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5-NUCLEOTIDASE ACTIVITY IN THE PLACENTA IN CYTOMEGALOVIRUS INFECTION AS A MARKER OF DEVELOPMENT OF PREECLAMPSIA

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5'-nucleotidase is an enzyme that catalyzes the phosphorylytic cleavage of 5'-nucleotides, including adenosine monophosphate, which is converted to adenosine. The world literature contains a large amount of works concerning the role of the enzyme in the cardiovascular, immune, nervous, digestive, respiratory and other systems. Nevertheless, despite the long study period, the features of the 5'-nucleotidase activity in various infectious processes have practically not been studied. The goal of our research was to study the activity of the 5'-nucleotidase enzyme in the placenta in reactivation of cytomegalovirus infection in the third trimester of pregnancy. We studied placenta samples obtained in childbirth from women with laboratory confirmed reactivation of chronic cytomegalovirus infection and a diagnosis of moderate preeclampsia in the third trimester of pregnancy, as well as from clinically healthy women with latent cytomegalovirus infection (control group). The method with the formation of lead phosphate by Wachstein and Meisel in a slight modification was used for the histochemical localization of 5'-nucleotidase activity. A significant increase in the intensity of the histochemical reaction to 5'-nucleotidase in the syncytiotrophoblast of the placental villi was revealed, indicating an increase in the enzyme activity in the reactivation of cytomegalovirus infection during pregnancy. The cytophotometric index in the syncytiotrophoblast of the placentas of the first group increased to 37.4 ± 2.23 relative units compared with the control group (29.2 ± 2.55 relative units, p <0.05). A significant increase in the intensity of the enzyme in the placenta was noted in cases where the reactivation of the cytomegalovirus process was accompanied by preeclampsia. The cytophotometric index increased by almost 40% (p <0.001) in comparison with the data of the control group and amounted to 42.2 ± 2.99 relative units. Thus, an increase in 5'-nucleotidase activity created a high level of adenosine, which, in our opinion, could contribute to the development of characteristic signs of preeclampsia in cytomegalovirus infection during pregnancy. As a reason for the development of this phenomenon, we assume that hypoxia, inflammation and a decrease in energy supply formed as a result of an exacerbation of the infectious process.

Keywords: cytomegalovirus infection, pregnancy, placenta, 5'-nucleotidase, preeclampsia.

Introduction. The 5'-nucleotidase enzyme was first cloned from the rat and human placenta [21], an active study of its biochemical properties and localization, isolation and purification began since the 1970s and 1980s [14, 20]. Despite this, studies on the role of the enzyme in the reproductive system and the pathogenesis of various pathological conditions are still ongoing. The specific features of 5'-nucleotidase activity in various infectious processes are practically not studied.

5'-nucleotidase is an enzyme that catalyzes the phosphorylytic cleavage of 5'-nucleotides, including: adenosine monophosphate (AMP), cytosine monophosphate, uridine monophosphate, inosine monophosphate, guanosine monophosphate, as well as nicotinamide mononucleotide and NAD, thereby regulating their availability. The most effective substrate for 5'-nucleotidase is AMP. In this case, AMP is broken down to adenosine. The earliest studies of the function of nucleotides and adenosine in the morphosis were discussed in terms of their role as a source of energy and an integral part of other compounds. It is now generally accepted that purines and pyrimidines have potent effects mediated by the activation of specific membrane receptors. Adenosine acts as a P1 purinergic receptor agonist. The second type of purinergic receptor, called P2, is selective for ATP / ADP [24]. However, an increase in adenosine production can lead to the development of pathological conditions during pregnancy. Since adenosine regulates the formation of blood vessels, its high concentration can inhibit the growth of the placenta in the early pregnancy [6]. It is also known that an increased level of adenosine causes vasoconstriction of the placenta vessels [7].

An increase in the concentration of adenosine is observed in women with preeclampsia [13,17,18,19]. A number of researchers have determined that an increase in adenosine in the placenta contributed to the development of characteristic signs of preeclampsia, including hypertension, proteinuria and intrauterine growth restriction [6,12,17,19]. The reason for the increase in the content of adenosine during pregnancy, according to experts, is an increase in the activity of the enzyme 5'-nucleotidase in the placenta [12]. This placental enzyme is considered as a candidate marker for the formation of adenosine in this organ during preeclampsia [6]. **The goal of research** was to study the activity of the 5'-nucleotidase enzyme in the placenta in reactivation of cytomegalovirus infection in the third trimester of pregnancy.

Materials and methods. Samples of placentas from 102 women obtained from childbirth at 37-40 weeks of gestation were studied. The criteria for the inclusion of patients in the first group (37 cases) were: the presence of laboratory confirmed reactivation of chronic cytomegalovirus (CMV) infection in the third trimester of pregnancy, age from 18 to 37 years, consent to the study; in the second group (35 cases) - laboratory confirmed reactivation of chronic CMV infection and diagnosis of moderate preeclampsia (ICD O14.0) in the third trimester of pregnancy, age from 18 to 37 years, consent to the study; in the control group (30 cases) – absence of reactivation of chronic CMV infection during pregnancy, age from 18 to 37 years, consent to the study.

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The exclusion criteria from both groups were: exacerbation of any extragenital and infectious diseases, apart from CMV infection, primary CMV infection, age under 18 and over 37 years, smoking, alcohol and drug use, lack of voluntary informed consent.

Laboratory methods were used: PCR on a DT-96 apparatus using a set of NPO DNA-technology (Russia) for the diagnosis of CMV DNA in material from buccal epithelium scrapings, blood serum and urine; ELISA on a spectrophotometer "Stat-Fax 2100" (USA) using a set of ZAO "Vector-Best" (Russia) in blood serum for diagnostics of the form (acute, chronic, primary) of CMV process.

Assessment of the severity of preeclampsia was carried out on the basis of clinical guidelines (treatment protocol) “Hypertensive disorders during pregnancy, childbirth and the postpartum period. Preeclampsia. Eclampsia", approved by the Ministry of Health of the Russian Federation on 07.06.2016. No. 15-4 / 10 / 2-3483.

For the histochemical localization of 5'-nucleotidase activity, we used the method with the formation of lead phosphate by Wachstein and Meisel [3] in a slight modification. The enzymatic reaction was carried out on cryostat tissue sections for 30 minutes at 37° C in 50 mM tris-maleate buffer (pH 7.4) supplemented with 5 mM MnCl2, 2 mM Pb $(NO3)_2$, and 2.5 mM levamisole as an alkaline phosphatase activity inhibitor and in the presence of 1 mM AMP as a substrate. The control was incubated in a medium without a substrate. The reaction was detected in the presence of 1% Na_2S. Then the samples were placed in a glycerol gel, analyzed, and photographed under a Meiji Techno light microscope (Japan). The slides were studied using the Scion Image software (USA) according to the method described in our previous works [2].

The results of the study were statistically processed using the "Statistica 10.0" computer program after the Lilliefors and Kolmogorov-Smirnov normality tests using the Student's t-test.

Results and discussion. Placental slides showed the activity of 5'-nucleotidase in the plasma membrane of syncytiotrophoblast (Fig. 1). No enzyme activity was detected in the villi stroma. In control sections incubated in a medium without a substrate, the enzyme activity was not determined (Fig. 2). A number of specialists suggest that 5'-nucleotidase is involved in the regulation of blood microcirculation in the placenta [23].

Adenosine formed as a result of the reaction can selectively modulate growth, proliferation, migration, invasion and differentiation of cells during embryonal development, and regulate fetal metabolism [6,7]. Its important role in the angiogenesis and vasculogenesis of the fetus and placenta is suggested. In vitro studies have shown that this nucleoside under physiological conditions stimulates a significant production of pro-angiogenic factors, such as vascular endothelial growth factor and membrane-bound fms-like tyrosine kinase-1, and simultaneously inhibits anti-angiogenic factors - soluble fms-like tyrosine kinase-1 [11]. The functional properties of adenosine include the regulation of vascular tone and nutrient transport [16].

In reactivation of CMV infection in the third trimester of pregnancy, an increase in the activity of the histochemical reaction to 5'-nucleotidase was found (Fig. 3). The cytophotometric index in the placental syncytiotrophoblast in the first group increased to 37.4 ± 2.23 relative units compared with the control group (29.2 ± 2.55 relative units, $p < 0.05$). Thus, the activity of the reaction increased by 25%. Our data are consistent with in vitro studies describing increased expression and enzymatic activity of 5'-nucleotidase in CMV infected endothelial cells compared to uninfected ones [10]. The most pronounced changes were noted in the material of the second group of the study. The cytophotometric index increased by almost 40% ($p < 0.001$) in comparison with the data of the control group and amounted to 42.2 ± 2.99 relative units.

The mechanisms leading to an increase in 5'-nucleotidase activity in the placenta are poorly understood. It is known that the enzyme is activated under conditions of hypoxia, in the presence of a number of pro-inflammatory factors (TNFα, IL-1β, interferons, prostaglandin E), as well as a weakening of energy supply [8]. Earlier, in active CMV infection during pregnancy, an increase in the content of HIF-1α [5], TNF-α, IL-1β [1,22] and a decrease in the intensity of energy metabolism [4] were revealed. We believe that the reactivation of the CMV process, which promotes the formation of conditions of hypoxia and inflammation, led to an increase in the activity of 5'-nucleotidase in the placental villi syncytiotrophoblast, and to the formation of a large amount of adenosine. Its high level is supposed to compensate for the negative effect of inflammatory components, depletion of energy supply, and counteracts the progression of further complications [7]. However, a long-term increase in the expression of 5'-nucleotidase can

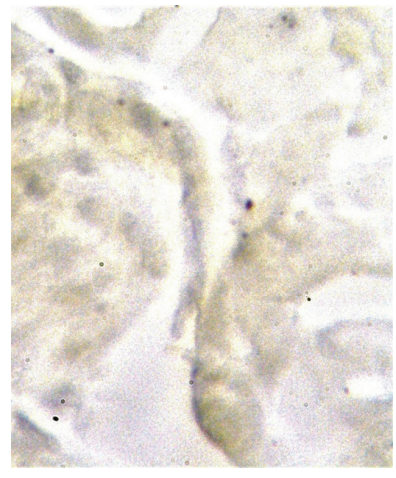

Fig. 1. Placenta from the control group. Dark brown deposits in micrographs correspond to 5'-nucleotidase activity in syncytiotrophoblast. Magnification 15×40.

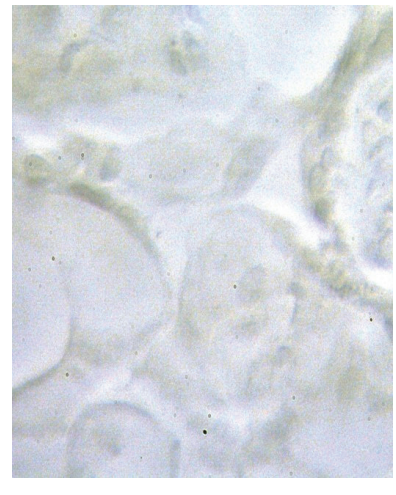

Fig. 2. Lack of 5'-nucleotidase activity in syncytiotrophoblast in control sections of the placenta. Magnification15×40.

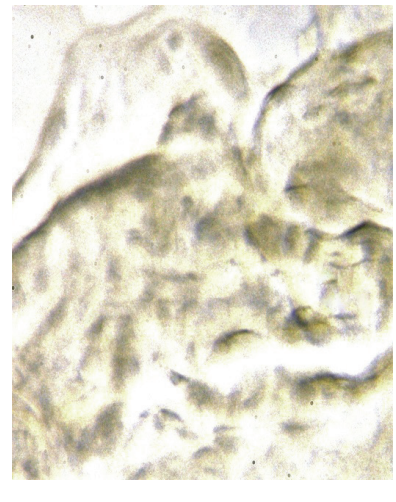

Fig. 3. Placenta from the group with reactivation of CMV infection. The 5'-nucleotidase activity is higher compared to the control group. Magnification 15×40.

lead to depletion of the pool of extracellular nucleotides, local formation of adenosine, activation of the corresponding purinergic receptors and induction of preeclampsia symptoms, which is one of the most serious complications of pregnancy, including associated with CMV infection [9,15].

Conclusion. A significant increase in the intensity of the histochemical reaction to 5'-nucleotidase in the placental villi syncytiotrophoblast was revealed, indicating an increase in the enzyme activity in reactivation of CMV infection during pregnancy. A significant increase in the intensity of the enzyme in the placenta was noted in cases when the reactivation of the CMV process was accompanied by preeclampsia. Thus, an increase in 5'-nucleotidase activity, in our opinion, could create a high level of adenosine, which contributed to the development of characteristic symptoms of preeclampsia in CMV infection during pregnancy. As a reason for the development of this phenomenon, we assume that hypoxia, inflammation and a decrease in energy supply formed as a result of an exacerbation of the infectious process.

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