Nitric Oxide and HSP70 Proteins during Normal Pregnancy, Gestosis, and Preclinical Gestosis

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We measured the content of NO metabolites in blood plasma and urine and concentration of HSP70 proteins in the plasma and leukocytes from 126 women with normal pregnancy, gestosis, and preclinical gestosis. In women with gestosis accompanied by NO deficiency the concentration of HSP70 proteins in the plasma increased more significantly than in leukocytes. These changes are an important marker of endothelial dysfunction and reflect the severity of cell damage during this disorder. The decrease in excretion of NO in the urine and increase in HSP70 protein content in leukocytes and plasma characterize endothelial dysfunction in women with preclinical gestosis.

Key Words: *pregnancy; gestosis; nitric oxide; stress proteins*

According to modern views, gestosis is a disease of adaptation that proceeds in stages typical of the general adaptation syndrome. The state of hemostasis and immune and neurohumoral systems responsible for the regulation of the vascular tone and microcirculation reflects the development of undesirable changes in pregnant women under the influence of stress factors. Systemic damage to vascular endothelium resulting in endothelial dysfunction plays a major role in the pathogenesis of gestosis. These changes promote the development of microcirculatory disturbances in vitally important organs and systems, which leads to polyorgan insufficiency.

Among various endothelial factors, nitric oxide (NO) serves as a criterion for endothelial function. NO is involved in many physiological processes, including regulation of smooth muscle tone, vasodilation, inhibition of platelet aggregation, development of immune reactions, and memory. Moreover, NO plays a role in some pathological processes [5,6,8]. The effects of NO are associated with its ability to affect the key mechanisms of the strain reaction and increase the potency of endogenous protective systems [2,5].

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The system of HSP70 proteins serves as a connecting link between the central mechanisms of the general adaptive syndrome and intracellular endogenous protection during stress [1-4]. HSP70 proteins are a marker of endothelial dysfunction. On the one hand, NO is involved in activation of HSP70 proteins. On the other hand, these proteins prevent overproduction of NO [1,2,4].

Here we measured the content of NO metabolites in the plasma and urine and concentration of HSP70 proteins in the plasma and leukocytes from women with normal pregnancy, gestosis, and preclinical gestosis.

MATERIALS AND METHODS

We examined 126 primigravid women in the second and third trimesters of pregnancy. The mean age of women was 26.2±1.7 years. Depending on the course of pregnancy and expected outcome of labor, the women were divided into several groups. Groups HII-P and HIII included 82 women in the second and third trimesters of normal pregnancy. Group GII-P consisted of 44 women in the second trimester of pregnancy; in the third trimester they were divided into groups GIII-O (28 women, O-gestosis, hydrops gravidarum)

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and GIII-OG (16 women, OG-gestosis, nephropathy of pregnancy). The control group included 16 healthy nonpregnant women (HNP) of the same age.

Activity of the NO system was determined by the total content of major NO metabolites nitrates and nitrites in the plasma and 24-h urine. Plasma and urine nitrates were reduced into nitrites in a Nitrate reduktor device (World Precision Instruments, Inc.) in the presence of 0.5 M NH_4OH as a buffer (pH 9.0). After reduction the urine or plasma samples were mixed with an equivalent volume of Griess reagent and incubated at room temperature for 10 min. The intensity of staining was evaluated spectrophotometrically at 540 nm. Nitrite concentration was estimated by the calibration curve. Total excretion of stable NO metabolites was calculated per 24-h urine volume [7].

For evaluation of HSP70 protein content in leukocytes, 10 ml venous blood was placed in a plastic tube with heparin, layered on 2 ml 6% ethylenediaminetetraacetic acid (EDTA), and incubated in a thermostat at 37°C for 60 min. After centrifugation in a cold centrifuge, 7 ml cold 0.9% NaCl was added to the pellet containing leukocytes, mixed, and centrifuged at 2000*g* for 5 min. NaCl (1.5 ml, 0.9%) was added to the pellet and vortexed. The solution was placed in a 1.5-ml Eppendorf tube, centrifuged at 2000*g* for 5 min, and frozen in liquid nitrogen.

For evaluation of plasma HSP70 concentration, 5 ml blood was placed in a plastic tube with 0.1 ml 7% EDTA, centrifuged at 2000*g* in a cold centrifuge at 4°C for 10 min, put in plastic Eppendorf tubes, and frozen in liquid nitrogen. The pre-isolated plasma was mixed 1:1 with cold lysis-buffer. Purified leukocytes were mixed with 50 ml cold lysis-buffer. Further lysis was performed routinely. The sample was incubated at 4°C for 30 min, and the pellet was removed by centrifugation at 12,000*g* and 4°C for 20 min. The supernatant was boiled with 2×SSC on a water bath (95°C, 7 min) and used for electrophoresis, immunoblotting, and HSP70 assay. Western blotting was performed with Bio-Rad reagents and devices. Test proteins were developed on a nitrocellulose membrane by enzyme immunochemiluminescence. After Western blotting the membrane was incubated in 5% Non-Fat Dry Milk in TPBS (0.2% Tween in phosphate buffered saline) at room temperature for 1 h to block the remaining binding sites. The blots were incubated with monoclonal antibodies against inducible HSP70 (SPA-810, Stress Gen Biotechnologies Corp.). Target antigens were visualized with ECL RPN 2108 Batch 76 kit. Exposure was performed on a Hyperfilm ECL film (Amersham Pharmacia Biotech). The film was developed and fixed using photo reagents. HSP70 content was estimated by the width and intensity of staining of antibody-binding band (Fig. 1). The concentration of HSP70 in leukocytes and plasma was determined by the density of spots (pixels) after computer data processing.

The results were analyzed by Student's *t* test (Statistica software).

RESULTS

In healthy pregnant women in the second and third trimesters of pregnancy (groups HIII and HII-P) the content of stable NO metabolites in the plasma did not differ from the control (Table 1). However, the urinary concentration of NO metabolites in the second and third trimesters of pregnancy 3-fold surpassed the control.

The content of HSP70 in leukocytes and plasma tended to increase in healthy women in the third trimester of pregnancy. It should be emphasized that in

TABLE 1. Content of Stable NO Metabolites in the Plasma and Urine and Concentration of HSP70 Proteins in Leukocytes and Plasma under Various Courses of Pregnancy in the Second and Third Trimesters

Parameter	HNP, control	Pregnant women, groups				
		HII-P	HIII	GII-P	GIII-O	HIII-OG
Plasma nitrates/nitrites (μmol)	13.8±1.4	14.2±1.3	14.6±1.8	14.8±1.3	12.2±1.4	9.7±1.4**++
	(16)	(27)	(28)	(29)	(19)	(12)
Urine nitrates/nitrites (μmol/liter)	76.8±12.3	210.1±13.9*	204.7±19.5**	175.1±18.1**	155.8±11.6**	89.8±10.3°°
	(16)	(27)	(28)	(29)	(16)	(12)
Leukocyte HSP70 (pixels)	104.2±6.9	107.6±4.7	121.3±7.6	128.6±6.8**	153.9±5.7*++	155.7±4.9*°°
	(16)	(30)	(26)	(26)	(13)	(14)
Plasma HSP70 (pixels)	78.2±6.9	97.1±8.7	99.4±10.8	136.4±7.1*	206.9±13.4*+	208.3±11.9*°
	(16)	(26)	(26)	(28)	(20)	(15)

Note. *p<0.01 and **p<0.05 compared to HNP (control); +p<0.01 and ++p<0.05, differences between GIII-OG and GII-P groups; °p<0.01 and °°p<0.05, differences between GIII-OG and GIII-O groups. Number of examined women is shown in brackets.

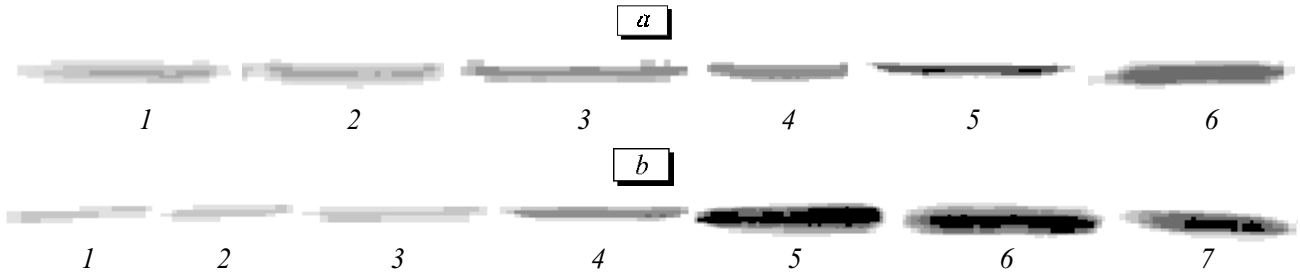

Fig. 1. HSP70 content in leukocytes (*a*) and plasma (*b*) under various courses of pregnancy. Healthy nonpregnant women (HNP, *1*), healthy women in the second (HII-P, *2*) and third trimesters of pregnancy (HIII, *3*), pregnant women with high risk of gestosis in the second trimester of pregnancy (GII-P, *4*), hydrops gravidarum (GIII-O, *5*), and nephropathy of pregnancy (GIII-OG, *6*), and molecular weight marker for HSP70 (*7*).

healthy pregnant women the increase in HSP70 content in the plasma and leukocytes was accompanied by an increase in NO metabolite concentration in urine. These changes in the content of stress proteins activate the adaptive mechanisms, which protects the organism from strain and promotes the adequate stress response to developing pregnancy. Previous studies showed that NO activates the synthesis of protective stress proteins.

The amount of stable NO metabolites in the plasma tended to decrease in group GIII-O women. In these women the concentration of NO metabolites in the urine was lower than in group HIII women (Table 1). The concentration of NO metabolites in the plasma and urine was minimum in group GIII-OG women (compared to healthy pregnant women). Moreover, the content of NO metabolites in the urine in these women was much lower than in group GIII-O women. Retrospective analysis showed that the amount of stable NO metabolites in the urine tended to decrease in group GII-P women with preclinical gestosis (compared to group HII-P).

The content of HSP70 in leukocytes and plasma from women of groups GIII-O and GIII-OG far surpassed that in group HIII women. The increase in HSP70 content in women of groups GIII-O and GIII-OG was accompanied by a decrease in NO concentration. These results indicate that intensive and prolonged stress reaction (gestosis) produced an adverse effect on organs and tissues. Therefore, the protective and adaptive response was converted into a pathological process. Retrospective analysis showed that HSP70 content in leukocytes from women in the second trimester of pregnancy increases compared to the control. The amount of these proteins significantly increased in the plasma and tended to increase in leukocytes (compared to group HII-P, Table 1).

Our observations indicate that normal pregnancy is characterized by high concentration of NO metabolites in 24-h urine and normal content of HSP70 proteins in leukocytes and plasma. The amount of NO metabolites in 24-h urine and plasma markedly decreases in women with gestosis, which is accompanied by changes in HSP70 content. High content of HSP70 proteins is an important marker of endothelial dysfunction during gestosis and NO deficiency. HSP70 content in the plasma increases more significantly than in leukocytes, which reflects the severity of cell damage during this disorder. Decreased urinary excretion of NO and increased HSP70 protein content in leukocytes and plasma characterize endothelial dysfunction during preclinical gestosis.

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