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Albumin Binding-Site Alteration in Melancholic Depression under Pharmacotherapy: Recording with the Use of Subnanosecond Fluorescence Spectroscopy

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Abstract—It is known that the conformation of human serum albumin binding sites is sensitive to pathological processes. In this work, changes in the physical–chemical properties of albumin binding sites in melancholic depression were studied. The K-35 fluorescent probe (dimethylaminonaphthalic acid *N*-carboxyphenylimide, CAPIDAN) was used as a reporter of these changes. It is shown that the fluorescence decay of K-35 depends on the state of the drug-binding sites of albumin. The kinetics of fluorescence decay were measured with a time resolution of approximately 30–50 ps. The parameters that characterize the fluorescence decay of K-35 in serum were reliably responsive to melancholic depression and the dynamics of its treatment. With melancholic depression, a decrease in the concentration of nonesterified long-chain fatty acids that are capable of affecting binding sites of serum albumin was observed. However, variations in the concentration of NEFA cannot be considered as a cause of the alterations of albumin binding sites. In addition to NEFA, other factors are likely to affect structural and physical-chemical properties of albumin in depression patients.

Keywords: depression, albumin, fluorescent probe K-35, time-resolved spectroscopy
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Depression is one of the most frequent and severe mental disorders. To date, there are no objective laboratory indicators in medical practice via which it would be possible to assess the severity of a patient's condition and the effectiveness of the therapy in the initial stages of depression. Therefore, the search for indicators, that is, biomarkers, that would "feel" the course of depressive states and the effectiveness of their therapy is rather relevant [1]. The authors of [2] concluded that the diagnostically important signs of mental illness might be found on the periphery, primarily, in the blood. Possible endogenous intoxication and probable damage to the main function of albumin (transport and detoxification) and exacerbation of endotoxicosis in patients with schizophrenia has been suggested [3–5]. The results of clinical studies carried out by the authors for several decades on a large clinical material [6–16] show that under stress, schizophrenia, and certain types of depression, the physicochemical properties of the binding centers of a transport protein, namely, albumin, change. The main research method was fluorescence spectroscopy in the subnanosecond range. The use of fluorescent probes [17, 18] revealed abnormalities in the binding centers of patients with the first episode of schizophrenia [3] and patients with anxious [19] and melancholic depression [14].

The purpose of this work is an attempt to find indicators that characterize the changes in albumin under melancholic depression during the treatment of patients, using subnanosecond fluorescence spectroscopy. Recording such changes in combination with clinical and biochemical data could make early individual assessment of the effectiveness of psychopharmacotherapy and prognosis of the development of the disease in depression more accurate.

MATERIALS AND METHODS

The K-35 fluorescent probe (dimethylaminonaphthalic acid *N*-carboxyphenylimide, CAPIDAN) was synthesized and kindly provided by B.M. Krasovitskii

Abbreviations: K-35, dimethylaminonaphthalic acid *N*-carboxyphenylimide, HSA, human serum albumin, and NEFAs, long-chain nonesterified fatty acids.

and coworkers from the Institute of Monocrystals, National Academy of Sciences of Ukraine, Kharkov, Ukraine. In the experiments, a lyophilized preparation of human serum albumin (HSA), free of fatty acids, from Sigma-Aldrich (lot no. A1887) was used. Human albumin was dissolved in a phosphate buffer solution (Sigma-Aldrich, United States) containing 0.137 M of sodium chloride and 0.01 M of sodium phosphate, pH 7.4, and palmitic acid (Sigma- Aldrich, United States, lot no. 61H24601). An aqueous solution of 1.4 mM of K-35 was added to an HSA solution in the buffer to the desired concentration.

The concentration of nonesterified fatty acids in sera was determined by the enzymatic (calorimetric) method using a set of reagents FA 115 (Randox, United Kingdom). The concentration of albumin in serum was carried out by the fluorescence method [8] and a method using bromocresol green.

Twenty-two patients with a diagnosis of melancholic (depressing) depression who were under treatment at the Moscow Research Institute of Psychiatry of the Ministry of Health of the Russian Federation were examined. The clinical piture and the criteria for inclusion and exclusion from the study were described in [14]. The control group included 54 healthy volunteers without somatic and mental pathologies, who were comparable in sex and age with the group of patients. Informed consent for participation in the study was obtained from all subjects. The study was conducted in accordance with the Helsinki Declaration on the ethical principles for conducting medical research involving people and the conclusion of a local ethical committee of the Moscow Research Institute of Psychiatry.

Blood samples were taken three times from each patient: before treatment (22 serum samples), after 15 days of treatment (20 serum samples), and after 30 days of treatment (17 serum samples) with Velafaxine. The blood serum of the patients was diluted 20-fold with a phosphate buffer solution. In each serum sample, a K-35 fluorescent probe was added to a final concentration of 30 μM. Approximately 95% of the total fluorescence intensity is emitted by K-35 molecules bound to albumin [15]. The amplitudes of the probe decay are normalized to the concentration of HSA in this serum, so that the K-35/HSA molar ratio was approximately unity.

The fluorescence decay of the K-35 probe bound to serum albumin was measured in nano- and picosecond ranges using a laser device developed at the Lebedev Physical Institute, Russian Academy of Sciences [14, 19]. Fluorescence was excited by a rapid laser flash ($7 \cdot 10^{-10}$ s). Using a personal computer based on the AMD Sempron processor, both the measurement process and the processing of experimental data (TimoHarp and FluoFit programs, Picoquant) were automated.

Before measuring each serum, the kinetics of fluorescence decay of the calibrator (a solution of 3-methoxybenzanthron in ethanol) were determined. This made it possible to compare the fluorescence intensities measured on different days and to use the calibrator as a standard of the amplitude to calculate the number of fluorescent molecules of the K-35 probe in sera [20].

Data for patients with depression were presented as the mean ± the error of the mean or as individual indicators for each patient. The significance of the differences was assessed using the Wilcoxon test (for coupled samples). The results were statistically processed using Statistics 6.0 and Excel 2003.

RESULTS AND DISCUSSION

Interaction of the K-35 probe with an albumin molecule. The decay of the fluorescence of K-35 bound to albumin at subnanosecond resolution is described by the sum of exponentials ([21], Eq. (1)):

$$F(t) = \sum_{i=1}^{i=3} A_i \exp(-t/\tau_i); \quad B = b\sum_{i=1}^{i=3} A_i\tau_i;$$
$$s_i = A_i\tau_i/B,$$

where t is the time after light absorption, $F(t)$ is the fluorescence intensity at time t, A_i are the amplitudes of the three exponentials, τ_i is the decay time of their fluorescence, the product of $A_i\tau_i$ is proportional to the total fluorescence flux emitted by the ith component, B is the total fluorescence intensity, b is the instrument constant, and s_i is the fraction of the total fluorescence flux in each exponent.

In HSA isolated from serum and defatted under physiological conditions (pH 7.4; ionic strength 0.159 M) the fluorescence decay times and the mean-square errors of their measurement (including fittings errors) are close to the following values: 7.7–9.5 ns for τ_1, 2.8–3.5 ns for τ_2, and 0.7–1.1 ns for τ_3 [21].

Decay of fluorescence in depression. Analysis of all fluorescence decay parameters of the K-35 probe in the serum samples of donors and patients before and after treatment with Velafaxine showed that before the treatment of the patients, the mean amplitudes A_1, A_2, and A_3 in the sera of these patients were significantly higher than in the group of donors. The application of the Wilcoxon test to independent samples of donors and patients showed a significant difference between these groups ($p < 0.025$). In the course of treatment, the fluorescence intensity of the K-35 probe (parameter B) decreases and it approaches the fluorescence of the donors.

The dynamics of changes in the mean values of amplitudes and the total fluorescence intensity of K-35 for patients before treatment and after 15 and 30 days of treatment is demonstrated in the table.

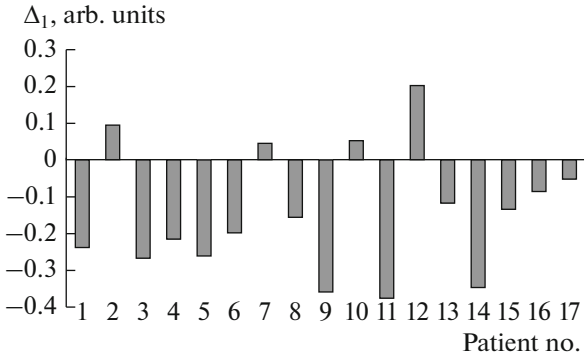

Fig. 1. The Δ_1 indicator (the relative change in the decay amplitude A_1) for patients with melancholic depression for 30 days of treatment with Velafaxine.

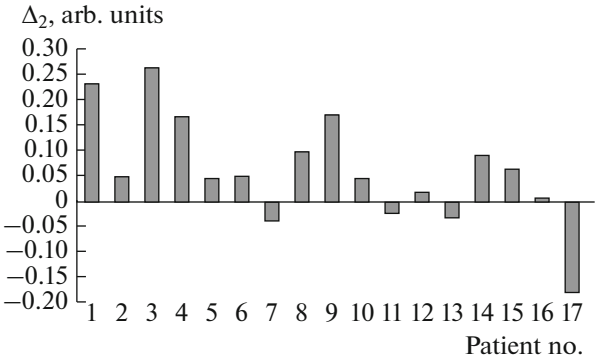

Fig. 2. The Δ_2 indicator (change in the ratio of decay amplitudes of K-35 A_3 and A_1) for patients with melancholic depression for 30 days of treatment with venlafaxine.

The relative (in comparison with the corresponding parameter for donors) changes of all three amplitudes during the treatment of melancholic depression are almost the same: the differences are within a few percent. The total intensity of fluorescence also decreases during treatment. Previous measurements in the anxiety depression group revealed changes only in the A_1 [19] parameter.

We introduce the parameters that characterize the relative changes in amplitudes during the treatment:

$$\Delta_1 \equiv \frac{A_{1\,\text{after}} - A_{1\,\text{before}}}{A_{1\,\text{before}}};\quad \Delta_2 \equiv \frac{\left(\frac{A_3}{A_1}\right)_{\text{after}} - \left(\frac{A_3}{A_1}\right)_{\text{before}}}{\left(\frac{A_3}{A_1}\right)_{\text{before}}},$$

where Δ_1 is the relative change in the amplitude of fluorescence decay of K-35; A_1 and $A_{1\text{before}}$ is the amplitude of fluorescence decay of K-35 for patients with melancholic depression before treatment; $A_{1\,\text{after}}$ is the amplitude of fluorescence decay of K-35 for patients after 30 days of treatment with venlafaxine; Δ_2 is the change in the ratio of the decay amplitudes of K-35 A_3 to A_1; $(A_3/A_1)_{\text{before}}$ is the amplitude ratio for patients before treatment; and $(A_3/A_1)_{\text{after}}$ is the amplitude ratio for patients after 30 days of treatment with Velafaxine.

Figures 1 and 2 demonstrate the indicators Δ_1 and Δ_2 for 17 patients with melancholic depression. It is seen that a decrease in A_1 and the growth of A_3/A_1 are typical results of treatment. The two-sided Student's *t*-test showed that, on average, for a group of 17 patients, the amplitude of A_1 significantly decreased with $p < 0.01$.

The indices τ_i and s_i did not change significantly.

Thus, it was possible to detect unidirectional changes in the structure of the albumin molecule in patients diagnosed with melancholic depression after therapy. Differences between the amplitudes of the fluorescence decay functions before and after treatment were 15–20% and significant at a level of 1%. The magnitude of the changes was reliably recorded. This led to the conclusion that this method can be considered as potentially useful for an objective evaluation of the effectiveness of pharmacotherapy, and the studied parameters can serve as potential biomarkers.

Change in the concentration of fatty acids in the sera of patients with melancholic depression. HSA is a carrier of long-chain nonesterified fatty acids (NEFAs) in the blood, and these acids affect the drug binding centers. K-35 is very sensitive to the amount of NEFAs bound to HSA: an increase in the molar ratio of NEFAs/HSA to three enhances the fluorescence intensity by one and a half times, while the A_3/A_1 ratio increases more than threefold [22].

The mean values of amplitudes ($A_{i\,\text{dep}}$) and the total intensity (B_{dep}) of fluorescence of K-35 for patients before treatment and after 15 and 30 days of treatment

	Number of people	$A_{1\,\text{dep}}/A_{1\,\text{don}}$, arb. units	$A_{2\,\text{dep}}/A_{2\,\text{don}}$, arb. units	$A_{3\,\text{dep}}/A_{3\,\text{don}}$, arb. units	$B_{\text{dep}}/B_{\text{don}}$, arb. units
Donors	54	1.00 ± 0.02	1.00 ± 0,03	1.00 ± 0.03	1.00 ± 0.03
Patients (before treatment)	22	1.23 ± 0.05	1.21 ± 0,04	1.19 ± 0.04	1.18 ± 0.04
Patients (15 days of treatment)	20	1.09 ± 0.06	1.09 ± 0,05	1.05 ± 0.05	1.09 ± 0.03
Patients (30 days of treatment)	17	0.97 ± 0.07	0.97 ± 0,07	1.03 ± 0.07	1.04 ± 0.02

All results are normalized to the average value of the corresponding indicator for donors ($A_{i\,\text{don}}$ and B_{don}); the data are presented as $M \pm m$.

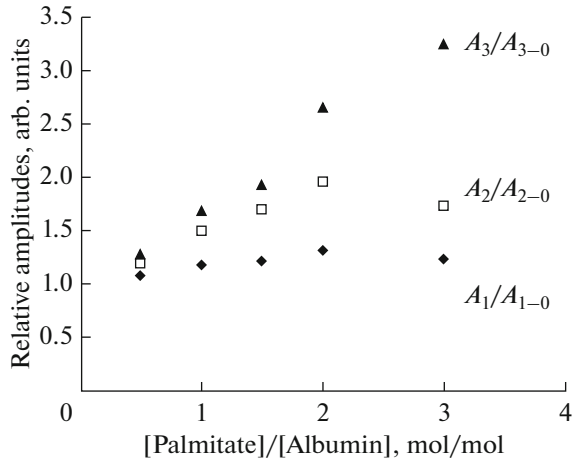

Fig. 3. The effect of palmitate on the fluorescence amplitude of he K-35 probe in defatted HSA. Concentrations of HSA and K-35 are 16 μM. A_{1-0}, A_{2-0}, and A_{3-0} are the amplitudes of the three components of the fluorescence decay of K-35 in the absence of palmitate; A_1, A_2, and A_3 are these amplitudes in the presence of palmitate. Abscissa axis, the molar ratio of palmitate/HSA; ordinate axis, the decay amplitudes of K-35, normalized to the corresponding amplitude in the absence of palmitate.

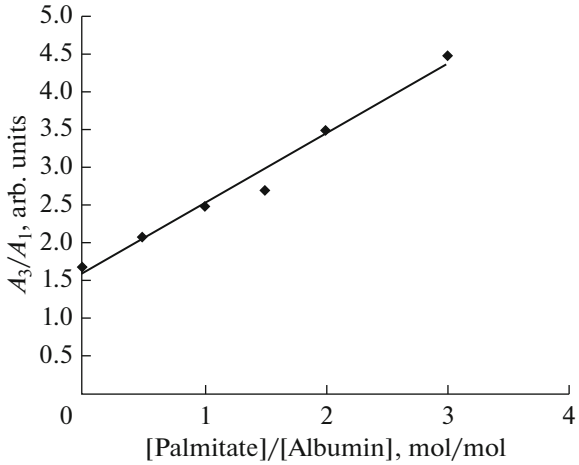

Fig. 4. The change in the ratio of the fluorescence amplitudes A_3/A_1 of the K-35 probe in defatted serum albumin upon addition of palmitate. Concentrations of HSA and K-35 are 25 μM. Abscissa axis, the molar ratio of palmitate/HSA; ordinate axis, the ratio of the amplitudes A_3/A_1.

To determine whether NEFAs are the cause of changes in fluorescence in depression, the values of NEFAs/HSA were determined in the serum samples from 26 donors and 18 patients with depression. In patients with depression, the number of fatty acids per albumin molecule is significantly lower, by almost two times, with respect to healthy objects.

Such a decrease in the concentration of NEFAs in serum could lead to a decrease in all three amplitudes

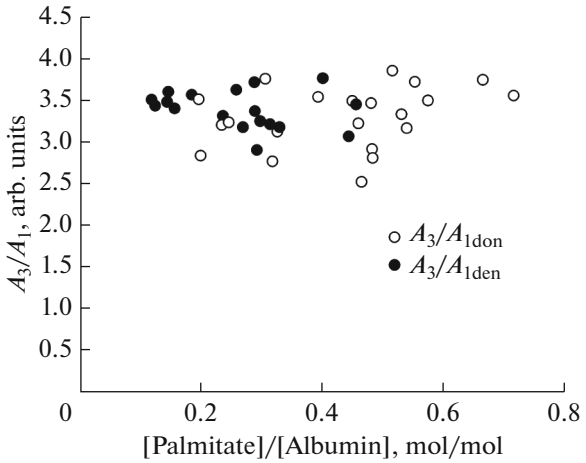

Fig. 5. The dependence of the ratio A_3/A_1 for donors ($A_3/A_{1\,\text{don}}$) and patients ($A_3/A_{1\,\text{dep}}$) with melancholic depression from the relative concentration of NEFAs.

of A_i [22]. In fact, it was shown using the model mixtures of NEFAs + HSA that the fluorescence factors are closely correlated with the ratio of NEFA/albumin (Figs. 3 and 4). The ratio of the A_3/A_1 amplitudes in the region of the molar concentrations of NEFA/albumin from 0 to 3 was approximated by a linear dependence with the value of reliability of the linear bond of $R_2 = 0.99$.

However, when studying the decay of K-35 in the serum samples of donors and patients with depression, no correlation was found between the fluorescence parameters of the probe and the concentration of fatty acids (Fig. 5).

Changes in the albumin molecule probably do not equate with variations in the concentration of NEFAs in serum; that is, some factors besides NEFAs affect the albumin molecule. These factors are disturbed by melancholic depression and change during treatment.

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