Hypoxic damage of cardiomyocytes during pregnancy and its experimental treatment

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ABSTRACT

BACKGROUND

The important role of equilibrium of environmental factors during the embryo-fetal period is undisputable. Women of reproductive age are increasingly exposed to various environmental risk factors such as hypoxia, prenatal viral infections, use of drugs, smoking, complications of birth or stressful life events. These early hazards represent an important risk for structural and/or functional maldevelopment of the fetus and neonates. Impairment of oxygen/energy supply during the pre- and perinatal period may affect at neuronal functions and induce cell death both in mother and fetus.

METHODS

The experiment was carried out on 22 three-month old female white Wistar rats, weighing 180-200 g, in accordance with the "Rules of work with experimental animals." Animals were divided into 2 groups of 11 animals in each. Throughout pregnancy the female groups I and II were daily intraperitoneally injected by sodium nitrite water solution $NaNO_2$ at a dose of 5 mg/100g body weight. Females of the second group after injection of sodium nitrite were administered by Cytoflavin treatment.

RESULTS

Examination of myocardial slices stained by HBFP method in the first test group after the administration of sodium nitrite showed us the evidence of interstitial edema and ischemic damage which manifested itself by diffuse localisation of reddish substrate located in the cytoplasm. However, in the second experimental group, where was applied cytoflavin correction, reddish substrate, was less pronounced, and we almost never revealed contracture damage in the contractile cardiomyocytes.

CONCLUSION

The administration of the drug "Cytoflavin" can reduce the violations of cardiomyocytes in pregnant animals due to its antihypoxic and membrane protective properties.

KEYWORDS: hypoxia, myocardium, sodium nitrite, pregnancy, rats

INTRODUCTION

Hypoxia during pregnancy, labor or early life stage is a major determinant of neurological and heart failure morbidity and mortality in the neonatal period. Many studies have been investigating neurological deficits following perinatal hypoxia, including seizures, cerebral palsy, mental retardation, attention deficit-hyperactivity disorder, anxiety as well as other mental diseases [1]. Insufficient delivery of the tissue energy reserves (oxygen, nutrients) to the developing brain threatens its function during entire life-span up to senescence, and it might be one of primary factors in the pathogenesis of neurodegenerative and heart diseases both in the mother and fetus [6].

Sodium nitrite has been formally assigned to pregnancy category C by the FDA. Sodium nitrite has caused fetal death in humans as well as animals due to formation of methhaemoglobin [1]. It was proved that exogenous nitrite when ingested contribute to the development of histotoxic hemic hypoxia which is not yet fully investigated. Sodium nitrite is used as a raw material for the production of caprolactam polymers and antioxidants for synthetic polymers[3]. It is used as a colour fixative and preservative in meats and fish. It is also used in: adhesives, binding agents, anti-freezing agents, cleaning/washing agents, disinfectants, colouring agents, construction materials additives, corrosive inhibitors, cutting fluids, fillers, food/foodstuff additives, heat transferring agents, intermediates, laboratory chemicals, lubricants and additives, non agricultural pesticides, oxidising agents, pesticides, pharmaceuticals, process regulators, reducing agents, stabilisers, surface-active agents [1, 3, 4].

The aim of the research was to investigate the morphological state of contractile cardiomyocytes of pregnant rats in terms of hemic hypoxia induced by sodium nitrite $NaNO_2$ with the subsequent correction of occurred hypoxic violations by cytoprotective drug Cytoflavin.

METHODS

The experiment was carried out on 22 three-month old female white Wistar rats, weighing 180-200 g, in accordance with the "Rules of work with experimental animals." Animals were divided into 2 groups of 11 animals in each. Throughout pregnancy the female groups I and II were daily intraperitoneally injected by sodium nitrite water solution $NaNO_2$ at a dose of 5 mg/100g body weight (dose causing hypoxia of moderate severity) [8]. Females of the second group after injection of sodium nitrite were administered Cytoflavin treatment (Polysan Company, St. Petersburg) at a dose of 0.5 ml/100g body weight of animal.

Pharmacological effects of Cytoflavin are based on the complex influence of the components that make up the drug. Succinic acid (SA) is endogenous intracellular metabolite of the Krebs cycle, performs in the body cells universalenergy synthesis. With the participation of coenzyme flavin adenine dinucleotide (FAD) succinic acid by mitochondrial enzyme succinate dehydrogenase become quickly transformed into fumaric acid and other metabolites which enter in the tricarboxylic acid cycle. SA also stimulates aerobic glycolysis and ATP synthesis in cells. The final product of succinic acid metabolism in the Krebs cycle is carbon dioxide and water. Succinic acid improves tissue respiration due to the activation of electron transport in the mitochondria.

Riboflavin (vitamin B2) is a flavin cofactor (FAD) activating enzyme succinate dehydrogenase and other redox reactions of the Krebs cycle.

Nicotinamide-dependent activating enzymes of Krebs cycle recquired to stimulate cellular respiration and ATP synthesis.

Inosine is a purine derivative, a precursor of ATP. It has the ability to activate a number of enzymes of the Krebs cycle by stimulating the synthesis of key enzymes, nucleotides: flavin adenine dinucleotide (FAD) and nicotinamide adenine dinucleotide (NAD). Thus, all components of Cytoflavin are natural metabolites and stimulate body tissue respiration.

On the 21 day of pregnancy after pericardiotomy and under ether full anesthesia the heart was removed and placed in the 0,9% solution of potassium chloride to reach its relaxation in diastole [6]. We stained sections of myocardium by HBFP method (hematoxyllin-based fuchsine-picric acid) to detect foci of ischemic damage. Preparation of material for ultramicroscopic study with lanthanum staining was done according to the standard method [2].

RESULTS

Examination of myocardial slices stained by HBFP method in the first test group after the administration of sodium nitrite showed us the evidence of interstitial edema and ischemic damage which manifested itself by diffuse localisation of reddish substrate located in the cytoplasm. However, in the second experimental group, on which cytoflavin had been applied, reddish substrate, was less pronounced, and we almost never revealed contracture damage in the contractile cardiomyocytes. That was indicating the antihypoxic properties of the applied drug Cytoflavin.

Electron microscopy has revealed ischemic and hypoxic damage in the cardiomyocytes of cytoflavin treated animals. In contractive cardiomyocytes it was observed reduction of glycogen granules, chromatin's granules condensation near nuclear membrane and a significant enlightment of the nucleoplasm. Focal and total lysis of mitochondrial cristae was accompanied by a vivid invagination of karyolemma which is an indicator of the intracellular edema. Expansion of sarcoplasmic reticulum, mitochondria lytic lesions were induced by irreversible damage of sarcolemma permeability. This observation was confirmed by the penetration of colloidal lanthanum particles through the sarcolemma into the intramitochondrial space. In most of contractile cardiomyocytes it was observed a decrease of the electron densityof myofibrils, numerous rigor complexes and deposition of Ca^{2+} in myofibrills. In addition, the pronounced ischemic damage was observed in the endothelial cells of capillaries which manifested in form of intracellular edema, focal lysis of the cristae of mitochondria along with increasing amount of pinocytotic vesicles of various types (Fig.1).

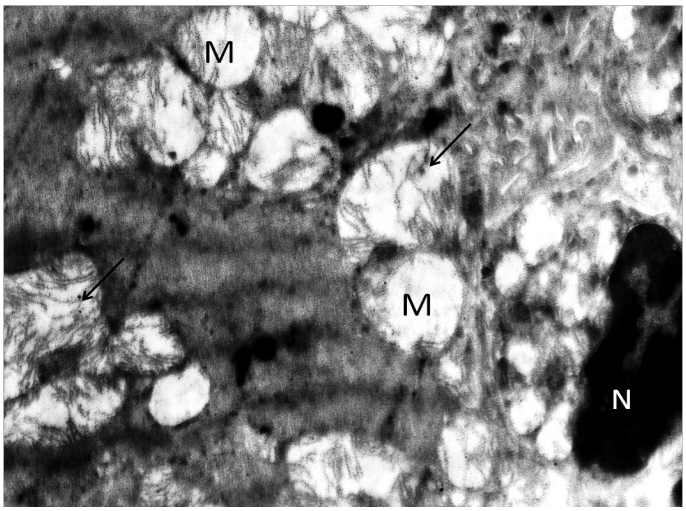

Fig.1.The ultrastructure of rat contractile cardiomyocyte (Group I without treatment). Lysis of mitochondrial cristae (M), invaginated kariolemma (N), lanthanum particles in the inner mitochondrial space and calcium deposits in myofibrills (arrows).TEM x 20000

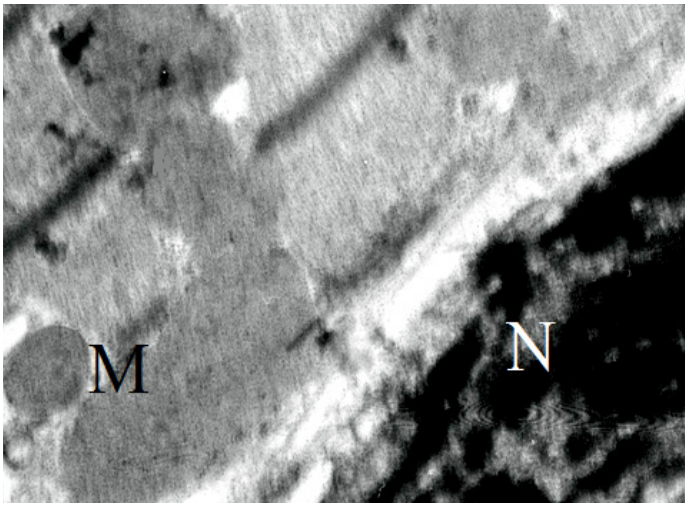

Fig.2. The ultrastructure of rat contractile cardiomyocyte (Group II with treatment). Normal structure of mitochondrial cristae (M) and myofibrills.TEM x 22000

Electron microscopy of rats' contractile cardiomyocytes in the 2 experimental group (with correction) revealed predominantly reversible lesions. Those changes were represented by uneven distribution of nuclear chromatin, focal lysis of mitochondrial crists but the vast majority of mitochondria still retained their normal structure without any colloid lanthanum granules in their matrix. Some contractive cardiomyocytes had reduced number of myofibrils and developed a large number of heterogeneous mitochondria, which were the evidence of compensatory processes (Fig.2). The penetration of colloidal lanthanum inside of sarcolemma was observed only in few isolated cardiomyocytes what demonstrated the membrane protective effect of the applied drug "Cytoflavin" (Polysan corp.). This positive effect is a result of the described metabolic antihypoxic and antioxidant activity of the drug due to the complementary action of succinic acid, inosine, nicotinamide and riboflavin. The visual amount of collagen fibers in the intercellular matrix compared to the first group was not abundant in comparison to the normal heart interstitium. Most capillaries were full-blooded reflecting compensatory processes in the myocardium.

DISCUSSION AND CONCLUSION

The important role of equilibrium of environmental factors during the embryo-fetal period is undisputable. Women of reproductive age are increasingly exposed to various environmental risk factors such as hypoxia, prenatal viral infections, use of drugs, smoking, complications of birth or stressful life events [1,5]. These early hazards including excessive consumption of nitrites represent an important risk for structural and/or functional maldevelopment of the fetus and neonates. Impairment of oxygen/energy supply during the pre- and perinatal period may affect myocardium functions and induce cell death. Several experimental studies have recently provided new insights into cellular events occurring in myocardium tissues following hypoxia. Membrane defects in cardiomyocytes of pregnant rats due to peroxidation of lipids, microvascular dysfunction and endothelial cell damage have been identified as factors playing a key role not only in single heart dysfunction in mother after combined hypoxia-pregnancy overloading but also can result in the development of multiple organ failure in newborns affected by hypoxia [6]. Cell swelling, the disruption of adherence junctions was shown to be responsible for the increased permeability of vascular endothelium, followed by leakage from the vascular bed to the surrounding tissues resulting in interstitial edema

In the present study, we have shown that both cardiomyocyte and endothelial damage may represent an important component of the response to hemic hypoxia in the myocardium of pregnant rats undergoing sodium nitrite influence. This finding may suggest a hypoxia-preganacy role for worsening endothelial damage with all the negative consequences in heart cell function

Based on our results, experimental administration of the drug "Cytoflavin" can help to reduce the violations

of cardiomyocytes in pregnant animals mainly due to its membrane protective properties which were revealed by lanthanum staining. In addition cytoflavin had no adverse effects on developing fetus and offspring. Further investigations require drug testing on the greater number of experimental animals considering correlation study between age of the fetus with the positive effect of cytoflavin in hypoxia prevention and the degree of hypoxia during pregnancy.

Conclusions:

1. Accumulation of nitrogen containing xenobiotics in the body of pregnant rat during pregnancy posses a vivid pathogenic effect upon contractile cardiomyocytes, which manifests itself in the form of ischemic and hypoxic violations along with membrane damage of contractile cardiomyocytes and endotheliocytes resulting in their death.

2. The administration of the drug "Cytoflavin" can help to reduce the violations of cardiomyocytes in pregnant animals due to its antihypoxic and membrane protective properties which were revealed by lanthanum staining.

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