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Plasmin–α_2-Antiplasmin Complexes in Acute Ischemic Stroke

Kompleksy plazmina–α_2-antyplazmina w ostrym okresie udaru niedokrwiennego mózgu

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Abstract

Background. In acute and chronic ischemic stroke, different hemostatic disturbances are described. Plasmin–α_2-antiplasmin complexes present in the blood reflect the formation of plasmin *in vivo*.
Objectives. The aim of the study was to determine the concentration of PAP complexes in the blood of patients with ischemic stroke during the first 24 hours after onset and the influence of patient age, extent of stroke, and some risk factors (hypertension, hypercholesterolemia, ischemic heart disease, atrial fibrillation, diabetes mellitus, and smoking) on the concentration of PAP complexes.
Material and Methods. The examined group consisted of 73 patients aged 42–90 years with ischemic stroke diagnosed for the first time. The control group consists of 30 healthy persons aged 40–65 years. The concentration of PAP complexes was determined in citrate plasma with ELISA and of plasminogen (PL) and α_2-antiplasmin (α_2-AP) with chromogenic substrates (Behring).
Results. The PAP complexes were significantly higher in the patient group than in the controls and increased with patient age and the extent of stroke. The risk factors for ischemic stroke had no influence on the PAP level.
Conclusions. In acute ischemic stroke, increased concentration of PAP complexes indicated higher plasmin generation *in vivo*. PAP increased with patient age and were significantly higher in extensive stroke. The risk factors for ischemic stroke had no influence on PAP level (**Adv Clin Exp Med. 2006, 15, 5, 797–801**).

Key words: ischemic stroke, plasmin–α_2-antiplasmin complexes.

Streszczenie

Wprowadzenie. W ostrym i późnym okresie udaru niedokrwiennego mózgu występują zaburzenia hemostazy. Dobrym wskaźnikiem plazminogenezy *in vivo* jest stężenie kompleksów plazmina–α_2-antyplazmina (PAP).
Cel pracy. Ocena stężenia tego wskaźnika we krwi chorych będących w pierwszej dobie udaru niedokrwiennego mózgu w porównaniu z grupą kontrolną oraz stężenia kompleksów PAP w zależności od wieku pacjentów, rozległości niedokrwienia mózgu (grupy Oxfordshire Classification) i obecności wybranych czynników ryzyka udaru, takich jak: nadciśnienie tętnicze, hipercholesterolemia, choroba niedokrwienna serca, migotanie przedsionków, cukrzyca oraz palenie papierosów.
Materiał i metody. Badaniem objęto 73 chorych w wieku 42–90 lat, u których objawy udaru niedokrwiennego mózgu wystąpiły po raz pierwszy w życiu. Grupę kontrolną stanowiło 30 zdrowych osób w wieku 40–65 lat. Stężenie kompleksów PAP oznaczono w osoczu krwi cytrynianowej metodą immunoenzymatyczną, a stężenie plazminogenu i α_2-antyplazminy na substratach chromogennych.
Wyniki. Stężenie kompleksów PAP było istotnie większe w grupie chorych w porównaniu z grupą kontrolną i zwiększało się wraz z wiekiem pacjentów. Stężenie tego wskaźnika było największe u chorych w grupie TACI (z najbardziej rozległym udarem mózgu)
Wnioski. W ostrej fazie udaru niedokrwiennego mózgu stwierdzono podwyższone stężenie kompleksów PAP świadczące o wzmożonej generacji plazminy *in vivo*. Stężenie kompleksów PAP zwiększa się wraz z wiekiem i rozległością zawału mózgu. Obecność czynników ryzyka udaru mózgu nie wpłynęła na stężenia kompleksów PAP (**Adv Clin Exp Med. 2006, 15, 5, 797–801**).

Słowa kluczowe: udar niedokrwienny mózgu, kompleksy plazmina–α_2-antyplazmina.

In acute and chronic ischemic stroke, hemostatic abnormalities change the balance towards coagulation by activating blood platelets, coagulation, and fibrinolysis. Plasmin is the most important enzyme in fibrinolysis activation. In the blood it is immediately inactivated by inhibitors, especially α_2-antiplasmin, and forms the inactive complex plasmin–α_2-antiplasmin (PAP). The PAP complex is a direct indicator of plasmin generation *in vivo*. It is possible to estimate the results of plasmin activity on fibrinogen and fibrin (FDP, D-dimers). In the literature there are more data about disturbances in fibrinolysis in coronary artery disease than in stroke [1–6].

The aim of this study was to determine the levels of PAP complexes in the blood of patients with ischemic stroke during the first 24 hours after onset and the influence of the patients' age, extent of stroke, and some risk factors (hypertension, hypercholesterolemia, ischemic heart disease, atrial fibrillation, diabetes mellitus, and smoking) on the concentration of PAP complexes. The levels of plasminogen (PL) and α_2-antiplasmin (α_2-AP) in the patients and the control group were also determined.

Material and Methods

The examined group consisted of 73 patients (36 women, 37 men) aged 42–90 years (mean age: 68 ± 11.6) who had been admitted to the Department of Neurology at Biziel Memorial Hospital in Bydgoszcz within 24 hours after the onset of their first-ever stroke. They had no symptoms of consciousness disturbances at that time. They had clinical signs of focal brain damage in the area of the anterior brain circulation. Reasons other than vascular for the neurological deficit were excluded by brain CT. Some of the risk factors for ischemic stroke in these patients included hypertension (73%), hypercholesterolemia (73%), ischemic heart disease (26%), atrial fibrillation (14%), diabetes mellitus (26%), and smoking (22%).

The patients were divided into three groups according to the age (< 55, 55–75, > 75 years old) and into three groups according to the extent of stroke (Oxfordshire Classification). Seventy-two percent of the patients suffered from partial anterior circulation infarct (PACI), 12% total anterior circulation infarct (TACI), and 16% lacunar anterior circulation infarct (LACI). There were no patients in the fourth Oxfordshire Classification group, i.e. posterior circulation infarct (POCI). The PAP complexes were determined in these groups.

The control group consisted of 30 healthy persons (15 men, 15 women) aged 40–65 years (mean age: 59.5 ± 7.6) who were healthy and without clinical indicators of atherosclerosis or prior history of neurological disease.

The parameters of fibrinolysis were measured at the Department of Pathophysiology Medical University in Bydgoszcz. The study was accepted by the local ethics committee.

Venous blood (4.5 ml) was withdrawn into a tube containing 0.5 ml of a 3.2% sodium citrate solution between seven and eight a.m. from the patients (this time being within 24 hours of stroke onset) and the healthy controls. The blood-samples were immediately centrifuged (3000 rpm for 20 min). The plasma was portioned and frozen at –20°C. The concentration of PAP complexes in the citric plasma was determined using an enzyme-linked immunosorbent assay (Enzygnost PAP micro, Behring, Marburg, range of method references: 99–369 µg/l), and plasminogen (PL) and α_2-antiplasmin (α_2-AP) by chromogenic substrates (Behring).

The results of the measurements were statistically analyzed using Microsoft® Excel 2000 and Statistica for Windows 5.0 by Statsoft®. A p value ≤ 0.05 was considered statistically significant. The values of the PAP complexes did not conform to the normal distribution. Their data are presented as the median (*Me*) and lower and upper quartiles (Q1, Q3). PL and α_2-AP values conformed to the normal distribution and their values are presented as the average (*X*) and the standard deviation (*SD*).

Results

The concentrations of PAP complexes and the levels of PL and α_2-AP (components of PAP complexes) in the patients and controls are shown in Table 1.

The concentration of PAP complexes in patients with an ischemic stroke within the first 24 hours after stroke onset was statistically significantly higher than in the control group. The level of PL was higher and the level of α_2-AP was lower in patients than in the control group. The concentrations of PAP complexes in the acute stroke patients according to age and the extent of stroke are illustrated in Figures 1 and 2.

The concentration of PAP complexes increased with patient age. It was significantly higher in patients over 75 years of age compared with younger patients.

The concentration of PAP complexes was the highest in patients with total anterior circulation infarct (TACI). It was significantly higher compared with patients with less extensive stroke (PACI, LACI).

Table 1. The examined parameters of plasminogenesis in stroke patients and in the control group

Tabela 1. Badane wskaźniki plazminogenezy u chorych na udar mózgu i w grupie kontrolnej

Parameter (Wskaźnik)	Ischemic stroke (Udar mózgu)	Control group (Grupa kontrolna)	Statistical significance (Istotność statystyczna)
	(n = 73) Me (Q1, Q3) X ± SD	(n = 30) Me (Q1, Q3) X ± SD	p
PAP (μg/l)	381 (226, 618)	180 (150, 240)	0.05
PL (%)	117 ± 25	87 ± 24	0.0001
α_2-AP (%)	80 ± 13	95 ± 11	0.0001

PAP – plasmin–α_2-antiplasmin.
PL – plasminogen.
α_2-AP – α_2-antiplasmin.
Me – median.
Q1, Q3 – quartiles.
p – level of significance of the differences between the examined groups.

PAP – plazmina–α_2-antyplazmina.
PL – plazminogen.
α_2-AP – α_2-antyplazmina.
Me – mediana.
Q1, Q3 – kwartyle.
p – poziom istotności różnic między badanymi grupami.

Table 2 shows the concentration of PAP complexes in patients with and without the risk factors hypertension, hypercholesterolemia, atrial fibrillation, diabetes mellitus, smoking, and ischemic heart disease.

There were no significant differences in the concentrations of PAP complexes in patients with and without the investigated stroke risk factors.

Discussion

Plasmin–α_2-antiplasmin complexes are a direct indicator of plasmin generation *in vivo*. These results showing that the concentration of PAP complexes was significantly increased in the first 24 hours of an ischemic stroke compared with the control group are similar to those of Kataoka et al. [7] and Ono et al. [8], whereas Tongi et al. observed that the concentration of PAP complexes was not increased during the first week of a stroke (it was only slightly elevated) compared with controls. Yamazaki et al. [5] measured the concentration of PAP complexes during the first seven days after stroke onset and according to the etiopathological type of stroke. They found significantly increased concentrations of this parameter only in embolic strokes.

In the present study the concentration of PAP complexes increased with patient age. The differences were statistically significant between the groups under 56 years old and above 75 years old and also between the group 56–75 years old and the group over 75 years old. Furthermore, there was no difference in its concentration in the group of patients under 56 years old compared with the control group. These results are similar to those of Ono et al. [8], who measured the concentrations of PAP complexes in patients divided into three groups (≤ 64 years old, 65–74 years old, ≥ 75 years old). They found significantly higher concentrations of PAP complexes in the group 65–74 year old and the group over 75 years old compared with the control group. Bom et al. [1] also found an increase in this parameter with patient age, while Meijer et al. [9] found no such dependency in their patients.

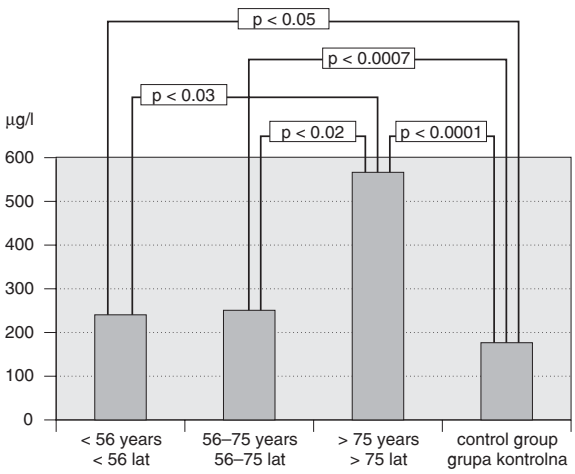

Fig. 1. Concentration of PAP complexes in three age groups of patients and control group

Ryc. 1. Stężenie kompleksów PAP u chorych w trzech grupach wiekowych i w grupie kontrolnej

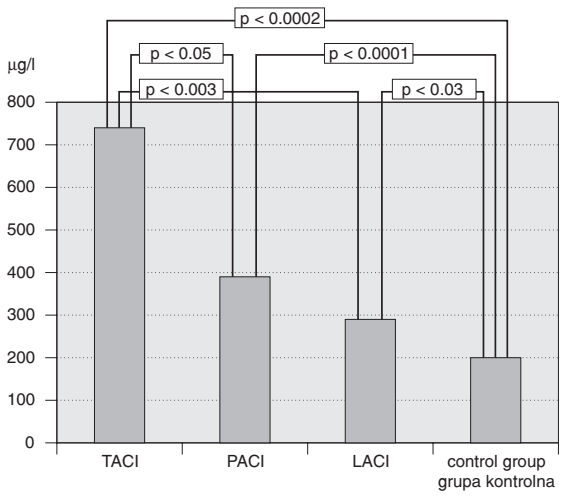

Fig. 2. Concentration of PAP complexes in patients according to extent of stroke and in control group

Ryc. 2. Stężenia kompleksów PAP u chorych zależnie od rozległości niedokrwienia mózgu i w grupie kontrolnej

Table 2. Concentration of PAP complexes in patients with and without the examined risk factors for ischemic stroke

Tabela 2. Stężenie kompleksów PAP u chorych w zależności od obecności lub braku danego czynnika ryzyka udaru niedokrwiennego mózgu

Risk factors (Czynniki ryzyka)	n	Median (Q1, Q3) (Mediana – kwartyle)	p
Hypertension (Nadciśnienie tętnicze)	46	379.3 (226.2, 597.9)	
Without hypertension (Bez nadciśnienia tętniczego)	17	453.6 (241.4, 728.9)	ns
Ischemic heart disease (Choroba niedokrwienna serca)	17	422.6 (181.8, 772.6)	
Without ischemic heart disease (Bez choroby niedokrwiennej serca)	46	379.3 (234.2, 586.53)	ns
Atrial fibrilation (Migotanie przedsionków)	8	487.82 (223.85, 795.75)	
Without atrial fibrilation (Bez migotania przedsionków)	55	376.9 (226.2, 597.9)	ns
Cholesterol level (Poziom cholesterolu) > 200 mg/dl	45	369.7 (234.2, 514.26)	
Cholesterol level (Poziom cholesterolu) < 200 mg/dl	13	422.6 (147.1, 728.9)	ns
Diabetes mellitus (Cukrzyca)	17	422.6 (266.5, 565.3)	
Without diabetes mellitus (Bez cukrzycy)	46	379.3 (226.2, 618.15)	ns
Smokers (Palący)	15	282.6 (226.2, 651.9)	
Non-smokers (Niepalący)	48	405.85 (215.33, 608.03)	ns

In the present study the concentrations of PAP complexes among the groups according to the extent of stroke were compared. It was significantly higher in the TACI group compared with the LACI and PACI groups (patients with a less extensive stroke). It was significantly higher in all groups compared with the controls. In the literature, no studies about the Oxfordshire Classification groups and the concentrations of PAP complexes were found. Toghi et al. [3] compared the concentration of PAP complexes in acute stroke with the size of the ischemic focus in brain CT. CT was done one or two weeks after a stroke. The concentration of this parameter was higher when the ischemic focus was larger than 10 mm. This was not studied here because the patients of the present study had CTs in the first hours of stroke and the ischemic focus was invisible. Toghi et al. [4] observed a coexistence of a higher concentration of PAP complexes and C-reactive protein in patients with ischemic stroke. This probably depends on the coexistence of cerebritis. This was not investigated in the present study.

The concentration of PAP complexes in patients with some risk factors for stroke (hypertension, hypercholesterolemia, ischemic heart disease, atrial fibrillation, diabetes mellitus, and smoking) compared with patients without these risk factors was not significantly different. Atrial fibrillation increases the risk of an ischemic stroke five to seven times. Some studies have tried to discover whether PAP complexes could be a good risk marker for an ischemic stroke in patients with atrial fibrillation. Feinberg et al. [10] found that elderly patients, especially women, and patients with left ventricular heart failure and in the beginning of atrial fibrillation were especially exposed to stroke. A high concentration of PAP complexes presented additional risk in these patients. However, Roldan et al. [11] found no significant differences between patients with atrial fibrillation and a control group without it.

The concentration of PAP complexes was increased in patients with diabetes mellitus [6, 12].

Some authors indicated that the concentration of PAP complexes was elevated in an acute stroke and it remained in a chronic stroke as well. The activation of fibrinolysis appears later than the activation of coagulation and is a secondary occurrence [3, 4, 7, 8, 13–15].

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