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THE STRUCTURAL FEATURES OF VASCULAR ENDOTHELIUM IN ACUTE CEREBRAL ISCHEMIA

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Abstract

The aim of the research was to study the number and structural properties of desquamated endothelial cells (DECs) in the peripheral blood in carotid ischemic stroke (CIS) and carotid transient ischemic attacks (TIAs) and its connection with the marker of endothelial dysfunction – endothelin-1.

We examined 35 patients with the first CIS, on days 1st and 10th, and also 34 patients with symptomatic carotid TIAs, on days 1st and 10th of the observation. Middle age of the examined patients with a CIS was $63,7 \pm 1,0$. Middle age of the examined patients with the TIAs was $54,7 \pm 1,0$. 25 practically healthy persons were examined as a group of control. Neurologic deficit was assessed with the National Institutes of Health Stroke Scale (NIHSS). DECs were estimated by CD34 immunobead capture in the peripheral venous blood of patients and persons of control group. We studied the level of endothelin-1 in the peripheral venous blood of patients and persons of control group using the enzyme immunoassay using the Biomedica (Austria) during the first 24 hrs and on day 10. Statistical processing of the obtained results was carried out using statistical analysis package Statistica. In this case, the mean value, the standard error and the correlation analysis were determined. Samples were compared using the Student's criterion (*t*) and the correlation coefficient (*r*).

During an examination of 35 patients in the acute period of CIS and 34 patients with carotid TIAs using the immunocytochemical method the number of DECs was studied in venous blood. The quantitative analysis of vascular endothelium in acute cerebral ischemias showed its statistically unreliable differences in CIS and TIAs.

A conclusion is drawn about the general mechanisms of endothelial dysfunction in CIS and TIAs. The number of DECs significantly correlates with the terms of disease. Regress of this indicator is noted in patients by the end of follow-up in both observation groups. During the first 24 hrs in patients with CIS and TIAs density of DECs of blood directly correlates with the level of endothelin-1 blood. The endothelin-1 level tends to decrease by the 10th day of observation and the correlation force with the DECs level is correspondingly reduced.

Keywords: carotid ischemic stroke, transient ischemic attack, endothelium, desquamated endothelial cells, endothelin-1.

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1. Introduction

Cardiovascular diseases are the number one cause of death worldwide [1]. The problem of cerebrovascular pathology and its consequences is the leading one for assessing the health status of the population in Ukraine [2]. Severe medical and social consequences of stroke are reflected in the indicators of disability of the population. In this regard, great importance is attached to the study of the pathophysiology of acute cerebral ischemia and the development of effective methods for correcting the revealed changes [3].

A transient ischemic attack (TIA) is currently defined as a transient neurologic disorder that is caused by focal vascular cerebral ischemia and is not accompanied by the formation of a heart attack according to neuroimaging methods [4]. TIA is not necessarily transient at the tissue level; approximately one third of traditionally-defined TIAs are associated with permanent ischemic tissue injury. The diagnostic utility of perfusion-weighted MRI currently remains to be confirmed in unbiased large datasets. Limited spatial resolution of currently available perfusion-weighted MRI techniques is also of concern. Improvements in perfusion techniques in the future may overcome these concerns by enhancing the reliability of diagnoses for punctate regions of ischemia that typically occur in TIA [5, 6].

Patients with TIAs have a high risk of ischemic stroke, therefore urgent examinations and preventive treatment are recommended, which significantly reduces the risk of stroke [7]. Patients with TIAs are at high risk of early stroke, and their risk may be stratified by clinical scale, vessel imaging, and diffusion magnetic resonance imaging [8]. Half of all recurrent strokes during the 7 days after a TIA occur in the first 24 hours highlights the need for emergency assessment. The ABCD2 score is reliable in the hyperacute phase shows that appropriately triaged emergency assessment and treatment are feasible [9].

The pathogenesis of TIA has no qualitative differences from that in ischemic stroke, the rapid regression of neurologic disorders in TIA is caused by the restoration of arterial patency due to thrombolysis. Based on the well-studied pathogenetic mechanisms of ischemic stroke, taking into account acute damage to the vascular wall under oxidative stress, cytokine activation, neutrophilic impregnation of vessels, hemorheological shifts [10], we suggested that acute cerebral ischemia is accompanied by an acute systemic irreversible reaction of the inner cellular layer of the blood vessels of various calibres, which leads to its desquamation, subsequent circulation in the bloodstream and gradual utilization. One of the key players in maintaining proper cardiovascular function is the endothelium, the inner layer of all blood vessels [11]. This monolayer of cells on one hand serves as a barrier between blood and the surrounding tissue and on the other hand regulates many aspects of vessel function. Therefore, it is not surprising that interventions reducing the risk for cardiovascular diseases improve endothelial function [12]. Endothelial dysfunction is a fundamental step in the atherosclerotic disease process. Its presence is a risk factor for the development of clinical events, and may represent a marker of atherothrombotic burden. Also, endothelial dysfunction contributes to enhanced plaque vulnerability, may trigger plaque rupture, and favors thrombus formation. The assessment of endothelial vasomotion is a useful marker of atherosclerotic vascular disease [13]. It was demonstrated significant associations between acute elevation of blood markers of endothelial cell and platelet activation and ischemic stroke and between acute elevation of blood markers of endothelial cell activation and ischemic stroke caused by large-artery atherothrombosis [14].

Under physiological conditions, the endothelium of large blood vessels has low proliferative activity, mainly due to their own elements by amitotic fission of endotheliocytes [15]. It is able to maintain the continuity of the cell layer by migrating and dividing intact cells. Desquamated endothelial cells are a novel and valuable marker of endothelial damage in a variety of vascular disorders [16]. There is an opinion that the content of DEC in peripheral blood vessels reflects the degree of damage that has been proven a higher value of this indicator in patients with acute stroke [17]. The extent of PB-MNC-CD34+ mobilization in each patient was directly related to neurological and functional recoveries as assessed by NIH Stroke Scale, and modified Rankin Scale respectively. The mobilization of PB-MNC-CD34+ cells might be predictive of neurological and functional recovery [18]. The number of endotheliocytes directly correlates with the index of endothelial dysfunction – von Willebrand factor, which indicates the diagnostic value of the definition of DEC in the blood [19]. We hypothesized that acute cerebral ischemias – CIS and TIAs – would be associated with impairment of vascular endothelium.

2. Aim of the research

The aim of the research was to study the number and structural properties of desquamated endothelial cells in the peripheral blood in carotid ischemic stroke and carotid transient ischemic attacks and its connection with the marker of endothelial dysfunction – endothelin-1.

3. Materials and methods

We examined 35 patients with the first CIS, who were hospitalized in 7 Kharkiv clinical hospital, on days 1st and 10th, as well as 34 patients with symptomatic carotid TIAs also in the 1st and 10th days of the observation. The average age of the examined patients with stroke was $63,7 \pm 1,0$ years. The average age of the examined patients with TIA was $54,7 \pm 1,0$ years. We evaluated the degree of neurologic deficit, according to scores on the National Institutes of Health Stroke Scale (NIHSS; range, 0 to 42, with higher scores indicating more severe deficits; scores of ≤ 6 indicate mild neurologic impairment; scores of ≥ 25 indicate very severe impairment). The study included patients with an NIHSS score of 7 to 24.

As a control group, 25 practically healthy persons were examined. This group is comparable by sex and age with the main group of patients. To verify the diagnosis of "Ischemic stroke", magnetic resonance imaging of the brain was performed.

DECs were estimated by CD34 immunobead capture in the peripheral venous blood of patients and persons of control group. An immunocytochemical reaction was performed on venous blood smears of patients and controls, after drying, using monoclonal antibodies CD34 conjugated with FITC (Caltag Laboratories). The finished preparations were viewed in a luminescence microscope MBI-15 (LOMO) using excitation filters SS, UVS, 3C optical filters with an increase in the eyepiece 7, of the objective 40. The quantitative density of the cells was determined in 5 fields of view.

We studied the level of endothelin-1 in the peripheral venous blood of patients and persons of control group using the enzyme immunoassay using the Biomedica (Austria) during the first 24 hrs and on day 10.

Statistical processing of the obtained results was carried out using statistical analysis package Statistica. In this case, the mean value, the standard error and the correlation analysis were determined. Samples were compared using the Student's criterion (t) and the correlation coefficient (r).

4. Results

The results of determining the quantitative density of endotheliocytes in patient's groups and in control are given in the **Table 1**.

As it can be seen from the data presented, the vascular endothelium undergoes structural changes in the onset of acute cerebral ischemia, which is manifested in a significant increase in its desquamated fraction ($p < 0,05$, compared with the control). In the dynamics of treatment in general in the group of patients with CIS there is a statistically significant decrease in this indicator – up to $5,45 \pm 0,21$. Thus, the quantitative density of DECs in the smear of peripheral blood on the first day of the disease was significantly higher than at the end of observation. This indicator directly depends on the terms of study, which indicates a gradual regression of vascular disorders in the dynamics of observation.

Table 1

Dynamics of quantitative density of DECs in patients with CIS and TIAs

Group of patients	Research terms	Quantitative density of DECs
CIS	1st day	$8,65 \pm 0,25$
	10th day	$5,45 \pm 0,21^*$
Carotid TIAs	1st day	$7,77 \pm 0,23$
	10th day	$4,37 \pm 0,23^*$
Control		$1,92 \pm 0,11$

Note: * $p < 0,05$ in relation to an initial level; all indicators of $p < 0,05$ in relation to control

This agrees with the previously obtained data [20] that ischemic stroke is accompanied by a change in vascular reactivity mediated by the expression of the endothelial vasodilator nitric oxide

and the potent vasoconstrictor endothelin-1 in the direction of predominance of the latter with the development of endothelial dysfunction. Similar results of a quantitative analysis of the structural state of the vascular endothelium were obtained by us in the group of patients with transient ischemic attacks in the carotid basins. So, on the 1st day of the disease the quantitative density of DECs reaches $7,77 \pm 0,23$, and by the 10th day of the disease it is $4,37 \pm 0,23$. Attention is drawn to the fact that in our study there was not obtained a significant difference in the numerical density of DECs in both clinical groups both on the 1st day and on the 10th day of the disease, which reflects the systemic character and severity of structural and functional changes of the vascular endotheliocytes. At the same time, during all observation periods, the significant difference of the studied index in the groups of acute cerebral ischemia is preserved in comparison with the control group.

The morphological picture of DECs was very polymorphic. Thus, whole cells with nuclear structures, non-nuclear endotheliocytes undergoing fragmentation, and separately lying fragments, the apoptotic bodies of endothelial cells, have been identified.

One of the most important indicators of endothelial dysfunction is the study of endothelin production. In all cases, compared with the control, a significant increase of vasoconstrictor agent endothelin-1 in the blood plasma was revealed (**Table 2**). So, on the 1st day of acute vascular catastrophe, the value of endothelin-1 reaches a significant growth in comparison with the control. These data show that in patients with CIS and TIAs already during first 24 hours of the disease, the endothelial release of vasoconstrictors is activated. The endothelin-1 level tends to decrease by the 10th day of observation, while a significant difference remains with the control at CIS and TIAs.

Table 2Dynamics of endothelin-1 in patients with CIS and TIAs ($M \pm m$, pmol/l)

Group of patients	Research terms	Quantitative density of DECs
CIS	1st day	$0,70 \pm 0,02$
	10th day	$0,66 \pm 0,01$
Carotid TIAs	1st day	$0,68 \pm 0,01$
	10th day	$0,62 \pm 0,01$
Control		$0,47 \pm 0,01$

Note: all indicators of $p < 0,05$ in relation to control

We identified strong correlation between endothelial dysfunction marker endothelin-1 and quantitative density of DECs in peripheral blood (CIS: $r = +0,47$; $p < 0,05$), (TIAs: $r = +0,44$; $p < 0,05$), which reflects the acute damage to the vascular endothelium. In the dynamics of observation between all these indicators, the direction of the correlation bonds persisted, but they did not reach statistically significant values ($p > 0,05$).

5. Discussion

In acute cerebral ischemia the release of proinflammatory cytokines, inflammatory mediators and free radicals with highly acute astrocytic proteins adversely affects intracellular structures surrounding the cell membrane and, importantly, the vascular wall [21]. Severe endothelial dysfunction was detected both in men and in women, with an intense vascular response following an acute stroke. High P-selectin level within the first 24 hrs that further increases during the first week after stroke suggests that vascular instability still exists and that patients with acute stroke are vulnerable and at higher risk during this period [22]. The important role of inflammation in ischemic stroke was also shown [23]. This causes damage to the basal vascular membrane, interendothelial contacts and the endothelial lining of the cerebral vessels, which is accompanied by an increase in the number of DECs in the peripheral blood flow [24]. Reducing the number of endothelial cells by the end of observation, apparently, involves, on the one hand, the weakening of the vessel wall damaging mechanisms, on

the other hand, with a favorable impact of ongoing medication events. The severity of the structural changes of the vascular endothelium in patients with TIAs was a confirmation of the thesis that the prevention of stroke is one of the most important areas of management of the patient who has had a TIA, it is based on non-pharmacological methods of prevention and drug therapy.

We examined the patients with CIS and carotid TIAs using the immunocytochemical method and monoclonal antibodies CD34, disorganized vascular endothelium, which was expressed as an increase in the number of DEC's in the peripheral blood flow, which is a promising direction in the diagnosis of cytochemical disorders in this patient population, a prerequisite for emergency medical care and prevention of acute cerebral ischemias.

The endothelin-1 level tends to decrease by the 10th day of observation, and the correlation force with the DEC's level is correspondingly reduced, which indicates the multifactorial genesis of endothelial damage and the possibility of their rapid recovery, in contrast to the biochemical markers of endothelial dysfunction. The revealed changes in the endothelial vasoregulation system (endothelin-1 data), endothelial structural changes allowed to conclude about endothelium-dependent mechanisms of the pathogenesis of ischemic stroke and transient ischemic attacks.

There is a link between damaging mechanisms in acute cerebral ischemia with structural and functional changes from the inner layer of the vascular wall, namely, the endothelium, as evidenced by the well-known correlation relationships. At the same time, there seems to be an acute reaction of the endothelial system to fast-acting processes of hypoperfusion, energy deficiency, free radical and mediator aggression in the onset of acute cerebral ischemia.

6. Conclusions

1. Patients with an acute CIS had significantly higher quantitative density of DEC's of blood ($p < 0,05$). The immunocytochemical method, using the monoclonal antibodies CD34, revealed changes in the endothelial system. On the 1st day of disease, a pronounced desquamation of endotheliocytes was revealed, as a fast and systemic reaction to an acutely developing cascade of pathological reactions in acute CIS.

2. The examination of patients with carotid TIAs also showed the presence of pronounced desquamation of endotheliocytes.

3. The index of the quantitative density of DEC's directly depends on the terms of the disease, the regress of this index was noted in patients at the end of the observation, which is a favorable trend, and, apparently, due to the weakening of the vascular wall-damaging mechanisms and the ongoing treatment measures.

4. During the first 24 hrs in patients with CIS and TIAs the endothelial release of vasoconstrictors is activated. The endothelin-1 level tends to decrease by the 10th day of observation, while a significant difference remains with the control at CIS and TIAs.

5. During the first 24 hrs in patients with CIS and TIAs density of DEC's of blood directly correlates with the level of endothelin-1 blood. The endothelin-1 level tends to decrease by the 10th day of observation and the correlation force with the DEC's level is correspondingly reduced.

6. The revealed changes in the endothelial vasoregulation system (endothelin-1 data), endothelial structural changes allowed to conclude about common endothelium-dependent mechanisms of the pathogenesis of ischemic stroke and carotid transient ischemic attacks, where the vascular endothelium is a primary target for the impairment.

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