EFFICACY OF HEMOPERFUSION IN MYASTHENIA PATIENTS MONITORED BY SERUM LASER CORRELATION SPECTROSCOPY

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Among the methods of pathogenetic treatment of myasthenia, since the 1960s various methods of extracorporeal detoxication of the blood have been extensively used, such as hemodialysis, lymphosorption, plasmapheresis, hemoperfusion, and immunosorption [1-8]. Nevertheless, experience of the use of sorption methods of treatment of patients with myasthenia, obtained in practice worldwide requires critical examination.

At present no satisfactory description exists in the literature of changes taking place in the human body associated with a positive clinical effect, or the causes of its absence. The result of this is either an unjustified widening of the indications or failure to utilize these techniques. Most frequently the main criterion for the use of methods of efferent therapy in myasthenia patients is a combination of the clinical picture of the disease and the physician's personal experience.

The aim of this investigation was to determine some of the precise mechanisms of the therapeutic action of hemoperfusion in myasthenia patients on the basis of the use of new biophysical methods of investigation of serum homeostasis and, in particular, of laser correlation spectroscopy.

EXPERIMENTAL METHOD

Hemoperfusion was a component of the combined treatment of 30 patients with myasthenia aged from 18 to 48 years, the average duration of the illness being 3.5 years. The diagnosis of myasthenia was based on the clinical symptoms, a positive pharmacological test, and the results of electrophysiological investigation. Thymectomy had been performed previously on 25 patients (83.3%). All the patients were taking anticholinesterase drugs (neostigmine, kalimin), and 22 of them were taking prednisolone (73.3%).

Hemoperfusion was conducted on the AT-196 apparatus. The absorbent was grade SKN-NS activated charcoal, from Kiev Research Institute of General and Inorganic Chemistry, and the capacity of the column was 400 ml. The type of perfusion was venovenous. The volume velocity of perfusion was 80-120 ml/min. In the course of hemoperfusion 20,000 U of heparin was injected, and neutralization with protamine sulfate was not used. In the course of one session from 5000 to 7500 ml of blood, i.e., 1-1.5 CBV, was perfused. Hemoperfusion was carried out twice in the course of 3 days. The only complications observed during hemoperfusion were transient shivering, in two persons (6.6%); no withdrawal syndrome connected with adsorption of therapeutic agents was observed.

During investigation of the blood serum by laser correlation spectroscopy (LCS) information (graphic and numerical) about the subfractional composition of molecular, supramolecular, and complex biological constituents, whose main contribution was due to various protein subfractions, immune complexes, and also (in the case of virus diseases and cancer) viruses, and RNP-

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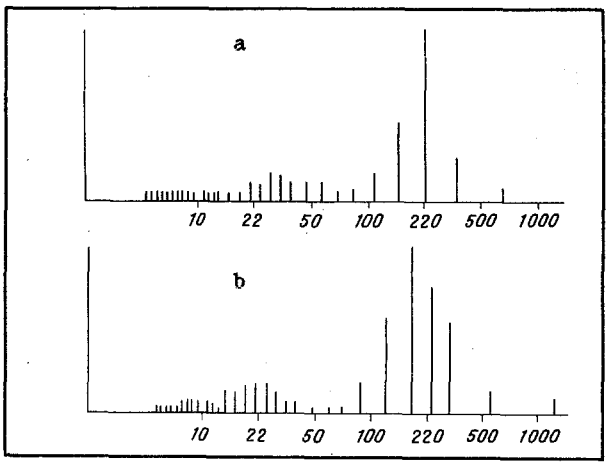

Fig. 1. Averaged character of LCS spectrum: a) in group of myasthenia patients before hemoperfusion; b) in group of myasthenia patients after two sessions of hemoperfusion. Abscissa, diameter of particles from 1 to 10^4 (in nm); ordinate height of columns of histogram is proportional to relative contribution of particles of the given diameter to the total spectrum of scattered light.

and DNP particles, was recorded in the final form. The basic physical principles, the apparatus used, and the method of mathematical analysis of the spectra were described in a recently published monograph [9]. Blood serum for LCS investigation was prepared as follows: 5 ml of venous blood, after retraction of the clot for 20-30 min, was centrifuged at 1500-2000 rpm for 10 min at 18-20°C. Next, 50-100 μl of serum was withdrawn into a glass test tube of Eppendorf type, in a volume of 1.5 ml, and frozen at –20°C. The samples could be kept up to 2 or 3 weeks in that form. Before measurement the sample was frozen and diluted 1:50 with sterile physiological saline, centrifuged at 5000 rpm for 15 min, after which 0.5 ml of the diluted and centrifuged sample was placed in the measuring cuvette of the spectrometer. The process of measuring the spectrum of scattered laser light took not more than 5 min. The cuvette of the spectrometer was then washed and filled as follows. By means of a specially drawn up program of regularization the integral spectrum of fluctuation of the intensities of scattered light was converted into a histogram, consisting of the distribution of particles of different sizes by their contribution to the total effects of the intensity of light scattering. Comparative evaluation of individual spectrograms was carried out by means of a specially devised classification program for computer analysis. The serum was investigated twice, before and after the end of the course of hemoperfusion.

To make an objective evaluation of the effect of hemoperfusion on the state of homeostasis of the blood serum in myasthenia patients in whom the duration of the disease differed, and in some cases with a history of thymectomy, we carried out additional tests on patients without thymectomy at different times from the beginning of the disease, and also a group of patients before the operation and at different times after thymectomy.

EXPERIMENTAL RESULTS

When the blood serum from myasthenia patients not subjected to thymectomy was tested at different times after the beginning of the disease, and when times from the beginning of the disease were taken as the basis of the systematizing criterion, no general principles were found in the distribution of the results after multiparametric evaluation of individual LCS spectrograms, by means of the classifying computer. Thus changes in homeostasis of components of the blood serum, discovered previously in a study of 91 patients with myasthenia and 30 healthy blood donors, were virtually independent of the duration of the disease. This result correlates in principle with the clinical data characterizing the course of myasthenia as intermittent.

Analysis of the results obtained after multiparametric assessment of individual LCS spectrograms with the aid of the classifying computer, when the systematizing criterion was based on absence or presence of thymectomy, performed at various times before this present investigation, with respect to the parameters studied these groups of patients also were found to be indistinguishable.

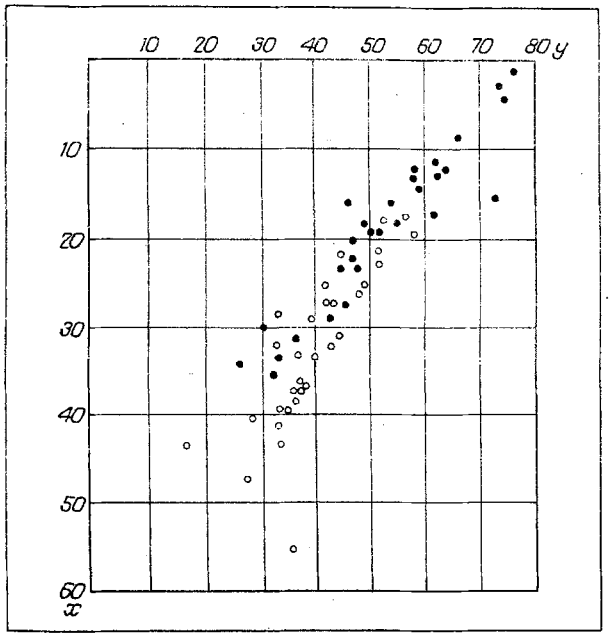

Fig. 2. Classification chart for comparing group of patients before and after two sessions of hemoperfusion. Filled circles — myasthenia patients before hemoperfusion (30 observations); empty circles — myasthenia patients after two sessions of hemoperfusion (30 observations). Here and in Fig. 3: abscissa and ordinate) conventional units.

The above results were necessary for a truly objective assessment of the effect of hemoperfusion if thymectomy or the duration of the disease had a significant influence on homeostasis of the blood serum, determined by the LCS method, in which case the time course of changes in the LCS parameters before and after hemoperfusion would have to be taken into consideration separately for the group of thymectomized and of nonthymectomized patients, allowing for the duration of the disease.

The averaged character of the LCS-spectrum (after the regularization procedure) is shown in Fig. 1, which demonstrates the contribution of particles of different sizes (from 1 to 10^4 nm) to the total effect of scattering of laser light both in the group of myasthenia patients before hemoperfusion and in patients after two sessions of hemoperfusion. By visual analysis, significant differences can be seen, namely that both the size of the "peak fractions" and the relative contributions of small particles to large particles change in the groups studied. Low-molecular-weight fractions reflect the contribution of monomolecular protein fractions (albumins and globulins) and of serum lipids, whereas high-molecular-weight fractions belong more to the supramolecular complex forms (including immune complexes). Precise identification of the peaks can only be done after special molecular-biological identification. Meanwhile, without more detailed identification, we are right to assert that the results characterize differences in the parameters of homeostasis of the serum components in myasthenia patients before hemoperfusion and after two sessions of hemocarboperfusion.

The results of multiparametric assessment of individual LCS-spectrograms of the blood serum of myasthenia patients before and after hemoperfusion, with the aid of a classifying computer, are given in Fig. 2. In most cases the blood serum of myasthenia patients before hemoperfusion was sufficiently clearly distinguishable compared with the group of patients after hemoperfusion. It must be pointed out that in all cases after hemoperfusion the parameters on the classifying computer are shifted from the zone corresponding to the more severe forms of myasthenia into the zone of milder forms, closer to the healthy blood donor group. For comparison we give the results of our investigation of 91 myasthenia patients with different degrees of severity of the disease (mild, moderately severe, and severe types of course) and a group of 30 healthy blood donors (Fig. 3).

Comparison of the results with the neurologic status is interesting. A multiparametric assessment of individual LCS-spectrograms with the aid of the classifying computer for the group of patients with clinical improvement after hemoperfusion closely resembles the distribution obtained on assessment of mild and moderately severe forms of myasthenia, and in most cases it overlaps the donor's group. In the absence of clinical improvement after hemoperfusion (in four of our patients) the results underwent minimal changes and remained similar to those for the group of patients before hemoperfusion. In these cases,

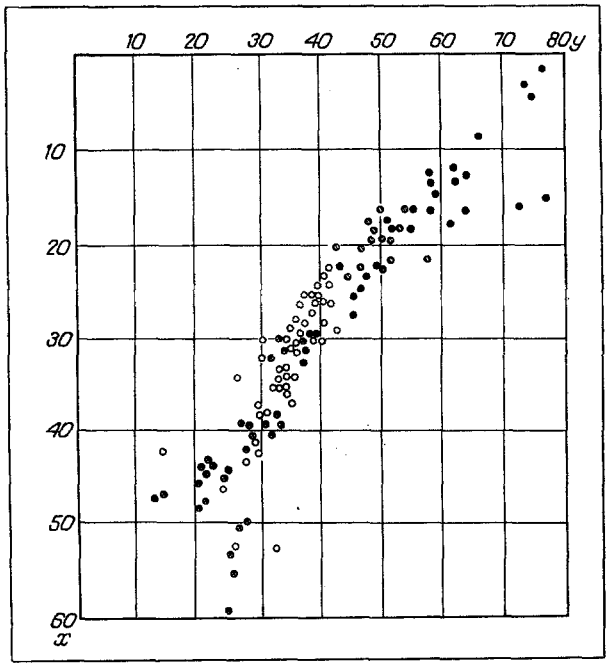

Fig. 3. Classification chart for comparing myasthenia patients by severity of the disease (91 patients and 30 healthy blood donors were tested). Filled circles — 27 patients with severe course of myasthenia, shaded circles — 16 myasthenia patients tested in period of exacerbation, empty circles — 48 myasthenia patients with mild and moderately severe types of course, circles with inscribed cross — 30 healthy donors.

no penetration into the group of patients with a milder course or the group of blood donors was observed. Clinical worsening, namely increased muscular weakness and an increased tendency toward pathological muscular fatigue, observed after hemoperfusion in one patient, also corresponded to the region of the classifier characterizing the group of patients before hemoperfusion. With the aid of the LCS data, processed by the classifying computer, the character of changes in the system of homeostasis of the blood serum of myasthenia patients under the influence of treatment can be judged. Analysis of the changes observed can be used as a test for developing objective criteria for the use of hemoperfusion in the treatment of myasthenia patients, and also to assess the efficacy of treatment given.

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