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INFLUENCE OF THE CHRONIC STRESS OF PREGNANT RATS ON THEIR OFFSPRING'S MORPHO-FUNCTIONAL LIVER CONDITION

Nikolayeva O., Kuznetsova M., Lytvynenko O.

Kharkiv National Medical University, Ukraine

Abstract

The morphofunctional state of the rats offspring liver, born from femal rats underwent chronic immobilization stress during pregnancy was studied. It has been revealed that chronic stress in female rats during pregnancy wields considerable influence over liver morphology and lipid-synthesizing function of their offspring. Pronounced discomplexing of the beam-radiary structure, increase of stromal - parenchymal index due to increase in stromal and decrease in the parenchymal components of the liver and decrease level of expression of endothelial synthase has been morphologically revealed. Biochemical study of the liver homogenate revealed signs of impaired synthesis, secretion and reuptake of lipids (increase of cholesterol, tryglyceerids and non-esterified fatty acids and decrease of phospholipids. In the offsprings blood serum dyslipidemia (increase of cholesterol and low-density lipoproteins level) was detected. All this is a risk factor for fibrosis, fatty hepatosis and level cirrhosis development.

Key words: chronic stress, hepatic morphofunctional state, pregnant rat, offspring.

Urgency. Diseases of the liver and biliary tract in modern society are becoming increasingly common. Thus, every year from 10 to 15% of people in industrialized countries

suffer from diseases of the hepatobiliary system [1]. Every year in the CIS countries 500 thousand to 1 million people suffering from liver diseases are registered [2]. In Ukraine over the past 10 years the prevalence of liver diseases has increased by 20.1% [2, 3]. Liver pathology occurs in 3 - 5% of pregnant women and is one of the most common and prognostically significant forms of gestational pathology [2]. According to statistical data, the prevalence of neonatal liver disease reaches 1 in 2500 live births [3, 4]. Of particular concern is that non-alcoholic fatty liver disease has become one of the most common liver pathologies in children and adolescents, due to an increase in the frequency of obesity and diabetes in this age group. Its prevalence in children ranges from 3 to 10%. Over the last ten years, this figure has increased from 2.6 to 5%, with boys 2 times more often involved than girls [5]. Thus, liver disease ranks a significant place among the causes of the general population early disability and mortality. This makes it necessary to study the mechanisms of liver damages at different conditions.

Stress takes the lead among many exogenous causes of liver damage. The study of chronic psychoemotional stress effect on the organism of female rats and their offspring [6] was conducted. Its influence was manifested by an imbalance of somatotropic and thyroid hormones in the blood of both pregnant rats and their offspring [7]. It has been established that chronic stress causes decrease of total T3 and T4 concentration in blood, as well as their free fractions in non-pregnant rats [8]. Immobilization stress in male rats was found to cause marked impairments of hepatic metabolism [9, 10].

It was also revealed that prenatal stress leads to intrauterine growth retardation in rats and contributes to the development of obesity and type 2 diabetes in the offspring in adulthood [10, 11, 12]. It has been established that the ability of hepatocytes to regenerate in young animals under stress is significantly higher than in animals aged 1.5-2 y.o. The use of the complex of dietary fibers and short-chain fatty acids normalizes the ratio of regeneration and damage of liver tissue in animals of both age groups under stress [13].

Despite the existence of numerous works devoted to the effects of stress on the body of mothers and their offspring, the characteristics of the effects of chronic stress exactly on the liver of the latter remain insufficiently studied.

Objective: to study the morphofunctional state of the liver of the offspring of female rats who were under the conditions of chronic stress during pregnancy.

Materials and methods. The morphofunctional state of the liver was studied in 76

randomly-unbreeding infant- rats of the WAG / G Sto population, of which 38 are offspring of mothers whose pregnancy proceeded under chronic stress. 11 newborn infant - rats constituted the 1st group, 15 one-month rats were included in the 2nd group and 12 two-month rats constituted the 3rd group. The control group consisted of 38 animals with the same number in the indicated age groups. Simulation of stress factor effect on rats was carried out with the use of an experimental model developed at D. E. Alperin Department of Pathological Physiology of Kharkov National Medical University [14]. Morphological study of the hepatic tissue was carried out according to generally accepted methods [15]. The preparations were stained with hematoxylin and eosin, according to van Gieson and Mallory methods. To assess the degree of liver's endothelial dysfunction, immunohistochemistry revealed the expression of markers of nitric oxide metabolism: endothelial nitric oxide synthase (eNOS) and inducible nitric oxide synthase (iNOS). Concentrated polyclonal rabbit antibodies (PRAB) of Thermo Scientific (Germany), Nitric Oxide Synthase inducible (iNOS) Rabbit Polyclonal Antibody at the dilution of 1: 100, Nitric Oxide Synthase endothelial (eNOS) Rabbit Polyclonal Antibody were used at 1:50 dilution. Fractional composition of lipids (cholesterol, phospholipids, tryglycerids, non-esterified fatty acids, NEFA) was studied by thin-layer chromatography on Silufol plates [16] and the glycogen content was measured using V. G. Asatiani spectrophotometric method [16]. Serum level cholesterol, thyroid, high-density lipoproteins (HDL) were determined with Olvex (Russia) reagent kits, low and very low-density lipoproteins (LDL, VLDL) were determined by calculation method [16]. The studies were carried out in compliance with the rules and international recommendations of the European Convention for the Protection of Vertebrate Animals used for experiments or for other scientific purposes (Strasbourg, 1986). Removal of animals from the experiment was carried out by decapitation. Statistical processing of the results was performed with the use of STATISTICA-10 program. To determine the reliability of differences Mann-Whitney's U test was used. Differences were considered significant at $p < 0.05$.

Results and discussion. Macroscopically, in the liver tissue of the offspring obtained from female rats under conditions of chronic stress during pregnancy, significant differences were found from the control group, which manifested themselves as brownish-yellowish color, afine-grained structure on the incision and uneven repletion of the hepatic veins. Microscopically in the liver tissue of newborns, 1-month-old and 2-month-old rat pups, histoarchitectonics disorders were revealed in the form of pronounced baloch-radar discomplexion, expansion of

sinusoids predominantly around portal zone of tracts and small outbreaks of extramedullary hematopoiesis (the latter only in the group of newborns). Hepatocytes had a foamy dischromic cytoplasm, uneven pyknosis of the nuclei and small optically empty fat vacuoles. This indicates the presence of hepatocyte fatty dystrophy developed most likely due to impaired uteroplacental circulation and chronic hypoxia during maternal stress. This indicates a high degree of damage of the progeny liver parenchyma, equally pronounced in all age groups.

Liver regenerative activity, reflecting parenchymatous component damage degree was estimated by the number of binuclear hepatocytes relative to their mononuclear forms (Table 1). It was established that in animals of all groups, the number of binuclear forms of hepatocytes significantly increased compared with the control. The highest percentage of binuclear hepatocytes is registered in the liver of the rat pups (the 1st gr.) and is almost 3 times more than in the control group and indicates a high intensity of liver damage. Then, as the age of the pups increases, there is a clear tendency for their number decrease: in 1-month-old animals (the 2nd gr.) the number of binuclear forms of hepatocytes is significantly less than in newborns, although it remains significantly higher compared to the control group pups; in 2-month-old animals (the 3rd gr.) the number of binuclear forms of hepatocytes was significantly less than in 1-month-old, but does not differ from that of the control group rats. This is an indicator of sufficient efficiency of the regenerative and compensatory-adaptive hepatic mechanisms in the postnatal period.

Table 1

The number of binuclear forms of hepatocytes in the liver of the offspring of rats which were under stress during pregnancy (M ± m)

index		Groups of infant rats		
		1 groups (newborns)	2 group (1-months-old)	3 group (2-months-old)
N of binuclear hepatocytes, %	Main	8.91±0.05**	6.93±0.02** *(p_1<0.01)*	3.86±0.04** (p1,2<0.01)
	Control	3.08±0.02	3.09±0.025	3.1±0.01

Notes: 1. ** - p <0.01 (comparison with the control group).

2. p_1 - comparison with the 1st group; p_2 - comparison with the 2nd group.

The degree of hepatic parenchyma damage was also estimated at the calculation of the

percentage ratio of stromal and liver parenchyma elements and calculating of the stromal parenchymal index (SPI) (Table 2). The higher is SPI, the higher are parenchymal losses in liver tissue and this indicates decrease of its functional activity.

Table 2

Morphometric data of the structural elements of the liver of the offspring of female -rats, under stress during pregnancy (M ± m)

Index		Groups of infant-rats		
		1 group (newborns)	2 group (1-month-old)	3 group (2 month-old)
Stroma, %	Main	32.8±1.1**	32.7±1.2**	32.8±0.9**
	Control	25.7±0.14	25.83±0.08	25.91±0.03
Parenchyma,%	Main	67.2±0.9**	67.3±0.8**	67.2±01**
	Control	74.3±0.15	74.17±0.08	74.09±0.03
Stromal-parenchymal index, %	Main	0.48±0.01**	0.48±0.01**	0.48±0.01**
	Control	0.34±0.003	0.35±0.001	0.35±0.02

Note: ** - p <0.01 (comparison with the control group)

As can be seen from table 2, a significant increase in the number of stromal and decrease in the parenchymal component of the liver was found in animals of all groups compared to the pups of control groups. The highest SPI index is recorded in rats of the 1st group – it is increased 1.4 times compared to the control. Then, as the progeny grows, this indicator does not change in any of the groups under observation and this indicates previously proliferated stroma state fixation and parenchymal losses due to gestational stress of animals.

Immunohistochemical study of endothelial dysfunction markers showed a decrease in the level of expression of endothelial nitric oxide synthase (eNOS) in all liver samples of rats of the main groups (the 1st, the 2nd and the 3rd gr.) compared to control.

This was manifested by the alternation of weakly stained areas of endothelial structures with areas of pronounced eNOS expression, the presence of stratification and desquamation foci of the endothelium in the central veins, as well as in the condensation of endothelial cells. The accumulation of inducible nitric oxide synthase (iNOS) in sinusoid endotheliocytes, muscle walls of blood vessels, stroma of portal tracts and hepatocytes was also observed. This indicates about

pronounced endothelial dysfunction and high parenchymal losses, and points at a decrease in the functional activity of the main groups rats liver.

The functional state of the rats liver was assessed according to a biochemical analysis of serum components and liver homogenates. The results are presented in Tables 3 and 4.

Table 3

Biochemical indicators of blood serum, characterizing functional state of the liver of the offspring of female - rats which were under chronic stress during pregnancy (M ± m)

Index	Groups of animals			
	1-month-old infant rats (the 2nd group)		2-month-old infant-rats (the 3rd group)	
	Control	Main	Control	Main
Cholesterol, mmol/l	3.93±0.05	5.04±0.04**	5.12±0.02	4.77±0.07
Low-density lipoproteins, mmol/l	1.07±0.03	3.11±0.03**	1.41±0.02	1.46±0.02 *(p_2<0.01)*
Very low - density lipoproteins, mmol/l	0.27±0.006	0.22±0.006**	0.49±0.009	0.48±0.02 *(p_2<0.01)*
High density lipoproteins, mmol/l	2.59±0.05	1.71±0.035**	3.22±0.002	2.82±0.05**
Tryglycerids	0.58±0.02	0.48±0.01**	1.09±0.02	1.11±0.04

Notes: 1.** - p <0.01 (comparison with the control group);

2. p_2 – comparison with the 2nd group

It was established that in newborns (the 1st gr.), 1-month-old (the 2nd gr.) and 2-month-old (the 3rd gr.) infant- rats, whose mothers suffered chronic stress, there are significant features of lipid fractions content in blood serum and liver homogenate (see tab. 3 and 4). In newborn rats in the liver homogenate, the content of cholesterol, tryglycerids, NEFA is increased, and FL and glycogen content is reduced. These data are consistent with the morphologically revealed decrease in the parenchymal component of liver and the presence of its fatty degeneration.

Table 4

Lipids and glycogen content in the homogenate of the offspring liver of female rats, which were under stress during pregnancy, (M ± m)

Indexes		Groups of animals		
		1st group	2nd group	3rd group
Cholesterol, mg/g	Main	0.47±0.02**	0.39±0.008** *(p_1<0.01)*	0.47±0.02** *(p_2<0.01)*
	Control	0.21±0.007	0.26±0.02	0.51±0.008
Phospholipids, mg/g	Main	15.15±0.25**	16.27±0.21** *(p_1<0.01)*	11.12±0.12** *(p_2<0.01)*
	Control	18.84±0.31	17.36±0.77	14.86±0.15
Tryglycerids, mg/g	Main	5.74±0.21**	7.9±0.08** *(p_1<0.01)*	10.19±0.16** *($p_{1,2}$<0.01)*
	Control	3.67±0.13	4.87±0.3	7.64±0.1
NEFA, mg/g	Main	10.8±0.37**	8.82±0.07** *(p_1<0.01)*	4.17±0.07** *($p_{1,2}$<0.01)*
	Control	7.65±0.25	5.7±0.193	3.83±0.08
Glycogen, mg/g	Main	12.05±0.35**	16.96±0.05** *(p_1<0.01)*	16.12±0.13 *($p_{1,2}$<0.01)*
	Control	14.5±0.23	19.62±1.08	15.77±0.14

Notes: 1.** - p <0.01;* - p<0.05 (comparison with the control group);

2. p_1 – comparison with the 2st group; p_2 – comparison with the 2nd group

Apparently, the features identified are explained by changes in the hormonal status of rat-mothers under the influence of stress and, therefore, changes in the activity of offspring lipid metabolism regulatory enzymes (the prenatal activity of most regulatory enzymes of the fetus is regulated by the hormones of the mother's body). Obviously, with a decrease in the number of actively functioning hepatocytes, the synthesis of apo-proteins and receptors for their transport forms is disrupted, which affects liver and serum lipid content (LDLP, cholesterol and TG - increase, while VLDLP and HDLP - decrease). Under the conditions of impaired TG transport (their accumulation in liver and decrease in serum), the use of carbohydrates for energy purposes

increases, as evidenced by the low glycogen content in liver.

In 1-month-old rats (the 2nd gr.) the direction of the changes detected remains. There was a significant increase in cholesterol level (by 48.64%), TG (by 61.98%), NEFA (by 33.36%) and decrease in the level of PL (by 6.26%) compared to control, but the severity of these changes is less than those in the newborns, which may be due to partial regeneration of hepatocytes and the beginning of the regulatory effects of the offspring's own hormones.

In 2-month-old pups (the 3rd gr.) positive dynamics was detected, compared to 1-month-old ones. Decrease in cholesterol level (by 8.33%) and PL (by 25.18%), as well as an increase in TG and NEFA (by 33.36% and 8.76%, respectively) in comparison with control group animals. These changes in the lipid spectrum may be associated with impaired synthesis, transport and inverse capture of lipids in the liver. The lipid spectrum of 2-month-old rats blood serum was significantly different from that in the other groups. A moderate decrease in cholesterol level, which may indicate an increased capture of its tissues, which is consistent with the results of morphological research (SPI increase compared with 1-month-old rats). The content of glycogen in the liver is significantly reduced in groups 1 and 2 (by 16.9% and 13.57%, respectively). The 3rd group animals its level increased by 2.22% compared with 1-month-old pups (the 2nd gr.), which indicates violations in the ratio between the processes of glycogen synthesis and breakdown (which, apparently, is due to increased glucose utilization with energy purpose).

In the liver tissue of 1-month and 2-month-old infant rats, an increase in cholesterol, TG and NEFA levels was detected. This indicates the accumulation of lipids in liver, which is confirmed by the results of a morphological study (moderate discomplexing of the beam-radiary structure, uneven nuclei's pycnosis, optically empty fat vacuoles) and the development of fatty hepatosis.

The persistent nature of metabolic disturbances indicates the epigenetic effect of prenatal stress of pregnant rats on the morphofunctional state of the offspring liver.

Thus, our studies indicate the possibility of epigenetic programming of metabolic disorders in the rat – mothers, underwent chronic stress during pregnancy, manifested by the development of dyslipidemia (with increased cholesterol, LDLP). This can be regarded as risk of hepatic organic pathologies development in the future .

Conclusions:

1. Chronic prenatal stress has a significant effect on the morphology of the liver of infant

rats, with the changes more pronounced in newborns and 1-month-old pups.

2. Morphologically, in the liver of newborns and 1-month-old animals a pronounced discomplexion of the beam-radiary structure, fatty hepatosis and increased volume of stromal component are revealed. This may cause the development of liver fibrosis and cirrhosis in the future.

3. The study of markers of endothelial dysfunction showed a pronounced decrease in the expression level of eNOS in newborns and 1-month-old rats and the relative normalization of this indicator by 2 months.

4. Biochemical study of the liver homogenate revealed signs of impaired synthesis, transport and reuptake of lipids (increased cholesterol, accumulation of TG and NEFA, and decreased level of PL), which is risk factor of fatty hepatosis.

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