Effects of IgG from the serum of ischemic stroke patients on hemostasis

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Abstract

Ischemic stroke is among the top diseases leading to mortality and disability in the world. The detailed investigation of the mechanisms underlying this pathology and especially mediating the tendency to relapse during the first year after stroke incident undoubtedly belongs to important tasks of modern medicine and biology. The current study aims to analyze the influence of IgG derived from the blood serum of ischemic stroke patients on some hemostasis factors. In total, 123 participants with IS, 62 with atherothrombotic ischemic stroke, 61 with cardioembolic ischemic stroke, and 57 subjects as control have been examined. The same patients have participated in the research a year after stroke. IgG from serum was isolated by affinity chromatography on protein A Sepharose column. The activity of key hemostasis factors under the influence of IgG was analyzed. Obtained results revealed that IgG of stroke patients but not healthy subjects caused the inhibition of the amidolytic activity of endogenously generated thrombin, protein C, factor Xa, and led to an increase in the degree of ADP-induced platelet aggregation. The reduction of clotting time in the test "Thrombin time" by IgG of patients at the acute phase of disease was also observed; IgG of healthy subjects mediated the opposite effect. In contrast to acute ischemic stroke IgG, IgG of patients one year after both atherothrombotic and cardioembolic ischemic stroke influenced only the activity of endogenously generated thrombin and factor Xa resulting in inhibition of their activities. It was also established that IgG of ischemic stroke patients, as well as healthy subjects, stimulated the secretion of tissue plasminogen activator by endotheliocytes.

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Key words: IgG; ischemic stroke; hemostasis.

Contributions: TK, wrote the article. NR, data interpretation and manuscript review. TH, carried out the experiments. TV, carried out the experiments.AZ, carried out the experiments. OK, statistical analysis. AK, statistical analysis. OS, experimental design. TA, experimental design. VM, supervised the study. LO, supervised the study.

Conflict of interest: The authors declare no conflicts of interest.

Received for publication: 2 January 2021.
Accepted for publication: 30 April 2021.

Journal of Biological Research 2021; 94:9582
doi:10.4081/jbr.2021.9582

Introduction

Stroke is among the leading causes of mortality and disability in the world.[1,2] Approximately 80% of stroke incidents occur as Ischemic Stroke (IS), which is a multifactorial disease, resulting from a complicated interplay between different factors. Over the past decades, numerous studies have contributed to major advances in the understanding of the complex mechanisms underlying the pathogenesis of ischemic stroke and stroke-related complications.[3,4] Patients surviving an initial stroke are known to be in a group of risk to meet stroke once again. The factors mediating significantly increased risk for recurrent stroke are even less studied than primary mechanisms of stroke. Therefore, there is an urgent need to study the reasons that could provoke stroke incidents in patients who have coped well with the acute phase of the disease.

In our previous studies,[5,6] it has been demonstrated that IgG of patients with multiple sclerosis as well as patients with stroke influenced the coagulation. It is generally known that hemostasis disorders namely the activation of coagulation and enhancement of platelet aggregation are an integral part of stroke pathophysiology. Moreover, there are some reports[7,8] that an increase in IgG concentration is associated with an increased risk of ischemic stroke. However, the possible mechanisms of IgG effect, whether they are a cause or a consequence are still unknown.

Taking into account all the above, we have hypothesized that the qualitative composition of IgG might undergo changes under the ischemic stroke, and it might be among the factors that affect the hemostasis. Thus, the main purpose of the current study was to isolate IgG from the serum of patients with IS and to examine their effects on key hemostasis factors.

Materials and Methods

Participants and experimental design

In our prospective study, we recruited 123 patients aged between 68 and 80 years old who were hospitalized to the neurological department of the Hospital №4 (Kyiv, Ukraine) with an established diagnosis of ischemic stroke. Ischemic stroke was confirmed by the treating neurologist and was based on clinical features and on results of computerized tomography or magnetic resonance imaging. Criteria for the exclusion of patients from the study were as follows: brain hematoma, other (non-vascular) diseases of the central nervous system, cancer, severe coronary heart disease, acute myocardial infarction, chronic pulmonary, renal, or hepatic failure, endocrinological disorders, active infection. There were 62 patients with Atherothrombotic Ischemic Stroke (AIS) and 61 patients with Cardioembolic Ischemic Stroke (CIS). One year after the stroke incident the same patients have invited to participate in the follow-up study. A total of 57 subjects (68-74 years old) without stroke and all listed above disorders were selected as a control group.

The protocol of the study was approved by the ethics committee of ESC "Institute of Biology and Medicine" (Kyiv, Ukraine) and carried out in accordance with the principles outlined in the Declaration of Helsinki. The patients and healthy donors gave written consent to participate after the explanation of the survey design.

Purification of IgG

The fasting elbow venous blood was collected from the AIS/CIS patients and healthy subjects, and then left still for 30 min. Afterwards, serum was separated by centrifugation at 900 g for 30 min. IgG was isolated by affinity chromatography on protein A Sepharose column.[9] Blood serum (1 mL) was diluted with equal volume of 50 mM Tris-HCl buffer containing 130 mM NaCl (TBS), pH 7.4 and applied to the column pre-equilibrated with the same buffer. Unbound proteins were removed by washing with TBS, pH 7.4. The bound IgG was eluted with 0.1 M glycine-HCl buffer, pH 2.2 and quickly neutralized with 1 M Tris to pH 7.4. The IgG concentration was determined by at 280 nm and calculated using an extinction coefficient 1.54. The homogeneity of isolated IgG was checked by polyacrylamide gel electrophoresis. The isolated IgG was dialyzed against TBS, pH 7.4, and lyophilized (TestarLyoQuest, Spain). To investigate the effect of IgG on hemostasis, the pool of IgG of the patients with AIS and CIS (7th day after IS and one year after stroke) was prepared. For this purpose, aliquots of lyophilized antibodies from each patient were mixed to obtain the antibody concentration of 12 mg per mL.

Chromogenic substrate spectrophotometric assays

The effect of IgG on generation and/or activity of key clotting factors was assayed using specific chromogenic substrates (Renam, Russia): H-D-Phe-Pip-Arg-*p*NA (S2238) for thrombin, Z-D-Arg-Gly-Arg-*p*NA (S2765) for factor Xa, and pyroGlu-Pro-Arg-*p*NA (S2366) for activated protein C. The degree of the activity was proportional to the color that developed in the result of the gradual release of *p*-nitroaniline from the chromogenic substrate. The reactions were monitored spectrophotometrically (μQuant, BioTek) at 405 nm against appropriate blanks to subtract spontaneous hydrolysis of the substrate. The activities of enzymes were calculated using a molar extinction coefficient of *p*-nitroaniline 9.96 $\mu M^{-1} \cdot cm^{-1}$.

To evaluate the effect of IgG on activities of thrombin (Renam, Russia) or factor Xa (Sigma, USA), the enzymes with final activity of 0.4 U/mL and 0.6 U/mL, respectively, were incubated in TBS, pH 7.4 in the presence of IgG pool obtained from AIS/CIS patients or healthy subjects (300 μg per 250 μL of incubation medium). After 5 min incubation at 37°C, 25 μL of the appropriate chromogenic substrate S2238 or S2765 (final concentration was 0.3 mM) was added to the reaction mixture. The final volume of the incubation medium was 250 μL. The change in absorbance was recorded for 30 min at a 405 nm wavelength.

To determine the effect of IgG on the activity of thrombin produced in the plasma, healthy subject's plasma was incubated with the pool of IgG obtained from AIS/CIS patients or healthy subjects (300 μg per 250 μL of incubation medium). After 5 min incubation at 37°C, the thrombin generation was induced by addition of prothrombin activator "Ecamylin." After 5 min incubation at 37°C, 25 μL of the chromogenic substrate S2238 (final concentration was 0.3 mM) was added to the reaction mixture. The final volume of the incubation medium was 250 μL. The change in absorbance was recorded for 30 min at a 405 nm wavelength.

The activity of protein C was studied followed the similar procedure by measuring the protein C mediated hydrolysis of chromogenic substrate S2366. In the first stage, healthy subject's plasma was incubated with the pool of IgG obtained from AIS/CIS patients or healthy subjects (300 μg per 250 μL of incubation medium). After 5 min incubation at 37°C, 25 μL of protein C activator derived from the venom of *Agkistrodon blomhoffi ussuriensis* was added. After 5 min incubation at 37°C, 25 μL of the chromogenic substrate S2366 (final concentration was 0.3 mM) was added to the reaction mixture. The change in absorbance was recorded for 30 min at a 405 nm wavelength.

Control samples for each experiment contained the appropriate components but equal volume of TBS, pH 7.4 instead of IgG.

Thrombin time test

To determinate the influence of IgG on the coagulation, "Thrombin Time" (TT) test was performed using a coagulation analyzer RT-2201C (Rayto, China) and the appropriate commercial kit (Renam, Russia). Analysis was done according to the manufacturer's instructions. Before the test, healthy subject's plasma was pre-incubated with the pool of IgG obtained IgG obtained from AIS/CIS patients or healthy subjects (final concentrations was 1.2 mg per 1 mL of plasma) at 37°C for 5 min. TT test was performed in triplicate using plasma from five different healthy subjects. Plasma incubated for 5 min with TBS, pH 7.4 (instead of IgG) was used as control.

Platelet aggregation assay

In order to investigate the influence of IgG on hemostasis, *in vitro* experiments were carried out using Platelet-Rich Plasma (PRP) and Platelet-Poor Plasma (PPP) of healthy subjects to recreate the model of physiological condition. PRP was obtained by centrifugation of healthy subject's blood at 150 g for 10 min at room temperature. PPP was prepared by further centrifugation of the remaining blood at 2500 g for 20 min at room temperature. PPP was stored at -20 °C until further analysis. To prevent platelet spontaneous activation, PRP was placed in a water bath at 37 °C for 30 min prior to aggregation experiments which were undertaken within the first 3h after blood sampling.

The effect of IgG on *in vitro* aggregation of platelets was assessed using a photo-optical aggregometer AT-02 (Medtech, Russia). Before the assessment, the platelet count in PRP was adjusted with PPP to about 250×10^3 cells/μL. PRP was pre-incu-

bated for 5 min with the pool of IgG obtained IgG obtained from AIS/CIS patients or healthy subjects (final concentration of IgG was 1.2 mg per 1 mL of PRP) at 37°C. To induce platelet aggregation, ADP (Sigma, USA) in the final concentration of 5×10^{-6} M was added to the samples. The aggregation process was monitored for 10 min.[10] The curve that represented the ADP-dependent aggregation after 5 min incubation of PRP with TBS, pH 7.4 (instead of IgG) was used as control. Data from five independent experiments were used for statistical analysis.

Endothelial progenitor cell culture

All experiments with cell culture were done in accordance with the bioethics and biological safety norms confirmed by the permission of Medical Company Ilaya® Bioethics Committee. All the donors have signed an informed consent before blood donation. Mononuclear Cells (MNCs) from human peripheral blood were isolated as described in Gubar *et al.*[11] Briefly, 20 mL of heparinized venous blood was centrifuged in Histopaque®-1077 density gradient (Sigma, USA) at 400g for 30 min at 4°C. MNCs were collected and washed twice in phosphate-buffered saline (PBS), pH 7.4 (Sigma, USA). The obtained MNCs were seeded onto collagen-coated 75 cm^2 culture flasks (SPL, Korea) in endothelial growing medium with the following composition: MCDB 131 medium (Gibco, UK); 5% FBS (Sigma, USA); 2 U/mL heparin sodium (Indar, Ukraine); 1 ng/mL VEGF; 10 ng/mL EGF; 2 ng/mL bFGF; 20 ng/mL IGF; 0.2 µg/mL hydrocortisone; 1 µg/mL ascorbate-2-phosphate (all manufactured by Sigma, USA). Cells were subcultured with a 0.1/0.02% trypsin/EDTA mixture in PBS. The seeding density was 3×10^3 cells/cm^2. Studies were performed on secondary cultures grown to confluence in 12-well dishes under the same conditions as primary cultures. To study the possible effects of IgG on tPA secretion by endothelial cells, the lyophilized IgG from were diluted with culture medium. Cells were treated with IgG for 24 hours. The final concentration of IgG in culture medium was the same for all experiments (1.2 mg/mL). The aliquots of culture medium were collected after the appropriate incubation period with IgG (30 min, 6h, and 24h), centrifuged at 3 000 *g* to remove cell debris, and frozen at −70°C until use for tPA measurements. The samples of control culture medium that was not exposed to any treatment were collected at the same time period to analyze the basal level of tPA secretion.

Tissue plasminogen activator assay.

Level of tissue Plasminogen Activator (tPA) in the culture medium was estimated by enzyme-linked immunosorbent assay according to the standard instructions. ELISA plate was coated overnight at 4°C with the samples of culture supernatants. The coated plate was washed three times with wash buffer (PBS, pH 7.4 that contained 0.05% Tween-20), blocked with 5% nonfat milk for 1 h at 37ºC, and washed again. After that plate was incubated for 1 h at 37ºC with monoclonal antibody to human tPA (Santa Cruz Biotechnology, USA). After being washed the plate was incubated for 1 h at 37ºC with corresponding secondary antibody conjugated to horseradish peroxidase (Bio-Rad, USA). After the washing step, the chromogenic mixture (3,3′,5,5′-tetramethylbenzidine (Sigma, USA) and H_2O_2 in 0.1 M sodium acetate, pH 4.5) was added. The reaction was terminated by the addition of 1N H_2SO_4. The absorbance was measured at OD (Optical Density) 450 nm.

Statistical analysis

All values were expressed as mean ± SD. Statistical analysis was performed using a commercially available software package (Statistica 8.0). The Kolmogorov-Smirnov test was used to verify the normal distribution of results. After normality test, the data were subjected to one-way Analysis Of Variance (ANOVA) followed by Duncan's Multiple Range test or Student's t-test. In all cases, the value $p<0.05$ was considered statistically significant.

Results and Discussions

In total, 123 participants with IS, 62 with AIS, 61 with CIS, and 57 subjects as control were included in the present study. Some peculiarities of the investigated groups are presented in Table 1.

In general, both groups of patients with stroke were comparable by most of the baseline characteristics. There were no significant variations between patients with AIS and CIS in gender, age, hypertension, and ischemic heart disease. The main difference was in terms of hyperlipidemia - the percentage of patients with hyperlipidemia was higher in the group of AIS (51.6%). Additionally, patients with CIS were characterized by a higher neurological deficit (NIHSS = 11.5±0.6) compared to patients with AIS (NIHSS = 9.1±0.6, p = 0,048).

At first, the concentration of IgG in the blood serum of patients with both subtypes of ischemic stroke was determined. The obtained data was summarized in Figure 1.

The increase in the concentration of IgG was revealed on the 7th day after AIS, while that of IgG level on the 1st day was the same as in the group of healthy subjects. The elevated level of IgG could be explained by the fact that under the ischemic stroke, the disruption of the blood–brain barrier might be the reason for the releasing of brain antigens into the bloodstream. As a result, the immune system of patients develops antibodies against various neuronal proteins. Additionally, some amount of IgG against the fragments of proteins may appear in the blood of patients with stroke as a consequence of enhancement of stroke-related proteolysis.[12] On the whole, our results are in keeping with work[13] that shows the increase in the level of IgG in the first week after atherothrombotic ischemic stroke. The highest concentration of IgG in the case of AIS was observed on the

Table 1. Baseline characteristics of patients with Atherothrombotic Ischemic Stroke (AIS) and Cardioembolic Ischemic Stroke (CIS)

	AIS (n=62)	CIS (n=61)	p
Age, years (M±SD)	73 ± 5	74 ± 6	0.652
Gender, male, n (%)	31(50.0)	29 (47.5)	0.714
Hypertension, n (%)	43 (69.3)	41 (67.2)	0.502
Hyperlipidaemia, n (%)	32 (51.6)	20 (32.8)	<0.001
Ischemic heart disease, n (%)	19 (30.6)	18 (29.5)	0.353
Baseline NIHSS (M±SE)	9.1±0.6	11.5±0.6	0.048

14th day after stroke incident and was 11.7±0.8 mg/mL comparing to 7.6±0.5 mg/mL in the group of healthy subjects. The concentration of IgG in the serum of CIS patients was at the level of healthy subjects in all tested terms. The concentration of IgG one year after AIS and CIS was found to be not different statistically from that in the control group.

The absence of the change in the concentration of IgG one year after stroke does not, however, exclude the possibility of changes in the qualitative composition of IgG. Even slight differences in the composition of antibodies can cause different effects of the total fraction of antibodies on hemostasis. Therefore, we tested IgG from the blood serum of patients at the acute phase of stroke and one year after the incident to find the difference in their effects. To investigate the influence of IgG of patients at the acute phase of ischemic stroke, we used IgG derived from the blood of patients on the 14th day after the stroke incident.

An increase in thrombus formation due to abnormal coagulation is often observed in patients with ischemic stroke.[3] Moreover, the high procoagulant status is considered one of the reasons for the risk of recurrent stroke. Considering the crucial role of thrombin in the blood coagulation process, it seemed of interest to determine the effect of IgG on the ability of thrombin to cleavage its specific substrate. To clarify this moment, in vitro experiments using active thrombin, its chromogenic substrate S2238, and IgG were performed. First of all, no change in the activity of thrombin under the influence of a healthy subject's IgG was revealed. But we observed an increase in the amidolytic activity of thrombin under the influence of IgG derived from the blood serum of patients at the acute phases of IS. As can be seen from the results in Table 2, IgG of patients at the acute phase of AIS and CIS caused an increase in thrombin activity by 33%, and 31%, respectively. On the other hand, incubation of thrombin with IgG obtained from the blood serum of patients one year after CIS and AIS did not affect the ability of the enzyme to cleavage of S2238.

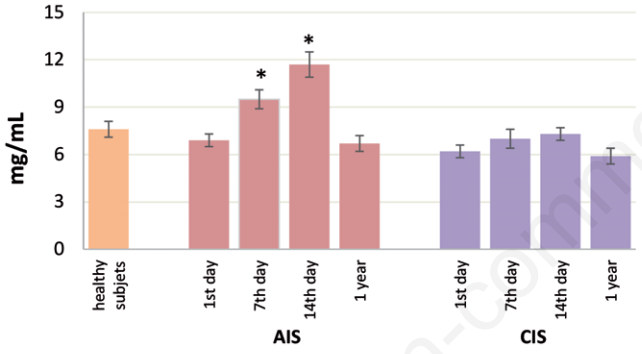

Figure 1. Concentration of IgG in the blood serum of patients with Atherothrombotic Ischemic Stroke (AIS) and Cardioembolic Ischemic Stroke (CIS); *p<0.05 significantly different from healthy subjects.

At the next stage, the influence of IgG on the activity of factor Xa was investigated. According to the data (Table 2), slight changes in the degree of substrate cleavage by factor Xa were found. We observed an inhibition of the activity of factor Xa by 18% and 15% after incubation with IgG of patients at the acute phase of CIS and one year after CIS, respectively. A similar tendency was detected in the case of IgG of patients with AIS - the activity of factor Xa decreased by 17% after incubation with IgG of patients at the acute phase and by 20% after incubation with IgG one year after AIS.

We also examined the effect of IgG on the activity of thrombin that was an endogenously activated in the blood plasma by a specific thrombin activator. The obtained results are shown in Table 2.

It was established that IgG of stroke patients caused a decrease in the amidolytic activity of thrombin activated in the blood plasma. The inhibition of thrombin activity by IgG of the patients at the acute phase of stroke was by 15% and 17% for AIS and CIS, respectively. Incubation of the plasma with IgG derived from the blood serum of patients one year after stroke resulted in a more pronounced inhibition of the amidolytic activity of thrombin. In this case, the activity of thrombin was decreased by 31% for AIS and 26% for CIS IgG.

Generally speaking, a multidirectional effect of IgG on thrombin activity might be explained by the fact that experiments were carried out at different conditions - using pure active thrombin and thrombin that was activated in the plasma. Taking into account the results regarding the influence of IgG on the activity of thrombin and thrombin activated in the blood plasma, we supposed that IgG of stroke patients can bind with the molecule of thrombin as well as with its precursor prothrombin. In the first case, it enhances the ability of thrombin to cleavage the specific substrate; in another one, the activation of prothrombin into thrombin could be disturbed as a result of binding of IgG to the appropriate sites on prothrombin.

There are few reports[14,15] that a low level of plasma protein C might be among risk factors contributing to recurrent thrombotic

Table 2. Effect of IgG on the cleavage of chromogenic substrates by active enzymes or endogenously generated enzymes (M±m).

The degree of chromogenic substrate cleavage (nmol pNA/min) caused by thrombin (S2238) or factor Xa (S2765) that was previously incubated with IgG

			AIS		CIS	
	Control (without IgG)	Healthy subject's IgG	Acute phase	One year after stroke	Acute phase	One year after stroke
S2238	21.6±2.0	20.8±1.5	29.1±2.2*	19.5±1.8	28.7±1.9*	22.9±1.7
S2765	26.2±1.7	25.8±1.6	21.6±1.6*	20.8±1.5*	21.2±1.7*	22.0±1.5*

The degree of specific peptide substrate cleavage (nmol pNA/min) caused by endogenously generated thrombin (S2238) or protein C (S2366) that were appeared after adding of the specific enzyme activators to plasma which was previously incubated with IgG

			AIS		CIS	
	Control (without IgG)	Healthy subject's IgG	Acute phase	One year after stroke	Acute phase	One year after stroke
S2238	27.0±1.6	28.3±2.5	22.9±1.5*	18.7±1.8*	22.5±1.2*	20.0±1.9*
S2366	12.5±0.9	11.6±1.3	9.5±0.9*	12.0±1.6	8.7±0.8*	11.6±1.5

*p<0.05 significantly different from control.

disease. This protein is recognized to exert an important role in anticoagulation.[16] To address whether IgG of stroke patients could affect the anticoagulant status, the activity of protein C was determined. For this purpose, the plasma of healthy subjects was pre-incubated with IgG of stroke patients and then a specific activator of protein C was added. According to the results, which are demonstrated in Table 2, a statistically significant change in the activity of protein C was found only in the case of IgG derived at the acute phase of ischemic stroke. The amidolytic activity of protein C was decreased by 26% for AIS IgG and by 33% for CIS IgG. Our results have revealed that IgG of healthy subjects and patients one year after stroke did not affect the activity of protein C.

To confirm the ability of IgG of the stroke patients to affect the blood coagulation, plasma clotting time in the test "Thrombin time" was assessed. This test is by far the most common screening test for coagulation abnormalities. Our results revealed (Figure 2) that the incubation of plasma with IgG of healthy subjects led to prolongation of plasma clotting times from 11.7±0.5 sec in the control to 13.9±1 sec. Treatment of the plasma with IgG of patients at the acute phase of stroke caused the opposite effect. Plasma clotting time was slightly shortened after incubation of plasma with IgG derived from the blood serum of patients with AIS and CIS.

It is well known that enhanced platelet aggregation is among the reasons for stroke incidents.[17] The high reactivity of platelets during the first year after stroke is strongly associated with an increased risk of stroke relapses. Due to a wide variety of platelet receptors, many factors can bind to platelets and influence their functional activity. We hypothesized that stroke IgG could be involved in the process of platelet activation either directly by interacting with receptors or indirectly via affecting blood factors, namely thrombin. Therefore, the next part of the research was aimed to investigate the effect of IgG on ADP-induced platelet aggregation. The results presented in Table 3 indicated the ability of IgG from the blood serum of patients with IS enhanced platelet aggregation. IgG of AIS patients seemed to be more effective than IgG of patients with CIS. The maximal degree of aggregation was 63±5% in the case of incubation of PRP with IgG of patients at the acute phase of AIS and 61±5% after incubation with IgG of CIS patients. IgG of patients one year after ischemic stroke did not influence statistically the degree of platelet aggregation.

We also addressed the possibility that IgG of patients with ischemic stroke can affect fibrinolysis. Considering that tissue Plasminogen Activator (tPA) needs for blood clot breakdown, we have evaluated the effect of IgG on the secretion of tPA by endotheliocytes. It was established that IgG derived from the blood serum of ischemic stroke patients as well as healthy subjects stimulated the secretion of tPA, but with different intensity (Figure 3).

Table 3. Effect of IgG on the ADP-induced platelet aggregation (M±m).

Group tested		Max. degree of aggregation, %
Control sample	42±5	
Healthy donors	42±8	
AIS	Acute phase	63±5*
	One year after stroke	51±5
CIS	Acute phase	61±5*
	One year after stroke	49±5

Values are expressed as mean±SD; *p<0.05 significantly different from control.

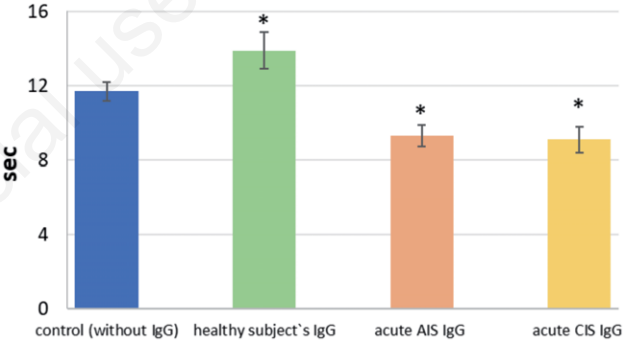

Figure 2. Effect of IgG on plasma clotting time in the test "Thrombin time"; *p<0.05 significantly different from control.

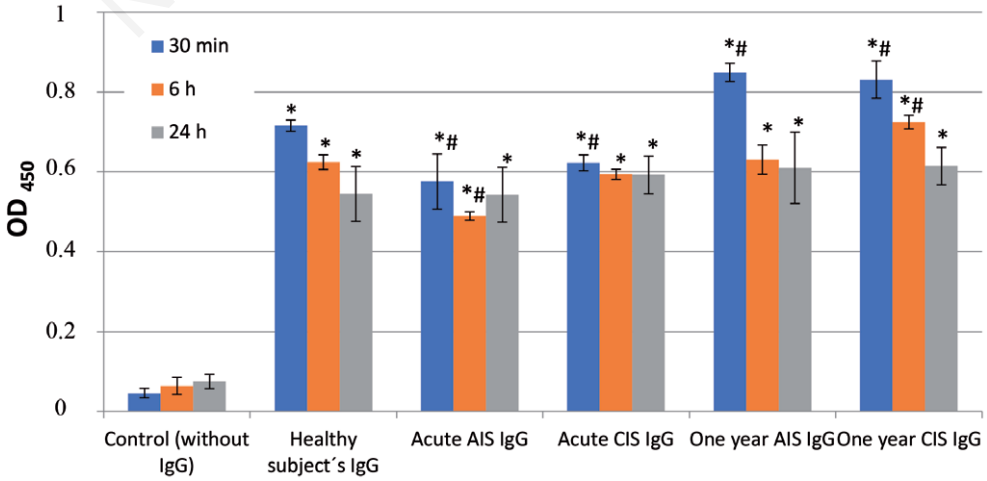

Figure 3. Effect of IgG on tPA secretion by endothelial cells; *p<0.05 significantly different from appropriate control; #p<0.05 significantly different from appropriate healthy subject's IgG. Data expressed as Optical Density of samples at 450 nm (OD450).

We should emphasize that endothelial cells do not release a significant amount of tPA in the absence of stimuli. Therefore, the basal value of tPA in control was low and stayed at the same level in all tested time points. The level of tPA in culture medium after 30 min incubation with IgG of patients at the acute phase of AIS or CIS decreased by 21% and 14% compared with the result in the group of healthy subjects. On the other hand, 30 min incubation of endotheliocytes with IgG derived from the blood serum of patients one year after a stroke caused an opposite effect on tPA level. tPA content increased by 18% for AIS IgG and by 15% for CIS IgG. A decrease of tPA level with a time of incubation (6 h, 24 h) might be evidence that IgG stimulates endothelial cells to secrete tPA but does not affect the tPA synthesis.

Conclusions

Thus, summarizing the data of the current study, we can assume that IgG of stroke patients might influence the hemostasis and its effects are multidirectional and depend on time after stroke incident. IgG of patients at the acute phase of the disease causes the increase in the activity of thrombin, enhances the degree of ADP-induced platelet aggregation, and decreases the activity of protein C. These results indicate that IgG possesses the potential to accelerate thrombus formation. On the other hand, the decrease in the activity of factor Xa, endogenously generated thrombin, and shortening the blood clotting time in the "Thrombin test" shows that IgG exhibits anticoagulant activity. Generally, the effect of IgG from the blood serum of patients at the acute phase of AIS was the same as the effect of IgG of patients with CIS. It was surprising to find that IgG of patients one year after stroke does not show prothrombotic effects on key hemostasis factors as they do not influence the activity of thrombin, protein C, and the process of platelet aggregation. Moreover, our findings indicate the ability of IgG to promote the enhancement of anticoagulation by decreasing the activity of factor Xa and endogenously generated thrombin. This statement must be confirmed in further studies and the underlying mechanisms need to be clarified. Obtained results may be potential of interest to design the strategies for the diagnosis and control of the treatment of ischemic stroke.

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