EXPERIMENTAL METHODS FOR CLINICAL PRACTICE

Study of High-Resolution H^1 Nuclear Magnetic Resonance Spectra of the Serum and Its Albumin Fraction in Patients with the First Schizophrenia Episode

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We studied high-resolution ^{1}H nuclear magnetic resonance spectra of the serum and serum albumin from patients with the first episode of schizophrenia and healthy individuals. A relative increase in signal intensities of CH_2 protons in serum LDL and VLDL in schizophrenia was demonstrated. Higher intensities of CH_2 and CH_3 protons of non-esterified fatty acids were found in ^{1}H nuclear magnetic resonance spectra of serum albumin. These data attest to an essential role of changes in lipid metabolism and changed ligand load of albumin in schizophrenia.

Key Words: *^{1}H nuclear magnetic resonance spectroscopy; serum; lipids; albumin; first episode of schizophrenia*

High-resolution nuclear magnetic resonance (NMR) spectroscopy in a potent magnetic field (14.09 T or 600 MHz frequency of ^{1}H NMR for protons) allows detection and even quantitative assay of important metabolites in biological fluids and changes in their composition in various pathologies and during therapy [2,4]. Plasma and serum are used for NMR studies. Signals of low- and high-molecular-weight compounds are present in ^{1}H NMR spectra of the plasma or serum: amino acids (Ala, Val), ketone bodies, sugars (Lac), creatine, creatinine (Cre), cholesterol, choline (Chol), lipoproteins, *etc.* [8]. Wide signals of proteins are suppressed by using specially selected pulse sequence.

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It was shown with a K-35 fluorescent probe (CAPIDAN) [1] that native conformation of binding centers in serum albumin is modified in chronic schizophrenia, which presumably modifies its ligand load. However, no conformation changes of binding center in serum albumin were detected by the fluorescent method in patients after the first episode of schizophrenia (FES) [3]. We attempted detecting changes in metabolism and ligand load of serum albumin by high-resolution ^{1}H NMR spectroscopy of the serum and serum albumin in FES patients.

MATERIALS AND METHODS

Patients (n=19, 6 women, 13 men, mean age 28.2±9.5 years) with the first psychotic episode were examined by clinical and biochemical methods. Detailed description of the clinical picture and criteria of group formation have been presented previously [3]. None of the

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patients received antipsychotic therapy before hospitalization at clinical department of Moscow Institute of Psychiatry. The patients and healthy volunteers gave written informed consent to participation in the study, which was carried out in accordance with the Helsinki Declaration. Control group consisted of 4 sex- and age-matched individuals referred by their clinical and biochemical parameters to a group of healthy subjects. Studies by ^{1}H NMR were carried out with the serum and serum albumin fraction isolated using polyethylene glycol as described previously [13].

High-resolution ^{1}H NMR spectra of blood serum and albumin fraction water solution were recorded in an Avance-600 device (Bruker) with water signal presaturation. Serum concentration calculated by serum albumin concentration was 50 mg/ml, albumin fraction concentration in water solutions was 25 mg/ml; the amount of D_2O with phosphate buffer dissolved in it added to serum and albumin samples was 10% v/v.

For suppression of potent water signal, all spectra were recorded using pulse sequence *zgcppr* [5] RD – 90° – t_1 – 90° – t_m – 90° – FID, where RD was relaxation delay, during which a selective radiofrequency exposure at the water signal frequency was carried out (256 scans, spectral width 15,000 Hz). The complex ^{1}H NMR spectrum of the serum was simplified by experiments with an extra pulse t_1 reducing the intensity of wide signals from macromolecular components and protein-bound compounds. The resultant spectra contained signals of low-molecular-weight metabolites and amino acid residues located on the surface of protein molecules and having mobility greater than the main globule.

RESULTS

Figure 1 presents serum spectra of a healthy individual and a FES patient. The narrowest signals are those from protons of some metabolites (in ppm): 2.92 (singlet, Cre), 1.35 (doublet, CH_3 Ala), 1.20 (doublet, CH_3 Lac), and 0.94 (quartet, CH_3 Val). A wide signal from LDL (L_1) and HDL (L_2) lipid CH_2 protons is located in the serum spectrum at 1.15-1.05 ppm, while LDL and HDL (L_1) CH_3 protons wide signal is located at

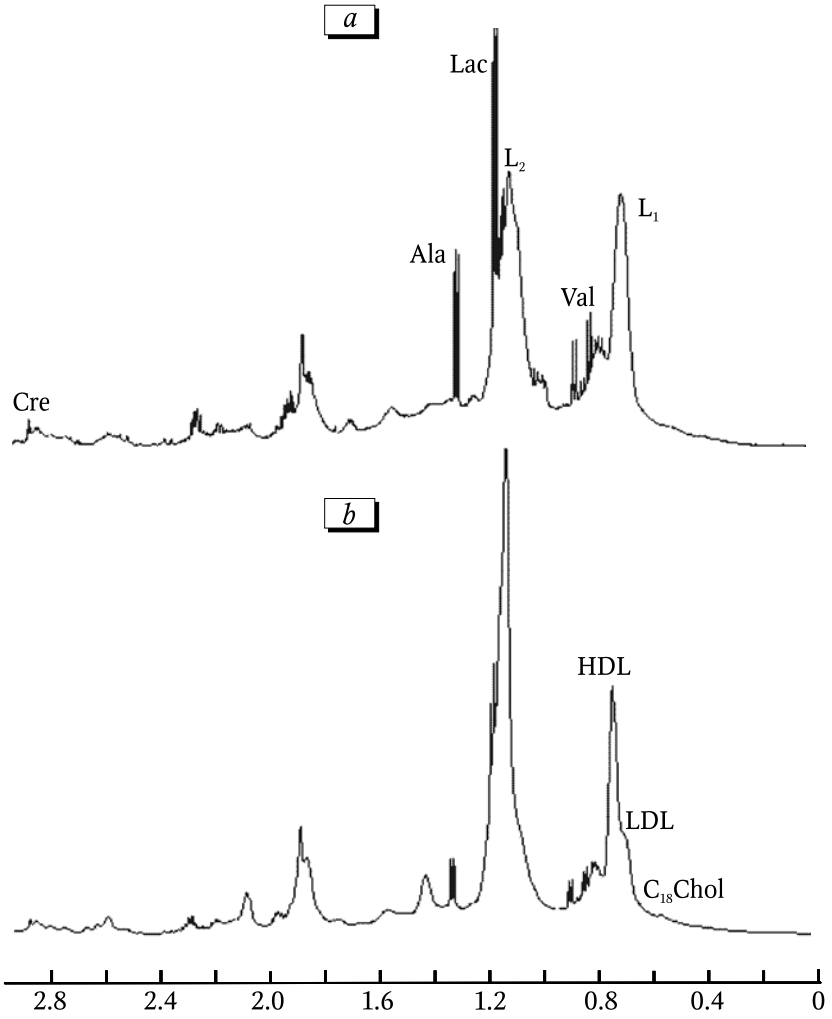

Fig. 1. ^{1}H NMR spectra recorded with water signal "pre-saturation" sequence for normal human serum (*a*) and serum from a FES patient (*b*). Here and in Fig. 2: abscissa: chemical shift, ppm.

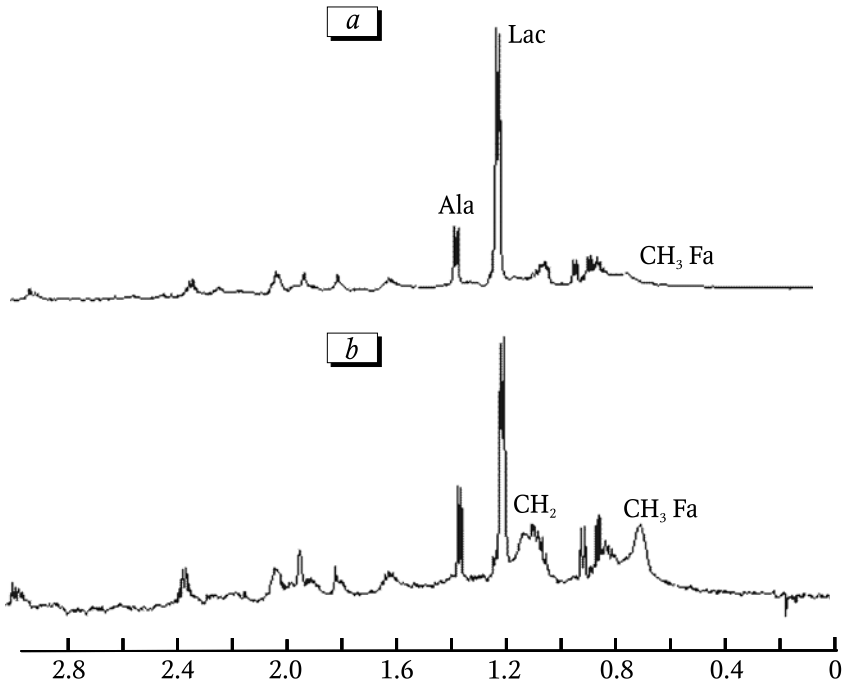

Fig. 2. ^{1}H NMR spectra recorded with water signal "pre-saturation" sequence for albumin isolated from normal human serum (*a*) and serum from a FES patient (*b*). fa: fatty acids.

0.8-0.6 ppm. Serum spectra of normal subjects were similar to those recorded previously [4,8].

Changes in the proportion of intensities of signals from N-acetylaspartate and creatine N-CH_3 protons shown by magnetic resonance tomography of the brain in the thalamus region [10,] were not characteristic of the high-resolution spectra of the serum, because of poor detection of these signals against the background of wide lines of other components.

High-resolution ^{1}H NMR spectra of the sera from donors and FES patients differed by the regions corresponding to lipid CH_2 and CH_3 proton signals (Fig. 1). Enhanced lipid CH_2 proton signal can be evaluated relative to the line intensities of minor molecules (for example, Ala or Val). The Lac CH_3 signal doublet is an arm of the wide L_2 signal. Therefore, summary intensity (I_{Lac}+I_{CH2}) intensity at 1.21-1.05 ppm was evaluated. The (I_{Lac}+I_{L2})/I_{Ala} ratio was higher in the spectra of FES patients (11.2±1.24) than in volunteer (6.5±0.5) at significance level of p=0.01. In addition, the wide signal of lipid CH_3 protons (L_1) in serum spectrum of FES patients has a clearly seen high field arm, presumably indicating changes in low density lipid fractions. It seems that the increase in signal intensity in the CH_2 proton region was caused by an increase in the amount of the long-chain lipid fraction in the patients. Increase of the intensity of the signal with δ=0.7 ppm belonging to C_{18} cholesterol methyl protons is worthy of note.

Changes of this kind were found in the lipid region of ^{1}H NMR spectra of blood plasma from twins suffering from schizophrenia [11]. Elevated lipid content was detected by biochemical methods in patients with acute schizophrenia [7].

Changes in this spectral region are not exclusively characteristic of FES patients. Similar changes were detected in the plasma of cancer patients [6] and patients with Alzheimer's syndrome [12].

For more ample characterization of changes in the serum and its proteins we studied ^{1}H NMR spectra of albumin fraction from the sera of FES patients and healthy donors. The protein isolation procedure in that case yields protein complexes with stably bound ligands. In addition to very intensive polyethylene glycol signal, signals of ligand protons of serum low-molecular-weight components predominated in the spectra of donor albumin water solutions, *e.g.* 1.35 ppm (Ala CH_3), 1.20 ppm (Lac CH_3), and 0.94 ppm (Val CH_3). These signals were classified according to the previous study [4,8] (Fig. 2).

The intensities of wide weak signals with chemical shifts of 1.15-1.0 and 0.8-0.5 ppm referred to signals from fatty acid CH_2 and CH_3 protons in the spectra of albumin fraction isolated from the sera of FES patients [9] sharply increased (in parallel with narrowing of the signal referred to CH_3 protons; Fig. 2). This could indicate an increase in the number of fatty acid molecules attached to albumin and greater mobility of fatty acid methyl groups. The mean ratio of relative integral intensity of the total wide signal of fatty acid and Lac CH_2 protons to the relative intensity of Ala signal (I_{CH2}+I_{Lac})/I_{Ala} differed significantly:

6.72±1.11 for patients and 3.90±0.32 for normal subjects (p=0.01).

Hence, high-resolution ^{1}H NMR spectra of the serum from FES patients indicated changed proportion between various lipid fractions bound to albumin, while high-resolution ^{1}H NMR spectra of albumin isolated from the serum of FES patients confirmed the hypothesis about different ligand load of albumin in healthy subjects and FES patients.

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