GENERAL PATHOLOGY AND PATHOPHYSIOLOGY

Effect of Routine Therapy and Selective Plasmapheresis on Electrical Properties of Cryoglobulins during Ischemic Stroke

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The charge properties of cryoglobulins were examined during the first 21 days of ischemic stroke (atherothrombotic and cardioembolic). Routine drug therapy produced no effect on the charge of cryoglobulins. Plasmapheresis significantly modified the electrokinetic parameters of cryoglobulins during atherothrombotic stroke and produced less pronounced effect on these proteins during cardioembolic stroke.

Key Words: *cryoglobulins; ischemic stroke; plasmapheresis; charge properties; electrophoretic mobility*

Recent studies greatly improved our understanding of the pathogenesis of ischemic stroke. Considerable attention is now focused on autoimmune mechanisms of cerebral tissue damage. Under conditions of damaged blood-brain barrier resulted from disruption of endothelium and astrocytic dysfunction, the brain, an "immunologically privileged organ", can be recognized by the immune system as a foreign agent [1]. Proteins with abnormal temperature-dependent solubility (cryoglobulins) appear in the serum of patients with ischemic stroke. Numerous studies of structural parameters of cryoproteins and similar immunoglobulins exhibiting no cryoactivity revealed no pronounced molecular features of cryoproteins [2]. Experimental data on physicochemical properties of cryoglobulins showed that the peculiarities of these proteins are greatly determined by their charge parameters depending on the existence of extra negatively charged residues on the protein surface [5].

Clinical studies demonstrated a positive effect of plasmapheresis decreasing blood level of cryoproteins on the recovery from ischemic stroke. Therefore, it is interesting to study the effect of plasmapheresis on the charge parameters of the cryoglobulins.

Our aim was to examine charge properties of cryoglobulins in patients with atherothrombotic and cardioembolic types of ischemic strokes and the effect of plasmapheresis on these properties.

MATERIALS AND METHODS

The study was carried out on patients with ischemic stroke (n=32) aging 42-78 years (18 men and 14 women) during acute period of the first onset of carotid ischemic stroke. The patients were hospitalized in Neurology Department of No. 20 State Clinical Hospital during the first 24 h of the disease. All patients received similar complex basic therapy aimed at correction of dysfunctions of the central

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and cerebral hemodynamics, improvement of hemorheological indices, and prevention and arrest the cerebral edema.

The patients of the main group (n=16, simple randomization) were subjected to plasmapheresis, the procedure used in the therapy of some inflammatory, degenerative, and autoimmune diseases. The applied method of efferent therapy is based on effusion of 1.0-1.5 volume of circulating plasma (on days 2, 4, and 6 of the acute period of the disease) followed by heparin cryoprecipitation with the first plasma substitution with isovolumic crystalloids and subsequent substitution with the supernatant (cryoprecipitate-depleted autoplasma). Blood substitutes and donor plasma were not used [4].

Cryoglobulins were isolated from the serum of patients with acute carotid ischemic stroke on days 1, 2, 3, 7, and 21 of the disease by repeated cold centrifugation followed with dissolution with heating [2]. The protein concentration was determined by photometry on a CARY-50 spectrophotometer (Bio) at λ=280 nm.

Electrostatic properties of cryoglobulins were assessed by comparison of electrophoretic mobility (EPM) of erythrocytes loaded and not loaded with cryoglobulins. EPM of erythrocytes was determined in an OPTON flow cytopherometer [3]. To exclude the effect of intrinsic erythrocytic EPM and temperature-dependent changes in viscosity of erythrocyte suspension on examined electrostatic properties, we calculated the relative EPM as the ratio of EPM of erythrocytes loaded and not loaded with cryoglobulins. The data were analyzed statistically using Statistica 6.0 software.

RESULTS

The data on the relative electrophoretic mobility for patients with atherothrombotic type of ischemic stroke are presented on Fig. 1 (5 and 37°C correspond to intact cryocomplex and its complete disintegration, respectively).

In patients not treated with plasmapheresis, the relative EPM did not significantly change during the entire period of observation. In all cases, the relative EPM measured at 37°C surpassed the values at 5°C.

The first plasmapheresis session on day 2 of the disease considerably affected charge parameters of cryocomplexes (Fig. 1). EPM significantly decreased, then increased by day 7 (p<0.05), and then decreased again to the end of the acute period. This pattern of changes in relative EPM was observed both at low (5°C) and normal (37°C) temperatures.

Thus, standard pharmacotherapy produced no effect on the charge parameters of cryoglobulins during atherothrombotic type of ischemic stroke. Selective plasmapheresis immediately decreased the charge of cryocomplexes.

Figure 2 shows the relative values of EPM in patients with cardioembolic type of ischemic stroke. In these cases, plasmapheresis slightly decreased EPM during the first three days of the disease at 5 and 37°C. To day 7, the relative EPM did not significantly differ from values recorded on day 1 after the onset of the disease. Then this index increased to the end of the acute period of the disease. On day 21, EPM measured at 5°C and 37°C temperatures significantly surpassed the corresponding values recorded on the day 1.

In cardioembolic type of ischemic stroke, the kinetics of relative EPM differed in patients treated and not treated with plasmapheresis.

At 37°C, EPM increased on day 3, returned to the initial level by day 7, and then did not vary significantly.

At 5°C, the relative EPM significantly decreased on days 2 and 3 of the disease, returned to the initial level on day 7, but then tended to decrease.

Comparative analysis of the kinetics of relative EPM during atherothrombotic and cardioembolic types of ischemic strokes showed that charge parameters of cryoglobulins did not significantly change in patients not treated with selective plasmapheresis. Therefore, in these cases routine therapy produced no significant effect on the electrokinetic properties of the cryoglobulins. Plasmapheresis in patients with cardioembolic type of stroke produced no significant effect on relative EPM. By contrast,

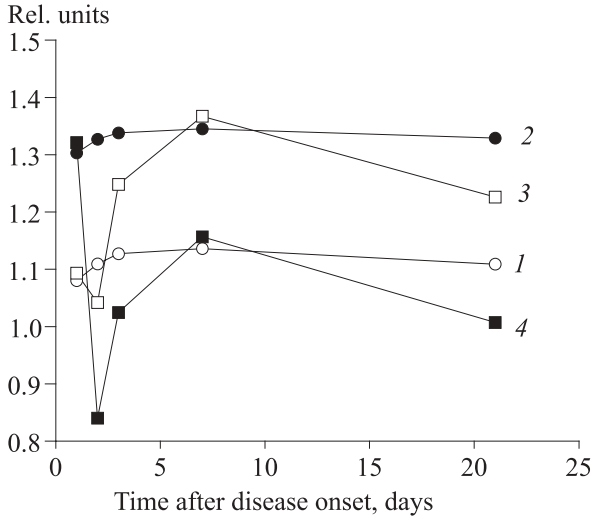

Fig. 1. Kinetics of relative EPM in patients with atherothrombotic type of ischemic stroke treated (*3*, *4*) and not treated (*1*, *2*) with plasmapheresis. EPM was measured at 5°C (*1*, *3*) and at 37°C (*2*, *4*).

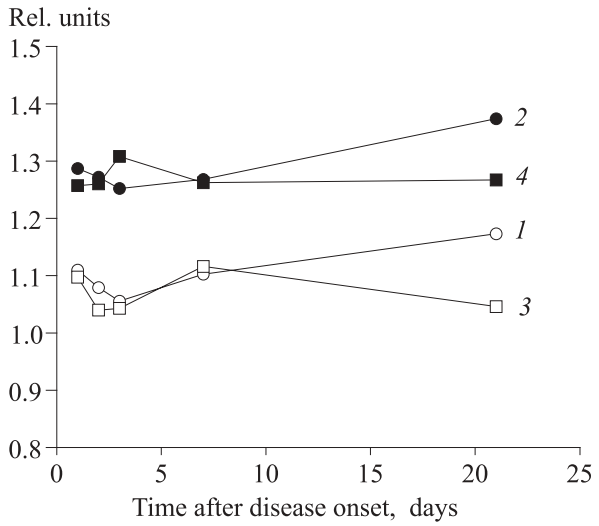

Fig. 2. Kinetics of relative EPM in patients with cardioembolic type of ischemic stroke treated (*3, 4*) and not treated (*1*, *2*) with plasmapheresis. EPM was measured at 5°C (*1*, *3*) and at 37°C (*2*, *4*).

plasmapheresis significantly decreased EPM of cryoglobulins during the entire observation period in patients with atherothrombotic ischemic stroke ($p<0.05$). In patients with cardioembolic stroke, significant changes were documented on day 21, *i.e.* by the end of the disease.

Thus, routine pharmacotherapy produces no significant effect on charge properties of cryoglobulins in patients with both types of strokes. In atherothrombotic stroke, plasmapheresis pronouncedly decreases the charge of the cryocomplexes after the first procedure. By contrast, in patients with cardioembolic stroke, plasmapheresis slightly decreased this charge only by the end of the acute period of the disease.

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