, reaction rate constant, Ellman reaction

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INTRODUCTION

Schizophrenia is one of the most severe and socially important mental illnesses; its careful analysis is one of the urgent problems of psychiatry. Early intervention after the first episode of schizophrenia (FES) accelerates of the onset of remission, reduces social losses and makes it possible to treat the neurocognitive deficiency observed in these patients and improve their quality of life at the initial stage of the disease [1]. Understanding the molecular processes in schizophrenia is a fundamental requirement for the development of diagnostic, differential-diagnostic, and prognostic methods.

All this puts the task of in-depth study of the pathophysiological mechanisms of the first episode of schizophrenia. Our knowledge of the pathogenetic mechanisms of FES, in spite of intensive study, still remains fragmentary [1, 2].

It has been shown that patients with the first episode of schizophrenia have significant metabolic disturbances, changes in enzyme activity with the accumulation of toxic products in the body, and impaired functional properties of blood albumin even before the start of therapy [3, 4]. In this respect, the state of protection systems against emerging intoxication and developing oxidative stress is of great interest. This problem is usually analyzed by measurements of ascorbic acid, antioxidant enzymes (SOD), etc. [5–7]. The concentration and reactivity of thiol groups of albumin, which are active participants in oxidative processes, were not previously determined in the blood serum for mental illnesses. However, the disturbance of this link in the redox processes in the mentally ill may play a role in the development of metabolic disorders, which were previously identified as a syndrome of endogenous intoxication [8].

The albumin molecule connects many important processes in the body: as a transport protein, it participates in the transfer of endogenous substances (fatty acids, bilirubin, and many metabolites) and drugs [9]. Serum albumin has one reduced SH group at the Cys-34 position. During termination of the free-radical oxidation chain, this thiol group is irreversibly oxidized. Under physiological conditions albumin thiols com-

[1] Corresponding author; address: ul. Poteshnaya 3, Moscow, 107076 Russia; phone: +7 (495) 963-1409; e-mail: uzbekovmg@gmail.com.

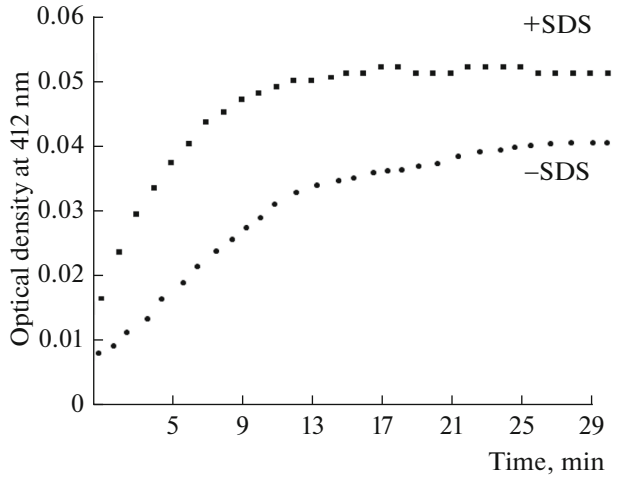

Fig. 1. The change in optical density at 412 nm as a result of the reaction of albumin thiols with dithionitrobenzoic acid (data from a typical experiment).

pose up to 80% of all detectable high-molecular-weight thiols of the plasma [10]. It appears that albumin is non-specifically involved in reactions with radicals; however, its involvement is determined by the fact that its concentration in the extracellular space is relatively high and its metabolism is relatively rapid (approximately 20 days) [11].

The performance of the antioxidant function by albumin depends not only on its reduced thiols, but also on the conformational state of the albumin molecule [12]. Thus, the state of the thiol group of albumin may act as a marker of its conformational changes, which will eventually affect albumin's transport functions. In this regard, the study of the concentration and reactivity of albumin SH-groups in various pathological processes is of considerable interest, not only as a factor of antioxidant protection, but also as an important link in the metabolic processes in the body.

The aim of this study was to study the changes in the concentration and reactivity of albumin thiol groups and their contribution to the development of endogenous intoxication in patients with their first episode of schizophrenia.

MATERIALS AND METHODS

The group of subjects was 21 people (mean age 28 ± 9). All patients entered the Moscow Institute of Psychiatry and were examined during the first attack of schizophrenia, before the treatment. The severity of the disorders before treatment was 75 ± 2 points on the PANSS scale, which corresponds to moderate severity of the disorder. The clinical picture and criteria for inclusion and exclusion from the study were previously described in [3, 4].

The control group consisted of ten people who, according to clinical and biochemical indicators, were assigned to a group of healthy people who were comparable in sex and age with the group of patients. The biochemical parameters in patients with their first episode were determined upon admission to the clinic, before the beginning of pharmacotherapy.

The concentration and reactivity of albumin SH groups was determined in the albumin fraction of the serum. Blood serum was obtained by a standard method. The albumin fraction was prepared by the method described in [13] by separation in a two-phase system with polyethylene glycol 4000 (Sigma). The SH-group concentration was determined in the classic Ellman reaction [14] of thiol disulfide exchange with a thiol-specific reagent dithio-bis-nitrobenzoic acid (DTNB, Sigma) (Fig. 1), in the presence of detergent sodium dodecyl sulfate (SDS, Acros Organics). The reactivity of the albumin SH groups was evaluated by modifying the Ellman reaction in the absence of detergent. Eighty μL of albumin fraction was mixed with 1 mL of 0.0002 M DTNB. The kinetics of changes in optical density at a wavelength of 412 nm were recorded. The duration of the measurement was 30 minutes with 1 minute increments (Fig. 1). In the reaction without SDS, the reaction rate constant was determined, whose magnitude was used to evaluate the reactivity of the albumin Cys-34 thiol. The optical density was measured on a Beckman Coulter Du-7 spectrophotometer.

Mathematical data processing was performed using the Guggenheim method for pseudo-first order reactions [15]. The increase of the optical density $A(t)$ with time t was approximated by the equation:

$$A(t) = A_0 + (A_\infty - A_0)(1 - \exp(-tK_V)),$$

where K_V is the rate constant for the interaction of albumin SH-groups with DTNB and A_0 and A_∞ are the optical density at the beginning and at the end of the reaction, respectively.

The transformation of this equation leads to a formula for K_V determination:

$$\frac{A(t) - A_0}{A_\infty - A_0} = 1 - \exp(-tK_V);$$

$$\ln\left(1 - \frac{A(t) - A_0}{A_\infty - A_0}\right) = -tK_V.$$

The constant K_V was determined for the time interval from 0 min to the curve exit to the "plateau." The concentration was calculated using the last point at 30 minutes. The rate constant for the reaction of albumin SH-groups with DTNB, K_V, was obtained (Fig. 1). K_V characterizes the rate at which unoxidized albumin thiols enter the reaction of thiol–disulphide exchange; a similar reaction occurs in the body during free-radical oxidation.

The reagents we used were PEG-4000 and 5,5'-Dithio-bis(2-nitrobenzoic acid) (Sigma);

Table 1. The mean concentration and reaction capacity of albumin thiols in the blood serum of patients with FES and the control group. The data are presented as the mean and standard error of the mean

Group	Concentration of albumin thiols in blood serum, [SH], μM	Reaction capacity of albumin thiols, K_V, min^{-1}
Control group	394 ± 37	0.25 ± 0.03
Patients with the first episode of schizophrenia	277 ± 32	0.17 ± 0.02
p Value	$p = 0.366$	$p = 0.017$

NaH_2PO_4, Na_2HPO_4, and Dodecyl sulfate sodium salt (SDS) were from Acros Organics (Belgium).

The statistical processing of the results was performed using the Statistica for Windows v.6 application software using the Mann–Whitney test for independent samples. The data in the text are presented in the form $M \pm m$.

RESULTS

A comparison of the concentration of reduced thiol groups of albumin in healthy individuals and patients with FES showed a tendency to decrease in the group of patients (Table 1).

Investigation of the reactivity of the SH groups retained in the albumin fraction of the initial blood serum revealed a significant decrease in the reaction rate constant (K_V) in the FES group: 0.17 ± 0.02 min^{-1} vs. 0.25 ± 0.03 min^{-1} in the control (Table 1), i.e., by 24%, $p < 0.05$.

Using a combined analysis of the concentration and reactivity of the thiol groups of serum albumin, it was shown that the areas of the points that relate to patients and healthy subjects differ significantly (Fig. 2).

The equation of the linear discriminant function is as follows: $Y_d = aX + b$.

In order to mathematically describe the division of the "healthy" group and the "patient" group in terms of a combination of two parameters, that is, the concentration and reactivity of the albumin thiol groups, the linear discriminant function $Y_d = aX + b$ was used, where Y is the reactivity of albumin thiol groups and X is the concentration of albumin thiol groups. The coefficients of the equation were chosen in such a way as to divide the "healthy" and "sick" groups with maximum probability. The linear discriminant function described by this equation is shown in Fig. 2. Thus, if a patient with a certain X value has a Y value greater than Y_d, then, according to the state of his albumin thiol groups, he may be related to the healthy group. If at his X value, the Y value is less than Y_d, then his state of albumin thiol groups corresponds to the state in the patients. The probability of relating a patient to the patient group using this linear discriminant function is 86%. If we divide these two regions using a linear discriminant function, then the sensitivity of the method (the probability of correct assignment to the patient group on the basis of results) is 85.7% and the specificity (the probability of correct attribution to the healthy group on the basis of the results) is 88.8%.

DISCUSSION

This study showed that the first episode of schizophrenia is accompanied by a change in the reactivity of serum albumin, which may affect the free-radical processes in the patient's body. Both the literature data [7] and our previous studies [16] point to an increase in the level of free radicals in schizophrenia and the serious consequences of these changes in the body.

Free-radical production in this disease may increase due to several causes. We have previously shown a dramatic increase in monoamine oxidase activity in chronic schizophrenia and FES [4, 17]; this enzyme is one of the main sources of free radicals in the brain [18]. As well, the formation of highly toxic free radicals that cause neuronal damage leads to hyperproduction of dopamine in some brain structures in schizophrenia and its further metabolism [19]. In turn, these processes that occur in the brain affect the characteristics of the blood plasma, including serum albumin, and increase the overall oxidative activity of the blood.

In addition, the activity of certain antioxidant systems of the body, such as superoxide dismutase (SOD), glutathione peroxidase, and catalase [20], decreases. These results indicate that redox processes are substantially disturbed in patients with schizophre-

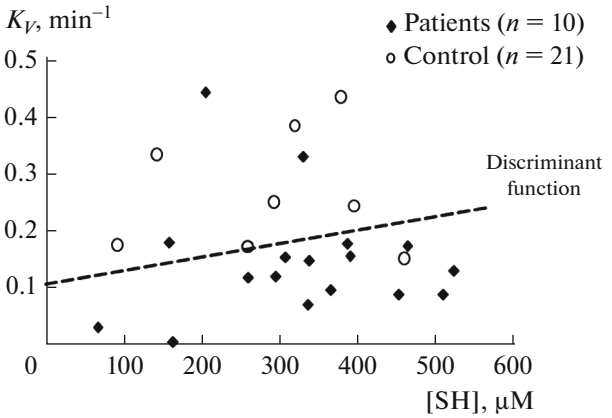

Fig. 2. The correlation between the concentration and reactivity of albumin thiols in patients with the first episode of schizophrenia and in the control group.

nia. This is expressed both in the intensification of oxidative processes and in the weakening of the protective antioxidant systems of the body. These processes have been observed not only in chronic patients but also in the first psychotic episode [21]. This suggests that the disturbance of redox processes is involved in the pathogenesis of the disease.

Since albumin accounts for up to half of all serum protein and participates in the transport of many compounds and in protection against free-radical oxidation, the change in its functions may have a considerable effect on the change in redox processes in the body.

However, the albumin antioxidant function depends not only on the fraction of its reduced thiols but also on the conformational state of the albumin molecule. The SH-group is located in an albumin molecule in a certain cavity (we will call this the "pocket") [9]. If at a given conformation of the molecule this "pocket" is not available for interaction with target molecules (for example, free radicals), then, in spite of the presence of the reduced SH group, the molecule will not participate in redox reactions. If the molecule is unfolded and the SH group becomes available for interaction at the smallest concentration of free radicals, then as a result, almost the entire pool of albumin thiols may be oxidized. In addition, the characteristics of the albumin thiol group may serve as markers of its conformational changes.

Previous and current studies have shown pronounced metabolic disturbances in patients with FES, which, in particular, affect the characteristics of the binding centers and the conformation of the serum albumin molecule.

CONCLUSIONS

In this study, it was found that before the start of therapy in patients with the first episode of schizophrenia the reactivity of the thiol group in the serum albumin molecule, as well as the concentration of these groups in serum, decreased ($p < 0.05$), which probably leads to changes in redox processes that occur with the participation of albumin thiol groups.

In our opinion, the most informative indicator of the state of albumin thiol group is the function that is a linear combination of the total concentration and reactivity of albumin thiol groups. Using a combination of these two parameters it was possible to distinguish the patients and the control group with 86% reliability.

CONFLICT OF INTEREST

The authors declared no conflicts of interest.

ETHICAL APPROVAL

All patients gave informed consent for participation in the study. The study was performed in compliance with the protocol approved by the ethics committee of the Moscow Research Institute of Psychiatry of the Ministry of Health of the Russian Federation on April 27, 2009, and in accordance with the Declaration of Helsinki for experiments involving people.

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