THE INFLUENCE OF RATS-MOTHERS FOOD RATION WITH NUTRIENT DEFICIENCY ON THE STRUCTURAL-FUNCTIONAL STATE OF THE LIVER OF THEIR TWO MONTHS OFFSPRING

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Abstract: The aim of this study was to determine the influence of rats-mothers food ration with nutrient deficiency on the morphology and functional status of the liver of their two months offspring.

Materials and methods. The experiments were performed on the offspring of WAG population rats, which was prenatal under nutritional deficiency. The offspring of rats (26 specimens) were divided into two groups, 50 % of the rats constituted the control group (i. e., their mothers received the vivarium baseline diet), the other 13 animals were in the 2nd (main) group. The young rats were removed from the experiment two months after birth by decapitation. Morpho-functional condition of the liver of the offspring rats treated with pregnancy deficiency of fat and carbohydrate diet was evaluated according to the results of immunohistochemical study of markers of liver vascular endothelium damage – endothelial synthase of nitric oxide (eNOS) and inducible nitric oxide synthase (iNOS) and biochemical study of liver tissue homogenates (studied fractional composition of lipids and organ glycogen).

Results. It was found that the nutritional deficiency of rats-mothers had a significant effect on the structural and functional state of the liver of their bi-monthly offspring, which was manifested by a decrease in the level of endothelial synthase expression, which indicated a high degree of damage to the liver endothelium. Biochemical study of liver homogenates revealed signs of impaired secretion and reuptake of lipids, which were manifested by an increase in CL, TG and EFAs accumulation, as well as a decrease in liver PL and glycogen, which indicated the presence of pronounced disorders of carbohydrate and lipid metabolism.

Conclusions. The obtained data allow us to establish the negative nature of the effect of deficiency of fats and carbohydrates in the diet of rats-mothers on the structural and functional state of the liver of their bi-monthly offspring. This condition was accompanied, both by signs of liver vascular endothelial damage and metabolic disorders, this all suggests that the identified disorders may be a risk factor for fatty hepatosis as well as liver cirrhosis in the future.

Keywords: liver, offspring, morphology, immunohistochemistry, homogenate, fat and carbohydrate deficiency diet, pregnancy.

1. Introduction

Digestive diseases occupy a leading position in the structure of morbidity and mortality among the population of Ukraine. Of greatest concern is the increasing number of children with chronic gastrointestinal tract disease every year. Among the factors that can adversely affect the liver is a poor diet with reduced protein, fat or carbohydrate intake [1]. According to a number of foreign experts, there is a close relationship between fetal conditions in which the fetus is present and the development of certain diseases, such as atherosclerosis, arterial hypertension, and others [2, 3]. Many studies have focused on the effects of either low-calorie or low-protein diets on various organs and systems, including the liver of pregnant rats. A number of functional disorders of the organ have been identified, which have been accompanied by changes in protein, lipid and carbohydrate metabolism [3, 5], but its effect on the offspring in the mother-fetus system has not been carefully studied.

The aim of this study was to determine the influence of rats-mothers food ration with nutrient deficiency on the morphology and functional status of the liver of their two months offspring.

2. Materials and methods

The studies were performed based on the Experimental Biological Clinic (Vivarium) of Kharkiv National Medical University, Ukraine. The experiment was conducted in the autumn-winter period 2015–2016 in order to reduce the degree of influence of seasonal variations on the studied indicators.

The study was performed on the offspring rats obtained from 13 random-born females of the WAG population, 50 % of whom were in the control group; the offspring rats in both groups received a basic vivarium diet and were removed from the experiment 2 months after birth by decapitation. The morphofunctional state of the liver was evaluated by immunohistochemical studies (the markers of vascular endothelial damage: endothelial nitric oxide synthase (eNOS) and inducible nitric oxide synthase (iNOS) were determined) [4] and biochemical homogenate studies. The fractional composition of lipids in tissue homogenates was determined by the thin layer chromatography method on Silufol plates, and glycogen by spectrophotometric method according to V. G. Asatiani.

All animal experiments were carried out in accordance with the rules and international recommendations of the European Convention for the Protection of Vertebrate Animals, which are used for experiments or for other scientific purposes (Strasbourg, 1986). Statistical processing of the results was performed using GraphPadPrism5. The U Mann-Whitney test was used to determine the significance of the differences.

3. Results

Immunohistochemical study of markers of endothelial dysfunction revealed a moderately pronounced decrease in the level of expression of endothelial nitric oxide synthase in all samples of liver of two-month offspring rats relative to the control group, which showed a moderate intensity of damage to parenchyma. There was a progressive decrease in inducible nitrogen synthase in sinusoid endothelial cells, the muscular layer of the vessel walls, portal tract stroma and hepatocytes.

The functional state of the liver of two-month-old rats was evaluated by the indicators of the fractional composition of lipids in the body homogenates, the results of which are presented in **Table 1**.

Analysing the fractional composition of lipids in liver homogenates of two-month-old rats (**Table 1**), the following data were obtained: cholesterol (CL) increased by 5.88 %, triglycerides (TG) by 20.16 %, and EFAs by 6.7 %, which indicated activation of the processes of synthesis, secretion and deposition of TG, resulting in impaired glycogen utilization. In addition, a decrease in the concentration (phospholipids) of PL – by 13.32 %, in turn, indicated a violation of the processes of their synthesis.

Table 1
Fractional composition of lipids and glycogen in liver homogenates of two-month rats Me [25; 75]

Indicators	Study groups	
	Group 1 (Control)	Group 2 (Main)
Cholesterol, mg/g	0.51 [0.49;0.54]	0.54 [0.52; 0.58]. p<0.0402
Phospholipids, mg/g	15.09 [14.43;15.27]	13.08 [12.52; 13.52] p<0.0001
Triglycerides, mg/g	7.64 [7.49;8.0]	9.18 [8.81; 9.83] p<0.0001
EFAs, mg/g	3.88 [3.71;3.94]	4.14 [3.95; 4.43] p<0.0110
Glycogen, mg/g	15.75 [15.38;16.21]	14.98 [14.57; 15.31] p<0.0039

Note: p – compared to the control group

4. Discussion and conclusions

Assessing the structural and functional state of the liver can be distinguished that the leading role was played by the violation of the integrity of the endothelium of the vessels of the organ. This, in turn, indicated damage to the liver parenchyma and decreased functional activity of the organ. Beside the liver performs a number of vital functions, it is also the central organ in which all types of metabolism intersect. Therefore, the study of metabolic indicators is equally important for assessing the functional state of the liver, especially when violations of the structure of the organ are detected.

In this study, metabolic and fat metabolism disorders were identified. This was accompanied by a tendency for the accumulation of lipids in the liver of bi-monthly offspring, which may indicate an intensification of the processes of their synthesis and accumulation in cells; and the decrease in glycogen content was associated with the activation of decay processes and a decrease in its synthesis, which are primarily caused by increased utilization of glucose for energy goals.

The literature analysis showed that, for the most part, many researchers are focused on studying the effects of partial starvation or low protein diets on the metabolism of male and female rats. Thus, it was found that during the first two days of fasting in the liver of rats remained high functional activity of the antioxidant system. On the third day of fasting in the liver, there were signs of activation of lipid peroxidation processes against the background of increased activity of catalase and glutathione [6]. It has been shown that the response to starvation in the body occurs through the expression of several thousand genes, whose profile is closely related to the duration of food restriction [7, 8]. It was determined that in protein-free diet, NADH-dehydrogenase activity of rat liver mitochondria decreased (on day 14), while the 4-week retention of rats under these conditions resulted in a 5.5-fold decrease in the investigated enzymatic activity compared to control. It was proved that the activity of succinate dehydrogenase did not change during the two-week retention of rats on a protein-free diet. However, the 4-week maintenance of animals in both protein and low protein diets results in a decrease in succinate dehydrogenase activity. However, if the low protein diet succinate dehydrogenase activity of the liver mitochondria is halved, then in the absence of protein in the diet – three times. Therefore, it was concluded that in the implementation of changes in the system of biotransformation of energy in mitochondria in the conditions of alimentary protein deficiency, there are primarily disturbances at the level of the respiratory chain complex I [8, 9]. It has been proven that reducing the caloric content of a complete diet (and not just any of the ingredients) activates cell proliferation. The number of reticulocytes increased almost threefold, which testified to the intensification of hematopoietic activity of bone marrow, the number of mitotically active cells in bone marrow and liver increased [7, 10]. It was found that limiting the caloric content of the mother's diet by 40 % for 28 days contributed to an increase in the number of hepatocytes and an increase in the nuclear-cytoplasmic index in the liver of their offspring, activating the physiological regeneration of the organ parenchyma [8–10]. It has been shown that the protein-deficient diet of rats during pregnancy causes an increase in blood pressure in F0 generation, development of endothelial dysfunction, insulin resistance, and contributes to impaired glucose homeostasis in F1-3 generations, despite the fact that they received a diet. As we studied the effect of a diet of deficient diets and carbohydrates of rats-mothers on the morpho-functional state of the offspring liver in the mother-fetus system, it should also be emphasized that two-month-old rats were not exposed to the above factor, so the results cannot be completely compared because they complement each other to some extent.

Study limitations. This study was limited by the duration of the harmful factor, that is, rats did receive a deficient diet a week before the males were fed and throughout their pregnancy, the offspring of these females received a vivarium baseline diet after maternal transplantation. There were age restrictions as well, as there was no further dynamics of change as the offspring matured. Investigation of only local effect of alimentary deficiency on the liver, insufficient study of the relationship between the influence of the factor on the mother's organs and mechanisms of injury to the offspring.

Prospects for further research. Within the chosen research topic, we consider it necessary to offer additional investigation of the mechanisms of liver damage by conducting biochemical and molecular genetic studies, in order to determine the leading mechanisms of pathogenesis of damage at the level of rat body cells, as well as the role of maternal malnutrition in the formation of structural and functional abnormalities in the liver of their offspring and track the subsequent dynamics of change as the offspring grows. We also consider it appropriate to propose to consider the possibility of identifying the mechanisms of epigenetic programming for the development of metabolic disorders under the prenatal influence of alimentary deficiency factor.

Given the data obtained regarding the effect of alimentary deficiency of fats and carbohydrates on the morphological functional state of the liver of bi-monthly offspring, we consider it necessary to recommend, based on this study, to develop recommendations on the qualitative composition of the diet of pregnant and lactating women. In reducing the body weight of the child associated with alimentary deficiency in the prenatal period, particular attention should be paid to the development of practical recommendations and diet intake in order to restore body weight and accelerate development.

In our study, the main emphasis was placed on the effect of maternal nutrition on the structure and functional state of the offspring liver in the fetal system; we did not conduct a separate study of this factor (i.e. fat and carbohydrate deficiency)

on the offspring. A possible drawback of our study is that we determined the effect of the alimentary factor on two-month-old rats, i. e., before puberty, when the effects of our own hormones can both offset the detected metabolic disorders and worsen them.

Thus, based on all of the above, we can conclude that a nutritional deficiency diet leads to moderately pronounced structural and functional disorders of the body of double rats, which can be considered as a risk factor for the development of various organic liver pathology in the future.

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