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Influence of peptide pools from the plasma of ischemic stroke patients on hemostasis

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ABSTRACT

Ischemic stroke is one of the most common causes of severe mortality and disability worldwide. The detailed investigation of the pathophysiological mechanisms underlying this disease is among the most important tasks of modern science. The current study aims to analyze the influence of the peptide pools from the plasma of ischemic stroke patients on key hemostasis factors. 25 patients with atherothrombotic ischemic stroke and 25 patients with cardioembolic ischemic stroke were examined. The same patients also participated in a research one year after the stroke incidence. The peptide pools were isolated from the plasma by sequential precipitation with perchloric acid and ethanol. The concentration of peptide pools was measured spectrophotometrically at 210 nm. The size exclusion chromatography on Sephadex G 15 column was applied to analyze the peptide pool composition. The effects of the peptide pool on the generation and/or activity of key clotting factors like thrombin, factor Xa, and protein C were assayed using specific peptide substrates. A significant increase in the concentration of the peptides at the acute phase of the stroke was established. The changes in the composition and the content of individual molecules within the peptides pools of patients with stroke were revealed. The peptide pools of stroke patients and healthy subjects decreased ADP-induced (adenosine diphosphate) platelet aggregation and stimulated tPA (tissue plasminogen activator) secretion by endothelial cells. The effect of the peptide pools on the amidolytic activity of key hemostasis enzymes was also revealed.

KEYWORDS: peptide pools, ischemic stroke, hemostasis.

INTRODUCTION

Stroke is one of the leading causes of mortality and permanent disability worldwide [1, 2]. Approximately 80% of stroke incidence belongs to the ischemic type, which is a multifactorial and complicated disease, resulting from an intricate 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 (IS) and stroke-related disorders [3]. Despite significant efforts to clarify the reasons and key triggers of this disease, the exact mechanisms of IS remain largely unknown and about one-third of the cases of cerebral ischemia have no apparent cause. Patients who survived the stroke are known to be at significantly increased risk for recurrent stroke compared with the general population. Therefore, there is a pressing need for a better understanding of the mechanisms of ischemic stroke. A comparative study of the pathogenesis of ischemic stroke at the acute phase and one year after the stroke incident could be important to improve diagnostic approaches and prevent relapses.

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The total amount of peptides in biological fluids and tissues has represented the peptide pools, which are involved in the maintenance of homeostasis. According to the literature data, the peptide pool exerts a modulation effect on nervous, endocrine, immune, and cardiovascular systems [4, 5]. The peptides within pools can be formed from the protein precursors during protein processing as well as represent the degradation products of tissue proteins. Considering that both the peptide composition and the level of individual molecules within the pool depend on the activity of proteolytic enzymes, the pathologies that are associated with impairment of proteolysis could be accompanied by the alteration in the peptide pools. Moreover, the changes in the qualitative and quantitative composition of peptide pools not only result from the metabolic disorders but also could lead to further complications under the pathological process. Therefore, the analysis of the peptide pools of patients is useful in providing the prognostic criteria for monitoring the disease progression and estimating the effectiveness of therapeutic strategies.

Based on the fact that ischemic stroke is linked to a proteolytic imbalance [6, 7], it was hypothesized that the tissue peptide pools can undergo changes under ischemic injury, and it likely might reflect the metabolic status of ischemic stroke patients. To examine this idea, the concentration of peptide pools in the plasma of patients with ischemic stroke, the peptide composition, and the influence of peptide pools on platelet function and the activity of key enzymes of coagulation were determined.

MATERIALS AND METHODS

Participants

A total of 50 ischemic stroke patients (mean age 78.5 ± 9.5 years) were selected for this study. The patients with acute ischemic stroke were admitted to the neurology department of Kyiv City Hospital №4 within 24 hrs of the incident. The diagnosis of IS was confirmed by neurovisualization with computed tomography and magnetic resonance imaging. The stroke subtype (cardioembolic or atherothrombotic) was classified according to the TOAST (Trial of Org 10172 in acute stroke treatment) classification. Patients with infections, immune diseases, or abnormal heart, liver and kidney functions were excluded. Patients who were on anticoagulant therapy at admission were also excluded from the study. There were 25 patients with atherothrombotic ischemic stroke (AIS) and 25 patients with cardioembolic ischemic stroke (CIS). A follow-up study was also conducted on the same patients one year after the stroke incidence. The control group comprised of 30 healthy subjects (mean age 76.3 ± 2.5 years) who hadn't taken antiplatelet drugs over the last 14-day period. The patients or/and their relatives were briefed about clinical research. The written informed consent was obtained from all participants. The study design was approved by the Ethics committee of Educational and Scientific Centre "Institute of Biology and Medicine" of Taras Shevchenko National University of Kyiv (Ukraine) and was conducted according to the Declaration of Helsinki. Thus, there were 5 experimental groups: 1) healthy subjects (n = 30); 2) patients at acute phase of cardioembolic stroke (CS) (n = 25); 3) the same patients one year after cardioembolic stroke (n = 25); 4) patients at acute phase of atherothrombotic stroke (AS) (n = 25); and 5) the same patients one year after atherothrombotic stroke (n = 25).

Sample collection

Blood samples of patients with IS and healthy subjects were obtained in collaboration with the Kyiv City Hospital №4. Plasma samples were prepared by collecting blood in vacuum tubes containing 3.8% sodium citrate. After centrifugation at 800 g for 40 min, the supernatants were taken and stored in small aliquots at −80 °C until analysis.

Peptide pool isolation

The peptide pool was obtained according to the [8]. The plasma samples were mixed with 1.2 M $HClO_4$ at 1:1 (v/v) ratio in order to precipitate the proteins. After centrifugation at 10000 g for 20 min at 4 °C the supernatants were neutralized by 5 M KOH to pH 7.0 and the samples were subjected to centrifugation step again. After ethanol was added to the final concentration of 80%, the samples were kept at 4 °C for 30 min and centrifuged. The optical density of the supernatants was determined using the spectrophotometer Smart SpecTMPlus (BioRad, USA) at 210 nm. The concentration of peptides was calculated using calibration curve

prepared with CBZ-glycil-glycine dipeptide of 0.26 kDa as a standard.

Analysis of peptide pools by size-exclusion chromatography

The peptide pools were analyzed by size exclusion chromatography on Sephadex G 15 column (Bio Rad, USA) pre-equilibrated with 0.05 M Tris-HCl, pH 7.4 containing 0.13 M NaCl. The samples were loaded and the corresponding peaks were obtained at a flow rate of 30 mL per hour. The areas under the peaks of chromatographic curves were calculated using the OriginLab (v 9.1). The molecular weight of peptides was estimated using the calibration curve. For this purpose, the column was previously calibrated with the standard marker solution containing lysozyme (14.3 kD), insulin (5.7 kDa), and vitamin B12 (1.35 kDa).

Platelet-rich plasma and platelet-poor plasma preparation

In order to investigate the influence of the peptide pool on normal hemostasis process, *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 for platelet analysis was obtained by healthy subject's blood centrifugation 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 of resting period prior to aggregation experiments, which were undertaken within the first 3 hrs after blood sampling.

Chromogenic substrate spectrophotometric assays

The effect of peptide pool on generation and/or activity of key clotting factors was assayed using specific peptide substrates with detectable leaving group (Renam, Russia): H-D-Phe-Pip-Arg-*p*NA (S-2238) for thrombin, Z-D-Arg-Gly-Arg-*p*NA (S-2765) for factor Xa, and pyroGlu-Pro-Arg-*p*NA (S-2366) for activated protein C. The production of p-nitroaniline (*p*NA) was monitored spectrophotometrically (μQuant, BioTek) at 405 nm (thermostatted at 37 °C) against appropriate blank to subtract spontaneous hydrolysis of the substrate. The activities of enzymes were calculated using a molar extinction coefficient of *p*-nitroaniline – 9.96 $\mu M^{-1} \cdot cm^{-1}$, and expressed as μmol of *p*-nitroaniline per 1 min.

To evaluate the effect of peptide pool on activities of thrombin (Renam, Russia) or factor Xa (Sigma, USA), the active enzymes with final activity of 0.4 $U \cdot mL^{-1}$ and 0.6 $U \cdot mL^{-1}$, respectively, were incubated in TBS, pH 7.4 in the presence of the peptide pool (300 μg per 250 μL of incubation medium) in the wells of 96-well plate. After 5 min incubation at 37 °C, 25 μL of the appropriate chromogenic substrate S-2238 or S-2765 (with the final concentration of 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 the peptide pool on the thrombin generation, healthy donor`s plasma (PPP) was incubated in TBS with the peptide pools (300 μg per 250 μL of incubation medium). After 5 min incubation at 37 °C, the thrombin generation was carried out by addition of 25 μL of prothrombin activator "Ecamylin". After 5 min of incubation at 37 °C, 25 μL of the chromogenic substrate S-2238 (the final concentration 0.3 mM) was added to the reaction mixture. The final volume of the incubation medium was 250 μL. The kinetic measurements were performed 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 S-2366. Protein C activator derived from the venom of *Agkistrodon blomhoffi ussuriensis* was used. Control samples for each experiment contained the appropriate components but equal volume of TBS, pH 7.4 instead of IgG.

Platelet aggregation assay

The effect of the peptide pool on *in vitro* aggregation of platelets in healthy donor's PRP 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-incubated for 5 min with the peptide pools (the final concentration was 1.2 mg per 1 mL of PRP) at 37 °C with continuous stirring at 600 rpm. To induce platelet aggregation, ADP (Sigma, USA) at the final concentration of 5×10^{-6} M

was added to the samples. The aggregation process was monitored for 10 min under continuous stirring at 600 rpm and 37 °C [9]. The curves that represented the ADP-dependent aggregation after 5 min incubation of PRP with TBS, pH 7.4 (instead of peptide pool) were used as controls for this experiment. Data from five independent experiments were used for statistical analysis.

Tissue plasminogen activator assay

Total tissue plasminogen activator measurement in the culture medium was done by enzyme-linked immunosorbent assay according to the standard instructions. ELISA (enzyme-linked immunosorbent assay) 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 37 ºC, and washed again. After that the plate was incubated for 1 h at 37 ºC with monoclonal antibody to human tPA (Santa Cruz Biotechnology, USA). The plate was washed and incubated for 1 h at 37 ºC with corresponding secondary antibody conjugated to horseradish peroxidase (Bio-Rad, USA). After washing, 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 to each well. The reaction was terminated by the addition of 1N H_2SO_4. The absorbance was measured by a microplate reader (μQuant^{TM}, BioTek Instruments, Inc) at 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 DISCUSSION

According to the concept of "tissue-specific peptide pool" [10], the sum of all peptides in the tissue is considered as the peptide pool, which actively takes part in the maintenance of homeostasis within the organ. Despite the sufficient stability of the peptide pool, the amount and repertoire of peptides undergo alterations under the pathological process. The change in the peptide pool is thought to be one of the factors to disturb homeostasis in response to disease progression. On the other hand, due to the broad spectrum of activities [11] peptides could be involved in tissue repair mechanisms and in the normalization of the metabolic state.

Considering that, the first step of our study was to determine the concentration of peptides in the plasma of patients with AIS and CIS. Our finding revealed a significant increase in the concentration of peptides only at the acute phase of stroke (Table 1). The concentration of peptides was 3.14 times and 2.55 times higher in the group of patients with AIS and CIS, respectively than that in the group of healthy subjects. As follows from the data, the concentration of peptides in the plasma of patients one year after AIS and CIS has returned to the level of healthy subjects.

The increase in the concentration of peptides partly could be due to the elevation of proteolysis. Evidence from clinical studies demonstrates that under ischemic stroke neuronal and inflammatory cells release enzymes like matrix metalloproteinases and cathepsins [12, 13]. Getting into the bloodstream these enzymes could cleave the plasma proteins or/and affect functional blood proenzymes leading to their activation. The normalization of peptide levels one year after both AIS and CIS compared to the results at the acute phase of diseases may be considered as evidence of the improvement of the metabolic status of patients. It is important to note that despite the absence of change in the concentration of peptides in patients one year after ischemic stroke, the qualitative composition of peptides within the pool may be different from the indicator in the group of healthy subjects. To check this idea, the composition of the peptide pool was analyzed by size exclusion chromatography. The comparative study of the peptide pool of patients at the acute phase of the disease and one year after the stroke incident potentially might be useful in searching the prognostic criteria for monitoring of disease state. As can be seen from Table 2, the peptide pool of healthy subjects consists of five fractions that correspond to peptides with the

Table 1. Concentration of peptides in the plasma of patients with IS and healthy subjects.

Group tested		**Concentration of peptides, mg·ml^{-1}**
Healthy subjects		0.068 ± 0.018
AIS	Acute phase	0.214 ± 0.054*
	One year after stroke	0.099 ± 0.023$^{\#}$
CIS	Acute phase	0.174 ± 0.047*
	One year after stroke	0.069 ± 0.021$^{\#}$

Values are expressed as mean ± SD (n = 25 for patients with IS and n = 30 for healthy subjects); *$p < 0.05$ significantly different from healthy subjects; $^{\#}p < 0.05$ significantly different from appropriate group of patients at the acute phase.

Table 2. Molecular weight (Da) and level of individual peptides (%) in the peptide pools derived from the plasma of patients with IS and healthy subjects.

		Number of fractions							
		1	**2**	**3**	**4**	**5**	**6**	**7**	**8**
Healthy subjects		1109 Da (9.6%)	953 Da (24.6%)	687 Da (30.0%)	637 Da (31.0%)	383 Da (4.8%)	-	-	-
AIS	Acute phase	1870 Da (0.4%)	1464 Da (5.0%)	889 Da (7.0%)	663 Da (6.0%)	640 Da (11.0%)	582 Da (16.5%)	540 Da (50.6%)	443 Da (3.8%)
	One year after stroke	1955 Da (1.1%)	1321 Da (3.5%)	997 Da (2.9%)	835 Da (4.4%)	765 Da (1.4%)	719 Da (3.7%)	569 Da (77.6%)	443 Da (5.0%)
CIS	Acute phase	1862 Da (0.8%)	1354 Da (5.0%)	1220 Da (12.2%)	990 Da (0.6%)	813 Da (3.8%)	545 Da (74.8%)	438 Da (2.5%)	-
	One year after stroke	1944 Da (4.5%)	1339 Da (14.0%)	1242 Da (1.7%)	987 Da (2.4%)	852 Da (4.5%)	756 Da (0.9%)	563 Da (68.9%)	438 Da (2.8%)

molecular weight in the range from 383 Da to 1109 Da. The level of peptides with molecular weight about 637 Da, 687 Da, and 953 Da was 31.0% 30.0% and 24.6%, respectively. The pathogenesis of IS both at the acute phase and one year after stroke was accompanied by the changes in quantity and quality composition of peptides compared to the result in the group of healthy subjects. The main difference was the increase in the number of peptide fractions and the appearance of peptides with higher molecular weight (up to 1955 Da) that has not been detected in the plasma of healthy subjects. In all cases, the major fraction consisted of peptides with the molecular weight near 540-569 Da.

In general, the appearance of peptides that were not found in the plasma of healthy subjects indicates the intensification of catabolism or/and processes associated with cell disruption. The slight difference in the profile of peptides detected at the acute phase of disease and one year after stroke incident might be explained by the difference in the activity of proteolytic processes. Analyzing obtained data, we can see that despite the normalization of peptide pool concentration (Table 1) in patients one year after IS, the peptide composition was not the same as in the group of healthy subjects.

Stroke incident and stroke-related complications are very often accompanied by hemostatic disorders [14, 15]. Moreover, the high procoagulant potential

of the plasma is considered one of the reasons for the long-term risk of stroke relapse. The high reactivity of platelets during the first year after stroke may account for a significant proportion of stroke relapses. Since platelets are affected by a variety of stimuli that leads to alteration of their functional activity, it can be that the peptide pool is among the factors that influence platelet aggregation. Therefore, the next goal was to investigate the effect of peptide pool derived from the plasma of patients with IS and healthy subjects on ADP-induced platelet aggregation. As seen in Table 3, the peptide pool of healthy subjects did not influence the platelet aggregation.

The treatment of platelet-rich plasma of healthy subjects with the peptide pool of patients with IS leads to inhibition of ADP-induced platelet aggregation. The maximum degree of aggregation was decreased by 19% after the influence of the peptide pool of patients at the acute phase of AIS and by 29% after the influence of the peptide pool of patients one year after stroke incident. The peptide pools of patients with CIS mediated the most pronounced inhibition of ADP-induced platelet aggregation – the maximum degree of aggregation was reduced to 48 ± 3% and 44 ± 3% after the influence of the peptide pools of patients at the acute phase and one year after stroke, respectively. The inhibition of aggregation by the peptide pool indicates the presence of molecules with anti-aggregation activity within the pool and can be considered as one of the mechanisms of recovery after a stroke. Such an effect of peptides may be explained, first of all, by their direct action on platelet receptors. Additionally, peptides can compete with other effector molecules for binding with receptors.

Considering that thrombin generation is the central event of coagulation [16], it seemed of interest to determine the effect of the peptide pool on thrombin. Two types of experiments were performed. First, we investigated the influence of the peptide pools on the amidolytic activity of thrombin using pure active enzyme and the relevant chromogenic substrate S-2238. The effect of the peptide pools on thrombin generation in plasma was also examined. For this purpose, the plasma of healthy subjects was incubated with the peptide pool, and after that specific prothrombin activator was added. The obtained results are presented in Table 4.

The results of the assay demonstrated that the peptide pools did not affect the amidolytic activity of thrombin - the activity remained at the control level under the influence of the peptide pool of AIS patients, acute CIS patients, and healthy subjects. Only the peptide pool from the plasma of patients one year after CIS leads to the decrease (by 19%) in activity of thrombin in comparison with the control result.

The peptide pool of AIS patients caused an increase in the amidolytic activity of thrombin generated in the plasma. The thrombin activity was increased by 10% after incubation of the plasma with the peptide pool of patients at the acute phase of AIS. The incubation of the plasma with the peptide pool of patients one year after

Table 3. The effect of the peptide pool of patients with IS and healthy subjects on the platelet aggregation.

Group tested		Maximum degree of aggregation, %
Control sample		61 ± 5
Healthy subjects		56 ± 4
AIS	Acute phase	51 ± 3*
	One year after stroke	47 ± 4*
CIS	Acute phase	48 ± 3*
	One year after stroke	44 ± 3*

Values are expressed as mean ± SD (n = 25 for patients with IS and n = 30 for healthy subjects); *p < 0.05 significantly different from control sample.

Table 4. Influence of the peptide pools of patients with IS and healthy subjects on the amidolytic activity of pure thrombin, factor Xa as well as endogenously generated thrombin and protein C.

Amidolytic activity, μmol *p*NA·min^{-1}·mg protein^{-1}						
	Control sample	Healthy subjects	AIS		CIS	
			Acute phase	One year after stroke	Acute phase	One year after stroke
Pure thrombin	0.42 ± 0.02	0.42 ± 0.01	0.45 ± 0.04	0.44 ± 0.01	0.45 ± 0.01	0.35 ± 0.02*
Factor Xa	0.39 ± 0.02	0.32 ± 0.02*	0.68 ± 0.02*	0.40 ± 0.01	0.59 ± 0.02*	0.35 ± 0.01*
Endogenously generated thrombin	0.71 ± 0.02	0.70 ± 0.02	0.78 ± 0.02*	0.82 ± 0.02*	0.80 ± 0.02*	0.66 ± 0.02*
Endogenously generated protein C	0.89 ± 0.02	1.06 ± 0.03*	0.76 ± 0.02*	1.05 ± 0.04*	0.85 ± 0.02	1.03 ± 0.03*

Values are expressed as mean ± SD (n = 25 for patients with IS and n = 30 for healthy subjects); *p < 0.05 significantly different from control sample.

AIS resulted in a more pronounced increase in the amidolytic activity of thrombin - this parameter was by 15% higher than in the control sample. As can be seen from the results (Table 4), the amidolytic activity of thrombin increased (by 13%) under the influence of the peptide pool obtained from the plasma of acute CIS patients and slightly decreased (by 7%) in case of the incubation with peptide pool of patients one year after stroke.

During the next stage of our study, the influence of the peptide pools on the activity of factor Xa (fXa) was investigated. Factor Xa is a part of the prothrombinase complex, which plays a pivotal role in blood coagulation cascade participating in the activation of prothrombin to thrombin [17]. The amidolytic activity of fXa was tested on the synthetic fXa-specific substrate, S-2765. According to the data (Table 4), the peptide pool of patients at the acute phase of IS leads to a significant increase in the amidolytic activity of fXa. The activity of fXa was increased by 75% and 52% after incubation with the peptide pools of patients with AIS and CIS, respectively. The increase in the activity of fXa under the influence of the peptide pool may be the evidence of the pathological potential of the peptides presented in the peptide pool of patients with stroke. The activity of fXa was at the level of control sample in case of incubation of fXa with the peptide pool of patients one year after AIS. Peptide pools of healthy subjects and patients one year after CIS exhibit an opposite effect - the activity of fXa was decreased by 13% and 11% in comparison with the control value. Such an effect of peptide pools can be considered as a part of the mechanism to prevent the activation of prothrombin in thrombin.

To comprehensively study the effect of the peptide pools on the hemostatic system, we investigated the effect of the peptide pools on the amidolytic activity of protein C. This protein is recognized to exert a potent anticoagulant activity by regulating the activities of FVIIIa and FVa - important cofactors in the activation of FX and prothrombin, respectively [18]. According to our result, the amidolytic activity of protein C decreased by 16% after treatment with the peptide pool of patients at the acute phase of AIS. No effect of the peptide pool from the plasma of patients at the acute phase of CIS on the activity of protein C was observed. An increase in the activity of protein C in response to the influence of the peptide pools of patients with AIS and CIS one year after stroke incident was found (Table 3). It should be noted that the peptide pool of healthy subjects also leads to the increase in the amidolytic activity of protein C. In this case, the activity of protein C was higher by 18% than the control value.

Table 5. Influence of the peptide pools of patients with IS and healthy subjects on tPA secretion by endothelial cells.

OD_{405}						
Time, hrs	**Control sample**	**Healthy subjects**	**AIS**		**CIS**	
			Acute phase	**One year after stroke**	**Acute phase**	**One year after stroke**
0.5	0.01 ± 0.001	0.18 ± 0.01*	0.25 ± 0.01*	0.17 ± 0.01*	0.34 ± 0.01*	0.21 ± 0.02*
6	0.01 ± 0.001	0.17 ± 0.01*	0.23 ± 0.01*	0.17 ± 0.01	0.35 ± 0.01*	0.22 ± 0.01*

Values are expressed as mean ± SD (n = 25 for patients with IS and n = 30 for healthy subjects); *$p < 0.05$ significantly different from control sample. tPA secretion is expressed as optical density of samples at 405 nm (OD_{405}).

Considering that tissue plasminogen activator (tPA) is actively involved in the maintenance of the balance between thrombolysis and thrombogenesis, we investigated the effect of the peptide pools on the secretion of tPA by endothelial cells. It was established that all tested peptide pools stimulate the secretion of tPA, but with different intensity (Table 5). Endothelial cells by themselves do not release tPA and the basal value remains at the same level at 0.5 and 6 hrs of the experiment. The level of tPA after 30-min incubation of endothelial cells with the peptide pools of AIS and CIS patients at the acute phase of disease was higher than after incubation with the peptide pools of patients one year after stroke. This tendency continued after 6 hours of incubation with peptide pools.

The same level of tPA at 0.5 and 6 hrs of the experiment might indicate that the peptide pools stimulate tPA secretion by endothelial cells but do not affect the synthesis of tPA.

CONCLUSION

The obtained data demonstrated that the peptide pools of patients with both types of ischemic stroke contain molecules that exhibit different effects on hemostasis. On one hand, the incubation of plasma with the peptide pool of patients with stroke leads to increase of the amidolytic activity of endogenous generated thrombin (the acute phase of AIS, CIS and one year after CIS) and activity of pure factor Xa (the acute phase of AIS and CIS). On the other hand, the peptide pools derived from the plasma of subjects with ischemic stroke caused the decrease of ADP-induced platelet aggregation, mediated the increase of the amidolytic activity of endogenous generated protein C (one year after AIS and CIS), and stimulated the secretion of tPA by endothelial cells. These results may be of interest to those designing strategies for the diagnosis and treatment of stroke and stroke-related complications. But with no doubt, more studies are needed to accurately clarify the results of this experiment.

CONFLICT OF INTEREST STATEMENT

The authors report no potential conflicts of interest relevant to this article.

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