PLATELETS AGGREGATION UNDER INFLUENCE OF THE IMMUNOGLOBULIN CLASS G SEPARATED FROM THE BLOOD PLASMA OF PATIENTS WITH ISCHEMIC STROKE

Introduction. Every disease that hit our organism leaves some proteins that continuously circulate in the bloodstream after each postponed disease. In addition, these proteins theoretically could play specific role during the illness emergence or development of complications. The most common of such specific proteins is immunoglobulin class G. In certain cases, immunoglobulins class G can be realized as autoantibodies [12]. In previous research we have shown that IgG, which appear in the bloodstream after stroke, are able to induce releasing of the proteins, fragments and protein complexes from the granules of the platelets [6]. Hence interaction between IgG and platelets surface causes modulation of the cellular response [7-9].

At the same time, stroke is one of the most pressing health and social problem which require urgent solution [4]. Full recovery of patients after stroke was not observed [1]. It is obvious that certain molecules or mechanisms exist that may be the reason for the disease recurrence or further complications. It is known that the leading mechanism of ischemic stroke realization correlated with hemostatic profile. Activation of blood clotting and thrombus formation were observed after stroke attack [3, 8, 14]. The origin of the primary thrombus formation is platelet's ability to aggregate [5].

Given the above, investigation of the potential impact of IgG, formed in the bloodstream after suffering a stroke, on platelet's ability to aggregate was of great importance [19]. It was showed that impact of immunoagglutinin class G was characterized by one-wave irreversible ADP-induced platelet aggregation. The maximum aggregation was shoved under influence of IgG fraction separated from the patients with AIS. This influence was on the 15 % more intensive in comparison with IgG fraction separated from the healthy donors. One year past disease all tested IgG fractions provoked inhibition of platelets aggregation up to 25 %. The maximum inhibition of healthy donor's platelets aggregation was provided by fraction separated from the patients with AIS one year past acute phase.

Key words: ischemic stroke, IgG, platelets aggregation.

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department of the Hospital №4 (Kyiv, Ukraine). The diagnosis of ischemic stroke was confirmed through computed tomography or magnetic resonance imaging. On the first day of admission to hospital, all patients received aspirin 325 mg orally, then the daily 100 mg aspirin. From the second day of hospital stay, patients received low molecular weight heparin in prophylactic dose. All donors and patients or their relatives had been warned about the conduct of clinical research and provide written agreement to participate in it. Current experiment was approved by the ethics committee from ESC "Institute of Biology"., Ukraine.

Blood samples were collected from the cubital vein with the addition of sodium citrate (38 g/l) in the final correlation 9:1. Platelet-rich plasma (PRP) was obtained by centrifugation of stabilized blood at 300 g for 10 min at 20 °C. Platelet-poor plasma (PPP) was obtained by further centrifugation of PRP at 1500 g for 30 min at 20 °C.

IgG was separated by affinity chromatography on protein A Sepharose [10,12]. Quality of immunoglobulin fractions was controlled by disk-electrophoresis in 10% PAGE with SDS-Na [6,7]. All separated fractions of IgG were lyophilized by (LyoQuest, Spain) and dissolved in the 0.05 M Tris-HCl buffer containing 0.13 M NaCl, pH 7.4 instead of tested fractions.

Platelet aggregation was measured during the first 2 hours of blood collected from the healthy donors on the photo-optical aggregometer AT-02 (Medtech, Belarus) in vitro. As PRP platelets in concentration 230-250 000 cells/µl was used. PRP from the healthy donors was incubated with tested fractions in a cuvette for 1 min at 37°C with constant stirring (500 rpm). As platelets aggregation inducer ADP in final concentration 2.5 mM was used. Aggregation analysis was performed according to the manufacturer instructions. Kinetics of the platelets aggregation was assayed during 5 minutes after addition of the aggregation inducer. Control platelet aggregation was carried out. Which instead tested molecules (IgG & peptide pool) equal volume of 0.05 M Tris-HCl buffer containing 0.13 M NaCl, pH 7.4 was added. All similar manipulations were conducted with a control sample as with an experimental samples.

Statistical processing of the results was performed using software Statistica 7. Value changes were considered significant at P < 0.05. Statistical processing and analysis of electrophoregrams was performed by the scanning of the computer program TotalLab 2.01.

**Results and discussions.** Fractions of immunoglobulin class G and were extracted from: plasma of healthy donors; plasma of patients with atherothrombotic ischemic stroke (AIS) and cardioembolic ischemic stroke (CIS) in acute phase of the disease; and plasma of the same patients past one year of acute phase of the disease.

Details of IgG fractions separations concentrations and characterization had provided in our previous research [6, 7]. Than incubation of the tested IgG fractions with the PRP obtained from the healthy donors was performed. The effect of each tested IgG fractions on the ADP-dependent healthy donors platelets aggregation was evaluated. Aggregatograms were compared with the control platelet aggregogram which include equal volume of 0.05 M Tris-HCl buffer containing 0.13 M NaCl, pH 7.4 instead of tested fractions.

IgG fraction obtained from the healthy donor's blood plasma did not influenced tested process meaningful and was similar to the result of the control aggregation (Table 1).

Spontaneous aggregation. The addition to the donors PRP of the majority fractions has not provoked spontaneous aggregation. This indicates an absence of initial activation of platelets, which were separated from the blood of healthy donors. However, it has been shown that after addition of IgG fractions separated from the blood plasma of patients with AIS as well as with CIS one year past acute phase the rate spontaneous aggregation was equal up to 5%.

One-wave irreversible aggregation was observed after incubation of each explored fractions with the donor's platelets rich plasma (figure 1).

![Fig.1. Typical platelets aggregation amplitude under the influence of IgG separated from the blood plasma of:](image)

1. Healthy donors (n = 35)
2. Patients with CIS in acute phase (n = 56)
3. Patients with AIS in acute phase (n = 66)
4. Patients with AIS one year past acute phase (n = 57)
5. Patients with CIS one year past acute phase (n = 57)

The maximum amplitude of platelet aggregation which were influence of the IgG fractions separated from the plasma of patients in acute phase of both stroke subtypes was in average on 15% higher comparengly with the control sample. Donor’s PRP incubation with the IgG fractions separated from blood plasma of patients one year past acute phase of stroke provoke maximum amplitude of platelet aggregation that was on the 26% for AIS and on the 20% for CIS lower comparengly with a control sample.

The rate of platelet aggregation after incubation of donor's PRP with the IgG fractions separated from the blood plasma of patients with AIS as well as CIS was slightly increased compared with the control aggregators. However according to the timing the rate of platelets aggregation was on the 8% per minutes higher after adding of IgG fraction separated from the patients with stroke one year past acute phase in comparison with the same effect of the IgG fraction separated from the acute stroke patients (Table 1).
Table 1. The rate and maximum amplitude of ADP-dependent platelet aggregation after incubation of the healthy donor’s platelets with the tested IgG fractions

<table>
<thead>
<tr>
<th>IgG fraction separated from the blood plasma of patients</th>
<th>Maximum amplitude of platelet aggregation, %</th>
<th>Platelet aggregation rate, %/sec</th>
</tr>
</thead>
<tbody>
<tr>
<td>Healthy donors</td>
<td>42,3 ± 8,2*</td>
<td>43,3 ± 3,1</td>
</tr>
<tr>
<td>Patients with acute AIS</td>
<td>63,6 ± 5,6*#</td>
<td>46,8 ± 6,1*#</td>
</tr>
<tr>
<td>Patients with acute C1S</td>
<td>61,1 ± 5,2*#</td>
<td>46,8 ± 6,1*#</td>
</tr>
<tr>
<td>Patients with past one year stroke AIS</td>
<td>51,8 ± 10,4*</td>
<td>54,0 ± 5,5*</td>
</tr>
<tr>
<td>Patients with past one year stroke C1S</td>
<td>48,4 ± 11,5*</td>
<td></td>
</tr>
</tbody>
</table>

* P<0.05 in comparison with aggregation amplitude and the platelet aggregation rate under the influence of IgG separated from the plasma of healthy donors.
# P<0.05 The aggregation amplitude and the rate of platelet aggregation under influence of IgG separated from the blood plasma of patients with AIS and C1S in acute phase in comparison with same parameters past one year after acute phase of ischemic stroke.

Moreover, IgG fraction separated from the plasma of patients with AIS activated donor’s platelet aggregation process an average on 5% more intensive comparatively to the IgG fraction separated from plasma of patients with C1S in the acute phase of stroke as well as one year past acute phase.

Results could be an evidence of the potentially different immunoglobulin fractions formation in the bloodstream of the patients with different pathology. Previously it was proved that cardioembolic and atherothrombotic ischemic subtypes of stroke involve different response of hemostasis system [1-4].

Conclusion. The results shows that atherothrombotic and cardioembolic ischemic subtypes of acute stroke accompanied by increased concentrations of immunoglobulin class G. But concentration of IgG for the patients one year past acute phase is close to IgG concentration of healthy donors. All investigated IgG fractions separated from the stroke patients are able to influence certain parts of the haemostasis system instead of IgG fraction separated from the blood plasma of healthy donor which effect was close to zero. In particular IgG separated from the plasma of patients with AIS and C1S in the acute phase have caused the activation of ADP-induced healthy donor’s platelets aggregation. In contrast, IgG separated from the plasma of patients with AIS and C1S one year past acute phase have caused inhibition of healthy donor’s platelets aggregation.

References

IgG fraction separated from the blood plasma of Maximum amplitude of platelet aggregation, % Platelet aggregation rate, %/sec
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Patients with acute AIS 63,6 ± 5,6*# 46,8 ± 6,1*#
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Patients with past one year stroke AIS 51,8 ± 10,4* 54,0 ± 5,5*
Patients with past one year stroke C1S 48,4 ± 11,5* 
АГРЕГАЦИОННАЯ СПОСОБНОСТЬ ТРОМБОЦИТОВ ПОД ДЕЙСТВИЕМ ИММУНОГЛОБУЛИНОВ КЛАССА G ПОЛУЧЕННЫХ ИЗ ПЛАЗМЫ КРОВИ БОЛЬНЫХ С ИШЕМЧЕСКИМ ИНСУЛЬТОМ.

Сталя присвячена изучению АДФ-взаимосвязи агрегации тромбоцитов здоровых доноров после инкубации с иммуноглобулинами класса G, которые были выделены из плазмы крови больных атеросклеротическим и кардиомиокардиологическим пациентами и использованы в виде сыворотки или плазмы для инкубации тромбоцитов здоровых доноров. В результате было показано, что агрегация тромбоцитов в плазме больных с здоровыми донорами происходит в присутствии АДФ, что свидетельствует о более высокой концентрации АДФ в плазме больных.

Идентификация збудників змішаної інфекції орхідних в колекції Ботанічного саду ім. О.Ф. Фоміна.

Вступ. Тропічні та субтропічні види орхідних культивуються в багатьох країнах світу і є однією з провідних рослин до "вторинного" ураження іншими хворобами.

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