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Plasma DNA Levels in Patients with Atherosclerotic Involvement of the Major Arteries of the Head and Lateral Amyotrophic Sclerosis

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Measurements of DNA concentrations by fluorescence of the DNA—bisBenzimide complex in the plasma of normal subjects, patients with disorders of cerebral circulation of different severity caused by atherosclerotic involvement of the carotid and/or spinal arteries (after the acute stage of brain stroke), and patients with lateral amyotrophic sclerosis showed a significant increase in its content *vs.* the norm. Electrophoresis of DNA isolated from plasma showed that in the patients it is represented not only by long fragments of at least 20,000 bp (which is typical of normal subjects), but also by shorter fragments.

Key Words: *DNA; fluorometry; bisBenzimide; cerebral circulation disorders; lateral amyotrophic sclerosis*

The blood-brain barrier is always involved in organic diseases of the nervous system; the degree of such involvement can vary from metabolic to mechanical. This involves the development of pathological process, including autoimmune reactions toward brain-specific proteins of neurons, myelin, and glial elements. There are no published reports about changes in the plasma level of DNA which may be caused by destruction of nervous tissue and by DNA excretion by activated lymphocytes [2] because of developing autoimmune reactions.

We compared plasma DNA levels in two types of nervous system involvement associated with destruction of brain structures of different extent: extensive in atherosclerotic involvement of the carotid and/or spinal arteries and less expressed and specific in lateral amyotrophic sclerosis (LAS). Blood plasma of 28 patients with cerebral circulation disorders (CCD) of different severity caused by atherosclerotic involvement of the major arteries of the head was examined after acute stage of ischemic stroke. The main vascular disease in this group was atherosclerosis with or without arterial hypertension. DNA content was also measured in the plasma of 6 patients with LAS suffering from expressed disorders of motor functions and a lesser extent of brain matter destruction than in CCD. The reference group consisted of 7 normal subjects under 35 years without probability of atherosclerosis and hypertension.

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MATERIALS AND METHODS

Blood was collected from patients and normal subjects into tubes with heparin (2 ml blood+0.2 ml

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heparin manufactured by Moscow Endocrinological Plant). Blood was centrifuged for 10 min at 2000 rpm and plasma was collected for measuring DNA. There were no fragments of destroyed blood cells in the plasma. Protein was removed from 0.1 ml plasma using an equal volume of 20% NaCl in boiling bath for 2-3 min. After cooling to room temperature, the specimens were centrifuged at 3000 rpm for 30 min.

DNA concentration in protein-free plasma was assessed by fluorescence of the DNA—stain complex (bisBenzimide, Hoechst No. 33258, Serva) in a TKO 100 device (Hoefer Scientific Instruments). The following buffer was used for measuring: 0.01 M Tris-HCl, pH 7.4, 0.001 M EDTA, 0.1 M NaCl. Ten microliters of the specimen was added to 2 ml buffer, exposed for 5 min at 20°C, and fluorescence was measured (excitation wavelength 335 nm, fluorescence wavelength 458 nm). DNA isolated from human lymphocytes was the reference sample. DNA concentration was measured at least twice in each plasma specimen.

For isolation of DNA from the plasma, Tris-HCl, pH 7.5 (final concentration 0.01 M), 0.5% lauroylsarcosine sodium salt, and 0.01 M EDTA were added to 5 ml plasma. DNA was extracted with phenol, phenol-chloroform-isoamyl alcohol mixture (25:24:1), and chloroform. Then it was precipitated in the presence of 2 M ammonium acetate by two ethanol volumes. DNA precipitate was dissolved in 1 ml 0.01 M Tris-HCl, pH 7.5, 0.1 M NaCl, 0.01 M EDTA, and treated with RNase (0.1 msec/sec) for 1 h at 37°C and then with proteinase K for 30 h. Then protein extraction and sedimentation were repeated as described above. After measuring the resultant DNA, the specimens were layered onto 1% agarose gel (1-2 μg per track) and electrophorezed. Gels were stained with ethidium bromide and photographed.

RESULTS

Plasma levels of DNA were significantly higher in all patients than in the controls (Table 1), in whom it was 1.08±0.11 μg/ml, which coincides with published data [1-4,7]. In LAS patients with neurological symptoms assessed as 3-5 points (by 5-score scale), the content of DNA in the plasma was significantly higher: 3.83±0.48 μg/ml. The content of DNA in patients with different CCD was even higher (7.82± 1.14 μg/ml) and neurological symptoms were less expressed than in LAS patients. Therefore, it can be suggested that plasma content of DNA in neurological patients correlates with the volume of destroyed nervous tissue, which is higher in CCD than in LAS, but not with the severity of neurological symptoms. Analysis of DNA levels in the plasma of CCD patients with different patterns of disease showed

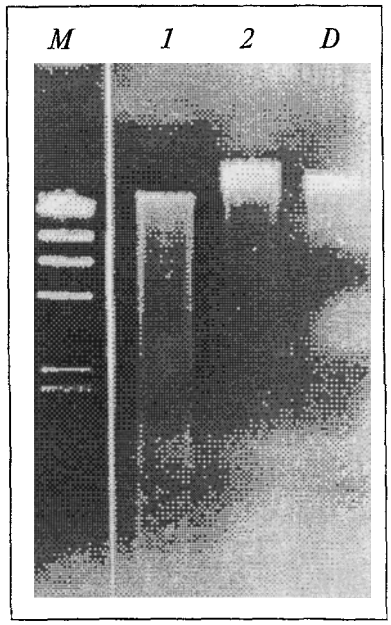

Fig. 1. Electrophoresis of DNA isolated from plasma of patients with cerebral circulation disorders (*1*, *2*) and donor (*D*) in 1% agarose gel. Ethidium bromide staining. *M*) marker of DNA length (Hind III — phage λ hydrolysate).

that the content of plasma DNA was the highest in patients with the most favorable clinical course (9.36±2.02 μg/ml). Patients with CCD running a stable course had lower plasma DNA levels (7.07± 1.58 μg/ml), as did those in whom the neurological status deteriorated during the next month (5.67±2.33 μg/ml). However, these differences were insignificant. Thus, an increase in plasma DNA level in neurological patients did not depend on the severity of clinical condition, as was described for systemic lupus erythematosus or lymphoid leukemia [1-4,7]. There was a tendency to inverse relationship: plasma DNA content was higher (although insignificantly) in the patients with a favorable course of CCD.

TABLE 1. DNA Content in Patients' Plasma (*M*±*m*)

Disease	DNA concentration, μg/ml	Number of patients
Patients with CCD:		
whole group	7.82±1.14*	28
with clinical improvement	9.36±2.02*	11
stable status	7.07±1.58*	11
clinical deterioration	5.67±2.33*	6
Patients with LAS	3.83±0.48*	6
Controls	1.08±0.11	7

Note. *$p<0.005$ *vs.* the control.

The severity of neurological symptoms is determined by the volume of brain matter involved and even more so by involvement of functionally significant compartments of the brain. In LAS patients, neurological symptoms can be rather grave and the involvement of motor neurons slight. In CCD the lesions of brain matter can be much more extensive, but not in the functionally significant compartments.

Electrophoresis of plasma DNA showed that it is represented mainly by 20,000 bp and longer fragments both in donors and patients. In the patients, shorter fragments (1000-20,000 bp) were present, as seen on electrophoregrams of plasma DNA of 2 patients and 1 donor (Fig. 1). Hydrolysate of phage λ with Hind III restrictase with fragments of 23,100, 9,400, 6,300, 4,600, 2,300, and 2,000 bp was used as the marker.

Thus, the total content of DNA isolated from patients can be represented not only by long, but also by short fragments. We believe that DNA fragments of different length can originate from different sources and result from degradation of nervous tissue elements and apoptosis [5,6].

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