

DECREASE OF FIBRINOLYTIC POTENTIAL IN THE OCCURENCE OF CEREBRAL ISCHEMIA

UTICAJ PADA FIBRINOLIZNOG POTENCIJALA NA NASTANAK CEREBRALNE ISHEMIJE

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Summary: One of the most present clinical manifestations of long and progressive atherothrombotic occurrences is the ischemic cerebrovascular insult, one of the leading causes of death and illness in the world. Lately, a growing number of scientists believe that disorders in the fibrinolytic mechanism function are the key to the occurrence of cerebral ischemia. The goal of this study is to investigate whether the disorder of the fibrinolytic mechanism has influence on the occurrence of ischemic cerebrovascular insult. Our study includes 90 examinees, 60 of which suffer from ischemic cerebrovascular insult and 30 are clinically healthy examinees forming the control group. The results of our investigation show that statistically a significantly larger number of patients has decreased fibrinolytic potential comparing with controls ($p < 0.01$). According to this, it has been noted that euglobulin lysis clot time in the patient group is significantly longer ($p = 0.005$). Statistically, no significant difference has been noted related to the activity of plasminogen ($p = 0.085$). Further on, the plasminogen activator inhibitor-1 values among the patients have been significantly higher ($p = 6.20 \times 10^{-11}$). Moreover, significantly higher values of tissue-type plasminogen activator antigen have been statistically noted in the patient group ($p = 5.20 \times 10^{-5}$). The results of this investigation impose the conclusions that the decrease in fibrinolytic potential affects the occurrence of ischemic cerebrovascular insult, that it is directly connected to the higher levels of plasminogen activator inhibitor-1 and that the growth of tissue-type plasminogen activator antigen concentration participates in the decrease of fibrinolytic potential among patients suffering from cerebral ischemia.

Keywords: fibrinolytic system, plasminogen, tissue-type plasminogen activator, plasminogen activator inhibitor-1

Kratak sadržaj: Jedna od najčešćih kliničkih manifestacija dugotrajnih i progresivnih aterotrombotskih zbivanja je ishemijski cerebrovaskularni insult. To oboljenje se danas nalazi među vodećim uzrocima morbiditeta i mortaliteta u većini zemalja. U poslednje vreme sve je veći broj naučnika koji smatraju da su poremećaji fibrinoliznog mehanizma ključ nastanka cerebralne ishemije. Cilj naše studije bio je upravo da ispitamo da li poremećaj fibrinoliznog mehanizma ima uticaja na nastanak ishemijskog cerebrovaskularnog insulta. U studiju je uključeno 90 ispitanika, od toga 60 bolesnika koji su doživeli cerebrovaskularni insult po tipu ishemije i 30 klinički zdravih osoba, koje su činile kontrolnu grupu. Rezultati našeg istraživanja ukazuju na statistički značajno veći broj ispitanika sa suprimiranim fibrinoliznim potencijalom u grupi bolesnika u odnosu na kontrolnu grupu ($p < 0,01$). U skladu s tim, ustanovljeno je statistički značajno duže euglobulinsko vreme lize koaguluma među bolesnicima nego među kontrolama ($p = 0,005$). Nije uočena statistički značajna razlika u aktivnosti plasminogena između te dve grupe ispitanika ($p = 0,085$), dok su vrednosti inhibitora aktivatora plazminogena-1 ($p = 6,20 \times 10^{-11}$) i antigena tkivnog aktivatora plazminogena ($p = 5,20 \times 10^{-5}$) statistički značajno više u grupi bolesnika. Ovi rezultati navode na zaključke da sniženje fibrinoliznog potencijala ima uticaja na pojavu ishemijskog cerebrovaskularnog insulta, da je ono u direktnoj vezi sa povišenim nivoima inhibitora aktivatora plazminogena-1, kao i to da i porast koncentracije antigena tkivnog aktivatora plazminogena doprinosi sniženju fibrinoliznog potencijala među pacijentima koji obolevaju od cerebralne ishemije.

Ključne reči: fibrinolizni sistem, plasminogen, tkivni aktivator plazminogena, inhibitor aktivatora plazminogena-1

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List of abbreviations:

t-PA – tissue-type plasminogen activator
PAI-1 – plasminogen activator inhibitor-1
u-PA – urokinase-type plasminogen activator
TAFI – thrombin activated fibrinolytic inhibitor
ELISA – enzyme-linked immunosorbent assay
mRNA – messenger ribonucleotid acid

Introduction

The basic role of the fibrinolytic system, as an integral part of the complex hemostatic system, is the disintegration of fibrin, both intravascularly and outside blood vessels, in the tissues where it accumulates. The purpose of this process is to provide the passage through a blood vessel, either by preventing the formation of a thrombus or by removing an already existing one (1, 2). This system comprises a large number of factors whose roles constantly interfere and whose common goal is to provide a stable interactive balance between the processes of blood clotting and coagulum disintegration (3). The central link in the fibrinolytic mechanism is the proteolytic enzyme plasmin, existing in plasma in the form of its inactive precursor, plasminogen. The activators of plasminogen, the so-called kinases, are responsible for the activation of plasminogen and formation of plasmin. They are released from damaged endothelial cells in cases of injuries to blood vessels, as well as by vasoactive amine actions, hyperthermia and physical labor. The main plasminogen activators are tissue-type plasminogen activator (t-PA) and urokinase-type plasminogen activator (u-PA) (4, 5). Plasminogen is also activated by coagulation factor XII, thrombin, as well as some bacterial enzymes (6). In order to provide stable homeostatic conditions in the human body, contrary to the above mentioned activators, but constantly balancing with them, there are numerous inhibitors of the fibrinolytic mechanism, acting as inhibitors of both plasminogen and its activators. The most important among them are α_2 -antiplasmin and plasminogen activator inhibitor-1 (PAI-1). The leading inhibitor of plasmin in the blood is α_2 -antiplasmin, while those in a thrombus are PAI-1 and thrombin activated fibrinolytic inhibitor (TAFI) (7–10).

The fibrinolytic process disorder can occur either in the sense of its increased activity – hyperfibrinolysis, or in the sense of its decrease – hypofibrinolysis which can have as a consequence the occurrence of atherothrombosis (1, 11).

One of the critical moments in long and progressive atherothrombosis is the ischemic cerebrovascular insult, defined as a state in which the blood flow is not good enough to satisfy the metabolic needs of brain tissue (12, 13). This disease is in the third place according to morbidity and mortality rates in the majority of countries nowadays, accompanied by heavy invalidity and high socioeconomic importance (14, 15). The occurrence of cerebral ischemia is influenced by a large number of seemingly different factors, among which one usually dominates. However, risk factors should never be observed in isolation, precisely due to the fact that their influences constantly interfere, as well as because it takes mutual activity of multiple factors to trigger this disease, almost as a rule. Evidence have been piling lately to support the thesis that the unavoidable link in the

chain of risk factors leading to the occurrence of ischemic cerebrovascular insult is the disorder of the hemostatic mechanism, especially its fibrinolytic part (16, 17).

Thus, references provide evidence about fibrinolytic capacity decrease caused by increased plasmin activity PAI-1 in patients suffering from ischemic brain disease (18–21). It is also important to point out the existence of evidence for the reduction of endogenous fibrinolysis induced by the increased concentration of t-PA antigen and decreased levels of this plasminogen activator in patients suffering from atherothrombotic insult (22). The fact that ischemic cerebrovascular insult nowadays more often affects a younger population, as well as that it requires frequent medical examinations and the engagement of multidisciplinary expert teams, supports the need for taking an active part in the early discovery of laboratory signs of risk factors for its occurrence.

The goal of this study was precisely to determine whether the fibrinolytic mechanism disorder, meaning the decrease in its potential, has influence on the occurrence of ischemic brain disease.

Material and Methods

Study design and subjects

The total number of examinees included in the one year long study (from January 2006 to January 2007) was 90. Sixty of them had an ischemic cerebrovascular insult and formed the case group, while the other thirty were clinically healthy and made up the control group. All patients were recruited during regular ambulatory work in the Department for Hemostasis and Trombosis Prevention at the Institute of Laboratory Medicine of the Clinical Center of Vojvodina.

The case and control groups had a mean age of 63.48 ± 9.62 years and 60.20 ± 7.96 years, respectively. Male to female ratio in case and control groups was 39:21 and 18:12, respectively.

The basic criterion for patients to be included in the study was that they had the ischemic type of cerebrovascular insult, while the insult diagnosis had to be verified by anamnesis, clinical examination and additional neurological diagnosis (computerized tomography or nuclear magnetic brain resonance). It is also important to emphasize the fact that the time which had to elapse prior to taking biological samples from the patients was at least one month from the clinical occurrence of insult, so that the influence of the acute phase response on the values of test parameters could be avoided. Moreover, all examinees had to be over 45 years old, so that the possible influence of thrombophilia and other genetic risk factors for the occurrence of early thrombosis could be reduced. The criteria for the exclusion of a patient from the

study were the following: an already verified hemostatic mechanism disorder of any kind (not related to the fibrinolytic system), diseases and conditions known to have some influence on the hemostatic mechanism, as well as the consumption of drugs with any possible influence on the hemostatic mechanism (with the exception of antithrombotic drugs). The identical criteria were valid for the control examinee group as well.

Ethical consideration

Prior to the study, an informed consent was taken from all the subjects. An Institutional Ethic Committee had approved the study protocol.

Laboratory methods

The estimation of fibrinolytic potential has been carried out in all the patients. In this study, fibrinolytic potential stands for the overall possibilities of the fibrinolytic system, that is, its complete activity. The activity of fibrinolytic process was examined using methods for determining the total activity of fibrinolytic system and methods for determining some of its individual components. The method thereby used to determine the total activity of the fibrinolytic system was euglobulin lysis clot time, while the chosen individual fibrinolytic system components were plasminogen activity determining, antigenic concentration of tissue-type plasminogen activator and levels of plasminogen activator inhibitor-1. Euglobulin lysis clot time was determined manually, in water-baths, according to Macfarlane and Pilling (23). Normal range for euglobulin lysis clot was from 120 to 240 minutes. Plasminogen activity was determined by the test with chromogenic substrate, on an automatic coagulometre ACL 200 (»IL«, Italy). Plasminogen results are reported in % activity. Normal range for plasminogen activity was from 73 to 127%. Values of t-PA antigen and PAI-1 were determined by the ELISA method, with an Asserachrom reagent for t-PA and PAI-1 (»Diagnostica Stago«, France). A plastic support coated with mouse monoclonal anti-human t-PA/PAI-1 antibody captures the t-PA/PAI-1 to be measured. Next, a second mouse monoclonal anti-human t-PA/PAI-1 antibody coupled with peroxidase binds to another antigenic determinant of t-PA/PAI-1, forming the 'sandwich'. The bound enzyme peroxidase is then revealed by its activity in a predetermined time on the substrate ortho-phenylenediamine in the presence of hydrogen peroxide. After stopping the reaction with strong acid, the intensity of the color produced bears a direct relationship to the t-PA/PAI-1 concentration initially present in the plasma sample (24). Normal values for PAI-1 levels were from 4 to 43 ng/mL and for antigenic concentration of t-PA from 1 to 12 ng/mL. The estimations of the fibrinolytic potential among the examinees have been made based on all these results, according to which they

were also put in categories of those with preserved and those with decreased fibrinolytic potential. Persons with longer euglobulin lysis clot time than normal, increased t-PA antigen and PAI-1 levels were classified in the group with decreased fibrinolytic potential, while the group of persons with preserved fibrinolytic potential was made from persons with normal values of these parameters.

Statistical analysis

The data were analyzed using the statistical software package »SMART LINE« (Smart Line Inc., NS). Statistics of all parameters were computed by classification on the basis of age and gender. For each fibrinolytic and lipid parameter we determined the average value and standard deviation. The significance of these parameters was determined by the t-test, which is applicable to data with normal distribution. However, in our study all the fibrinolytic and lipid parameters for the case and control groups were normally distributed. Furthermore, we also applied the F-test on each pair of case and control groups for all parameters. For parameters with a significant difference in the variance of case and control groups we applied a modified t-test with Welch's correction. Correlation between different parameters was obtained by calculating correlation coefficients, which theoretically lie between +1 to -1.

Results

We divided examinees into those with preserved and decreased fibrinolytic potential, based on the criteria cited in Material and Methods. The patient group included 31 (52%) persons with preserved and 29 (48%) persons with decreased fibrinolytic potential, while the control group included 24 (80%) persons with preserved and 6 (20%) with decreased fibrinolytic potential. In testing the statistical significance of the difference between the percentage of examinees from the patient group and those from the control group with decreased fibrinolytic potential, the result obtained was $p < 0.01$, which shows a statistically very significant difference. Further testing of the statistical significance of the differences between the subgroups with preserved and decreased fibrinolytic potential related to the sex shows a statistically significant difference between the patient group and control group, both in case of men and women (in both cases $p < 0.05$) (Figure 1).

The average value of euglobulin lysis clot time was 219.75 ± 78.77 minutes in the patient group, but 183.50 ± 58.22 minutes in the control group, which is statistically significantly longer ($p = 0.005$) (Figure 2).

The average noted value of plasminogen activity in the patient group was $92.67 \pm 11.37\%$, while in the control group it amounted to $96.87 \pm 9.48\%$,

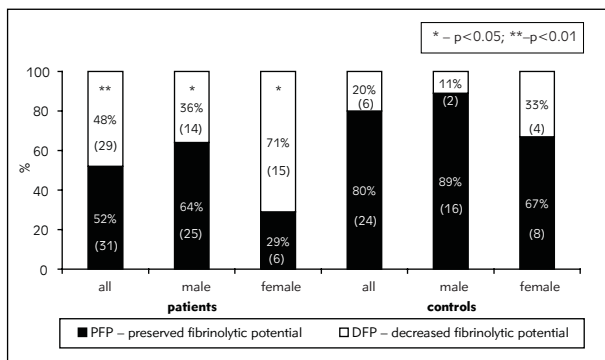


Figure 1 Percentage ratio and statistical significance of differences between preserved and decreased fibrinolytic potential among patients and controls in relation to sex.

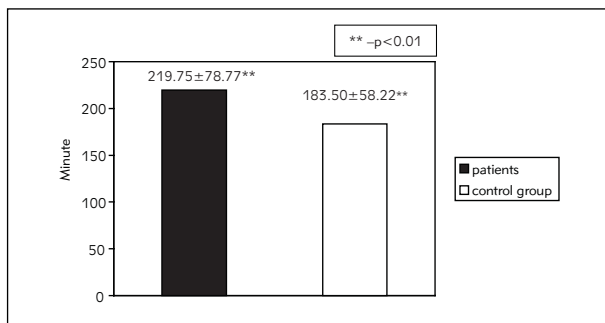


Figure 2 Statistical significance of differences between euglobulin lysis clot time in patients and control group.

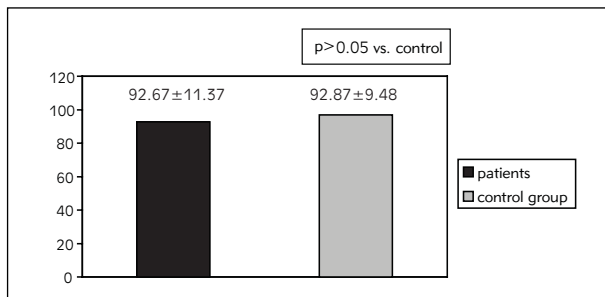


Figure 3 Statistical significance of differences between plasminogen activity in patients and control group.

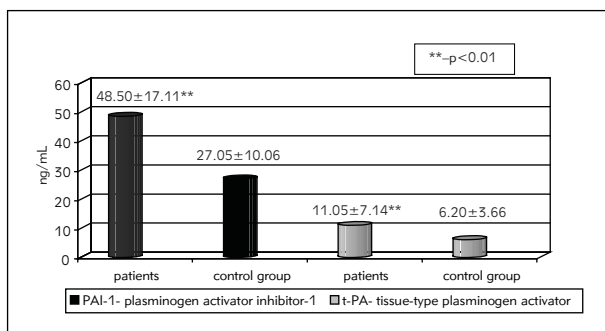


Figure 4 Statistical significance of differences between PAI-1 and t-PA antigen in patients and control group.

meaning that no statistically significant difference was noted ($p=0.085$) (Figure 3).

The testing of statistical significance of the differences related to the values of PAI-1 shows that there are statistically significantly higher values of this leading fibrinolysis inhibitor among the patients than among those from the control group ($p=6.20 \times 10^{-11}$). Namely, the average PAI-1 value in the patient group was 48.50 ± 17.11 ng/mL vs. 27.05 ± 10.06 ng/mL in the control group.

Besides that, statistically significantly higher t-PA antigen values were noted among the patients, contrary to the control group ($p=5.20 \times 10^{-5}$), meaning that the average value of this parameter in the patient group was 11.05 ± 7.14 ng/mL vs. 6.20 ± 3.66 ng/mL in the control group (Figure 4).

Discussion

In our study, the differences between the two groups related to the preservation of fibrinolytic potential are obvious from the very beginning, i.e. fibrinolytic potential decrease is statistically proved in a significantly larger number of patients than controls ($p < 0.01$). Therefore, the obvious conclusion is that the insufficient efficiency of the fibrinolytic mechanism due to a decrease in its potential plays an important role in the occurrence of cerebral ischemia. The main evidence which supports this assertion lies with the statistically extremely significant extension of euglobulin lysis clot time in the patient group when compared to the control group ($p=0.005$). Other studies share the same conviction, proposing that patients with an acute cerebral infarction do have a fibrinolytic disorder and suggesting that these abnormalities in the hemostatic mechanism can be considered a risk factor for ischemic brain disease (25, 26).

Comparing plasminogen activities between the two examinee groups, no statistically significant difference was noted ($p=0.085$). However, when the examinees from both groups were divided according to their sex, a statistically significant difference occurred between the diseased and healthy men ($p=0.017$), while there was no such difference among women. To conclude, in the examinee group plasminogen activity is statistically significantly lower among the diseased men than among the healthy ones. In Folsom and associates' study it is noticed that the risk of coronary heart disease increases with the increase of plasminogen concentration, which may seem illogical at first, but the authors explain it by a probable compensatory response to decreased plasminogen activity with people predisposed to this disease (27), while in the field of cerebral ischemia such difference has not been noticed.

The testing of statistical importance of differences in PAI-1 concentration brings out the conclusion that a very important difference does exist in this fibri-

nolysis inhibitor concentration between the patients and the the control group members ($p=6.2\times 10^{-11}$). When the examinees are divided according to their sex, the situation is the same both among women and men ($p=3\times 10^{-7}$; $p=2\times 10^{-5}$). With the verified fibrinolytic potential decrease in people who have suffered from ischemic cerebrovascular insult, in relation to the healthy examinees, it is obvious that the decrease has been provoked by the highly increased values of PAI-1, the leading fibrinolysis inhibitor. The results obtained by other authors are in accordance with these. For example, Olah and coauthors in their study on 53 patients with cerebral ischemia confirm decreased fibrinolysis and increased PAI-1 values with 23 patients (28).

The testing of the statistical significance in t-PA antigenic concentration between the group of patients and control group shows that there is a statistically very important difference in the concentration of this antigen between the two contrasted groups ($p=5.2\times 10^{-5}$), i.e. the concentrations of t-PA antigen are statistically significantly higher in the patient group. Many studies confirm the statistically important connection between t-PA and coronary disease (29). There are some new studies dealing with the issues of polymorphism of t-PA and PAI-1. Their results show that t-PA – 7351 C>T and PAI-1 – 675 4G>5G polymorphism affects transcriptional activity and that these two variants are connected to myocardial infarction with an increased risk in the case of T and 5G alleles. However, in the case of cerebral ischemia this connection does not occur, so it assumed that the role of t-PA and PAI-1 is more complex in brain than in heart (30). Contrary to these, other

studies consider the plasminogen activator system to be the one which plays the key role in the pathogenesis of cerebral ischemia (31). Namely, it is well known that the endothelium of cerebral capillaries *in vivo* produces functionally active t-PA and PAI-1. Therefore, beside the free t-PA's pool, the presence of t-PA originating from brain capillaries in an inactive form of t-PA–PAI-1 complex is also important. The relation between the free t-PA and t-PA–PAI-1 complex in brain microcirculation is 1:3.4. Moreover, there is a relatively large pool of PAI-1 in this part of circulation. The relation between the free PAI-1 and t-PA–PAI-1 complex is 1:6.3. The factors responsible for the existence of the t-PA–PAI-1 complex in brain microcirculation *in vivo* are still unknown, but the fact that there is even a pool of free t-PA and PAI-1 in brain capillaries leads to the assumption that brain endothelium itself probably provides a significant contribution to fibrinolytic mechanisms in brain microcirculation (32).

In the end, based on the results of this study it can be concluded that the fibrinolytic mechanism disorder, i.e. its insubstantial efficiency due to decrease in potential, has influence on the occurrence of ischemic cerebrovascular insult. The conclusion follows that fibrinolytic potential decrease with those suffering from ischemic cerebrovascular insult is directly connected to increased concentrations of PAI-1, the leading fibrinolysis inhibitor. Finally, the final conclusion is that increased t-PA antigen concentration is a factor which participates in the decrease of fibrinolytic potential with those who have suffered an ischemic cerebrovascular insult.

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