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Remote Ischemic Preconditioning Correction in Adma-Like Gestosis Model.

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ABSTRACT

Simulation of ADMA-like gestosis model was performed by means of administering L-NAME to rats during the period from the 14th to the 20th day of pregnancy. The animals demonstrated increase of arterial pressure, proteinuria, placental microcirculation disorders, vascular control disorders and destructive changes in placenta having ischemic genesis. Reproduction of short ischemia-reperfusion episodes resulted in apparent correction of the morphofunctional disorders appearing during gestosis model simulation. Therefore the results of the carried out experiment give ground for the further research over ischemic preconditioning for correction of gestosis and search of new dug preparations with the basic mode of action lying in biological processes taking place in the course of ischemic preconditioning.

Keywords: rats, N-nitro-L-arginine-methyl ether, endothelium dysfunction, gestosis model, ischemic preconditioning.

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INTRODUCTION

Late pregnancy gestosis is the most frequent disease of the pregnant women and heads the list of reasons of maternal and perinatal mortality. Currently a lot of authors mark placental dysangiogenesis as a factor playing a significant role in pathogenesis of this disease [1, 2]. Enlarged space between the spiral arteries and the chorionic villi as well as immaturity of the spiral arteries themselves result in trophoblast ischemia and increase of the fetoplacental barrier permeability [3, 4], which through the sequence of consecutive events induces development of endothelial dysfunction and gestosis [5, 6]. Due to the above investigation of the influence of remote ischemic preconditioning having anti-ischemic effect on the course of ADMA-like gestosis model is regarded to be of current concern.

METHODOLOGY

The experiment was carried out with participation of 40 Wistar albino rat females with the weight from 200 to 300 g. ADMA-like agent – non-selective inhibitor of NO-synthase N-nitro-L-arginine-methyl ether (L-NAME) was administered intraperitoneally by daily doses of 25 mg/kg during the period of seven days (14-20 days of pregnancy). The endothelial dysfunction was evaluated by correlation of endothelium-dependent and -independent vascular response [7, 8]. The pregnant females were divided into panels (n=10): I – non-involved; II – daily administration of L-NAME from the 14^{th} to the 21^{st} day of pregnancy; III – administration of L-NAME and reproduction of the 10-minutes' ischemic episode of the right-hand rear extremity on the 21^{st} day of pregnancy 90 minutes prior to the functional tests; IV – administration of L-NAME and daily reproduction of the 10-minutes' ischemic episode of the rear extremities alternatively within the period from the 10^{th} to the 20^{th} days of pregnancy. The ischemic episode was reproduced by means of the 10-minutes' femoral artery cross-clamping through application of a cuff onto the proximal third of femur. Microcirculation studies were performed at the uterine horn outer surface at the distance of 1 mm from the visible rim of the placental disk. Morphological examination of the placentas along with the uterine horn implantation area was carried out by means of hematoxylin and eosin staining of the samples.

INVESTIGATION RESULTS

Inhibition of NO-synthase induced by the 7-days' administration of L-NAME resulted in disturbance of interrelations between vasodilatation and vasoconstriction vascular control mechanisms which was evidenced by increase of endothelial dysfunction index (EDI) from 1.28±0.23 with the non-involved pregnant animals to 3.06±0.32 ($p<0.05$). Besides a significant raise of systolic and diastolic arterial pressure was observed: from 125±6.3 and 82.0±5.8 to 183.1±9.4 and 136.7±7.4 mm Hg correspondingly. Administration of NO-synthase inhibitor resulted in considerable placental microcirculation indicator decline: from 425.90±39.55 to 210.00±21.08 ($p<0.05$), as well as in reduction of stable NOx metabolites content in serum: from 2.35±0.21 µmol/dL to 1.33±0.09 µmol/dL ($p<0,05$). During microscopic examination of placenta there were observed uneven blood filling of the spongious layer, hydropic degeneration of trophoblast giant cells, focal necrosis at the boundary of trophoblast giant cells and decidual tissue, dystrophic changes and anemia of decidual layer. Reproduction of a single ischemic episode 90 minutes prior to the tests resulted in normalization of interrelationship between the vasodilatation and vasoconstriction response at time of experimental preeclampsia, evidenced by decrease of EDI up to 1.52 ± 0.09 and increase of microcirculation indicators up to 327.3±17.2 PU (Table 1). The biochemical serum analysis showed that the level of stable NOx metabolites had not statistically significant difference as compared to the same indicator for the animals with ADMA-like gestosis. Histologic examinations of placenta at time of the ischemic episode reproduction 90 minutes prior to the functional tests were not performed.

The panel of animals with 10-fold reproduction of ischemic preconditioning also demonstrated normalization of interrelationship between the vasodilatation and vasoconstriction response as evidenced by decline of EDI up to 1.56±0.13. Besides the 10-fold reproduction of ischemic preconditioning resulted in statistically significant decrease of arterial pressure: systolic up to 141.6±5.5, diastolic up to 104.2±5.7 mm Hg ($r<0.05$). Examination of placental microcirculation showed its comparable improvement by contrast to the previous panel of the treated animals nevertheless it hadn't reached the targeted level (Table 1). The biochemical serum analysis detected statistically significant anti-decrease effect in the stable NOx metabolites content, level of which made 1.92±0.18 µmol/dL. Microscopic examination of placenta at time of simulation of ADMA-like gestosis with 10-fold preconditioning revealed evident improvement of gistologic pattern which

was expressed in even blood filling of the spongious layer, absence of damage to the trophoblast giant cells layer and the decidual membrane.

Table 1: Results of correction of ADMA-like gestosis in rats (M±m)

Indicator / Panel	SAP, mm Hg	DAP, mm Hg	EDI, c.u.	Microcirculation PU	Concentration of nitrite ions (NOx), µmol/l
Non-involved	125.0±6.3^{U}	82.0±5.8^{U}	1.28±0.23^{U}	425.9±39.6^{U}	2.35±0.21
L-NAME	183,1±9.4*	136.7±7.4*	3.06±0.32*	210.0±21.1*	1.33±0.09*
L-NAME+ischemia-reperfusion (1)	177,1±9.8*	124.9±8.4*	1.52±0.09^{U}	327.3±17.2^{U}	1.11±0.09*
L-NAME+ ischemia-reperfusion (10)	141.6±5.5^{U}	104.2±5.7^{U}	1.56±0.13^{U}	339.6±20.4^{U}	1.92±0.18*

Remark: SAP, DAP – systolic and diastolic arterial pressure (mm Hg); EDI – endothelial dysfunction index (c.u.); placental microcirculation (perfusion units); concentration of nitrite ions (NOx); *- $p < 0.05$ as compared to non-involved animals panel; ^{U}- $p < 0.05$ as compared to L-NAME panel.

The mechanism of positive effect of the ischemic preconditioning has been not yet comprehensively investigated. Most probably the ischemic impetus induces system-level readjustment and change of endothelial function is just a particular manifestation in a common sequence of the ischemic disorders prevention which (endothelial function) is initiated simultaneously.

At the early stage ("classic" or "early" preconditioning) a whole range of humoral factors (triggers) is being secreted: adenosine, bradykinin, opioids, free radicals etc. It worth mentioning that all of these factors at time of secretion in the ischemia-affected tissues varying in structure, genesis and functional tasks are characterized by obvious vascular dilatation activity. Even if their final objective lies in developing collaterals in the ischemic foci it is being accomplished by means of endothelium.

Time length of the protective effect of the early stage ischemic preconditioning according to different authors varies from 5 minutes to 12 hours [9]. That's why by performing examinations 90 minutes after a single ischemic episode we knowingly involve its activity period. It is possible to suggest that under the NO deficiency conditions the triggers after entering endothelium overcome damage to the vasodilatative mechanisms. This favors balancing of the vascular control mechanisms and improves microcirculation. But a single ischemic impetus is not sufficient for arterial pressure recovery under chronic pathology conditions.

Time length of the protective effect of the delayed stage of or "late" preconditioning according to different authors varies from 12 to 96 hours [10]. Notwithstanding the formal resemblance of the ischemic episodes reproduction methodology the mechanisms of their preconditioning effect and positive influence on the endothelial cells in the both cases are considerably different [11].

During the delayed ischemic preconditioning stage genome is being activated. Induction of synthesis of nitrogen oxide, superoxide dismutase and other antioxidative enzymes as well as heat shock chaperones which are involved in cytoskeleton stabilization is being initiated [12]. Improvement of endothelial function at this stage can be explained by induction of synthesis of the powerful vasodilating agent – NO (NO-synthase expression) and of antioxidative enzymes synthesis which involves oxidative stress reduction.

FINDINGS

Therefore the results of the carried out experiment give ground for the further investigations of ischemic preconditioning for the purposes of gestosis correction and search of new dug preparations with the mode of action forming the basis for the ischemic preconditioning effect.

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