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The Influence of Experimental Chronic Antenatal and Acute Postnatal Hypoxia on the Morphological State of the Liver Stromal Component

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Abstract

BACKGROUND: At present, the asymptomatic course of liver pathology in early childhood remains an urgent problem.

AIMS: The objective of the study was the detection of morphological features of the stromal component of the liver of rats, which were at different stages of postnatal ontogenesis and were exposed to chronic antenatal and acute postnatal hypoxia (APH).

MATERIALS AND METHODS: The study material was rat liver tissue of the control group, the APH group, and the chronic antenatal hypoxia (CAH) group.

RESULTS: From day 1 to day 35, CAH leads to an increase and growth of the stromal component in the liver of offsprings due to the development of sclerotic changes caused by activation of collagen formation with a predominance of mature collagen Type I over immature collagen Type III on day 1 and day 14 of the experiment and predominance of collagen Type III over collagen Type I on day 35 of the experiment, increasing the expression of fibronectin. Starting from day 14, APH leads to the development of sclerotic changes in this organ which increase to day 35, less pronounced compared to the detected sclerotic changes in CAH, and manifest in increased expression of fibronectin, activation of collagen formation with a predominance of collagen Type I over collagen Type III on days 14 and 35 of the experiment.

CONCLUSION: CAH from day 1 and APH from day 14 of postnatal life lead to the development of sclerotic changes in the liver of offsprings, which are more pronounced in cases of simulation of CAH and increase with age.

Introduction

The unsatisfactory health of children in Ukraine today is primarily caused by the increase in chronic pathology and disability as evidenced by epidemiological studies [1]. It should be noted that children's health is a fundamental basis for the formation of public health and labor potential of the country [2].

One of the significant problems of modern medicine is perinatal pathology, the mechanism of which is complex and often caused by disorders of fetoplacental blood circulation. According to modern ideas, the leading place among the causes of perinatal pathology is held by antenatal and intranatal hypoxia of the fetus which develops on the background of maternal pathology [3]. It is believed that a number of common diseases of the gastrointestinal tract in adults have their origins in childhood [4]. In the structure of gastroenterological pathology in children, there is a predominance of chronic inflammatory diseases, characterized by rejuvenation, , and significant number of cases are liver pathology [5].

Asymptomatic course of liver pathology in early childhood remains an urgent problem at the present stage [6]. The hepatobiliary system pathology in children is increasingly attracting the attention of pediatricians in connection with the prevalence and formation of severe chronic forms of the disease [7].

Our analysis of data from national and international resources did not reveal any information on the effect of hypoxia, as the most common damaging factor acting in the antenatal, intranatal, and postnatal periods [8], on the morphofunctional state of the liver of children, in particular its stromal component.

The aim of this study was to identify the morphological features of the stromal component of the rat's liver at different stages of postnatal ontogenesis, which were exposed to chronic antenatal and acute postnatal hypoxia (APH).

Materials and Methods of Research

The study used WAG and spontaneously hypertensive rats (SHR), which are kept in the

experimental biological clinic at Kharkiv National Medical University.

In this study, three groups were formed: Group C (control group), which included 33 WAG rats born from females with physiological pregnancies; APH group (APH simulation), which included 37 WAG rats born from females with physiological pregnancy, which on day 1 of postnatal ontogenesis were exposed to alpine hypoxia by placing them in a pressure chamber for 15 min in conditions characterized by rise at height of 3500 m and corresponded to a pressure of 493 mmHg; chronic antenatal hypoxia (CAH) group (CAH simulation) with 47 SHR, which developed in conditions of CAH due to arterial hypertension in their mothers. The rats of all the groups were removed from the experiment on days 1, 14, and 35 of postnatal ontogenesis.

The study material was the liver of experimental animals obtained during autopsies. In each case, two pieces of tissue were dissected from the liver, and then, the resulting material was fixed in 10% formalin solution. Tightening of the tissues fixed in formalin was reached by placing them in alcohol of increasing concentration, celloidin, chloroform, and pouring in paraffin. 4–5 μm serial sections were prepared from the prepared blocks for further staining. The obtained micropreparations stained with hematoxylin and eosin, picrofuchsin according to van Gizon, and according to Mallory were studied under microscope "Olympus BX-41." A morphometric study was also performed to determine the percentage rate of parenchymal, stromal, and vascular components, and then, the stromal-parenchymal coefficient was calculated [9].

Immunohistochemical study was performed on 5–6 μm thick paraffin sections following Coon's indirect method according to M. Brosman's procedure implementing monoclonal antibodies to collagen Types I and III, fibronectin (Novocastra Laboratories Ltd, UK). The preparations were studied in a fluorescent microscope "Axioskop 40." The optical density of immunofluorescence was determined by G.I. Gubina-Vakulyk co-authors' method [10].

Indicators in the groups were compared using student's t-test. The results were considered reliable at $p < 0.05$. Statistical processing of the study results was performed using Microsoft Excel 2016.

Results

Microscopically, the liver surface of C, APH, and CAH rats groups within all experimental terms was covered with a connective tissue capsule, the fibers of which when stained with picrofuchsin solution acc. van Gieson had different shades from light red to dark red, whereas when stained acc. Mallory the predominance of blue collagen fibers over elastic red fibers was observed in the connective tissue. The connective tissue fibers penetrated from the capsule into the liver and divided it into lobes, which are known to be the parenchyma of this organ.

The stroma of the liver, which was more distinctive in the portal tracts, was represented in van Gizon's picrofuchsin staining from light red to dark red connective tissue fibers, whereas Mallory's staining was dominated by blue collagen fibers over elastic red fibers. A small amount of polymorphic cell infiltration was detected between the connective tissue fibers. In the interlobular connective tissue in the area of the portal tracts were identified vessels of the portal vein, hepatic artery, and bile ducts, which formed the triads (Figure 1).

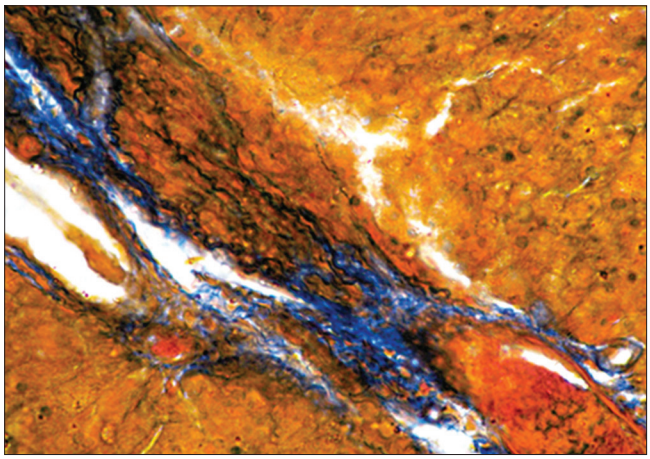

Figure 1: The liver of the chronic antenatal hypoxia group rat on day 35 of the experiment. The expressed collagenization of a portal tracts stroma. Mallory's staining ×200.

Stromal-parenchymal parameters of the liver of rats of groups C, APH, and CAH in all experimental terms had the following values (Table 1).

Table 1: Stromal-parenchymal correlation of the rat off springs' liver in the research groups (M ± m)

Group name	Subgroup name	Indications		
		Parenchymal volume, %	Stromal volume with vessels, %	Stromal-parenchymal coefficient
C	C_1	77.8 ± 6.3	22.2 ± 2.9	0.28 ± 0.01
	C_2	75.6 ± 5.8	24.4 ± 2.7	0.32 ± 0.01
	C_3	71.4 ± 5.9	28.6 ± 2.7	0.40 ± 0.01
APH	APH_1	77.7 ± 6.2"	22.3 ± 2.8"	0.28 ± 0.01"
	APH_2	68.6 ± 4.2	31.4 ± 2.6*"	0.45 ± 0.01*"
	APH_3	65.8 ± 4.3	34.2 ± 2.4*"	0.51 ± 0.01*"
CAH	CAH_1	64.4 ± 3.8*°	35.6 ± 2.1*°	0.55 ± 0.01*°
	CAH_2	62.1 ± 3.3*	37.9 ± 2.6*°	0.61 ± 0.01*°
	CAH_3	60.3 ± 3.8*	39.7 ± 2.9*°	0.65 ± 0.01*°

*p < 0.05 compared to a similar group C indication, °p < 0.05 compared to a similar group APH indication, "p < 0.05 compared to a similar group CAH indication. APH: Acute postnatal hypoxia, CAH: Chronic antenatal hypoxia.

During immunohistochemical study in the liver capsule, interparticle connective tissue, in perivascular and periductal spaces was detected; the luminescence of mature collagen Type I and young, immature collagen Type III, the ratio, and the luminescence intensity of which were different in C, APH, and CAH groups (Table 2).

Table 2: Optical luminescence density of collagen Type I та III of the rat offsprings' liver in the research groups (M ± m)

Group name	Subgroup name	Collagen	
		Type I (ODU)	Type III (ODU)
C	C_1	0.327 ± 0.09	0.413 ± 0.06
	C_2	0.396 ± 0.07	0.436 ± 0.07
	C_3	0.419 ± 0.08	0.448 ± 0.03
APH	APH_1	0.327 ± 0.04"	0.413 ± 0.04"
	APH_2	0.623 ± 0.03*"	0.562 ± 0.03*"
	APH_3	0.646 ± 0.02*"	0.576 ± 0.02*"
CAH	CAH_1	0.634 ± 0.04*°	0.555 ± 0.03*°
	CAH_2	0.724 ± 0.05*°	0.639 ± 0.03*°
	CAH_3	0.752 ± 0.02*°	0.785 ± 0.04*°

*p < 0.05 compared to a similar group C indication, °p < 0.05 compared to a similar group APH indication, "p < 0.05 compared to a similar group CAH indication. APH: Acute postnatal hypoxia, CAH: Chronic antenatal hypoxia.

The optical density of fibronectin luminescence in C, APH, and CAH groups on day 1 of the experiment was 0.443 ± 0.03 of conventional ODU, 0.446 ± 0.03 of conventional ODU and 0.597 ± 0.02 of conventional ODU, respectively. The optical density of fibronectin luminescence in C, APH, and CAH groups on day was 14 - 0.451 ± 0.02 of conventional ODU, 0.537 ± 0.02 of conventional ODU, and 0.649 ± 0.02 of conventional ODU, respectively. The optical density of fibronectin luminescence in C, APH, and CAH groups on day was 35 - 0.514 ± 0.04 of conventional ODU, 0.634 ± 0.02 of conventional ODU, and 0.737 ± 0.02 of conventional ODU, respectively. The analysis of the fibronectin optical luminescence density in all groups revealed a significant ($p < 0.05$) increase with rising experiment time which is caused by age-related changes in Group C, and in groups of APH (Figure 3), and especially CAH by both age and sclerotic changes.

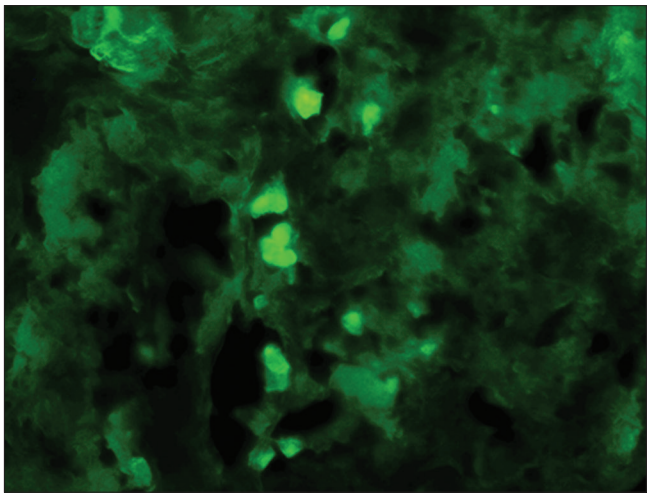

Figure 2: The liver of the chronic antenatal hypoxia group rat on day 35 of the experiment. Excessive expression of collagen Type III in the portal tract. Indirect Coon's method with MAB to Type III collagen ×400.

Discussion

It should be noted that in the APH group on days 14 and 35 of the experiment and in the CAH group at all stages of the experiment in Mallory's stained liver stroma were found fields of view (FoV) with determined

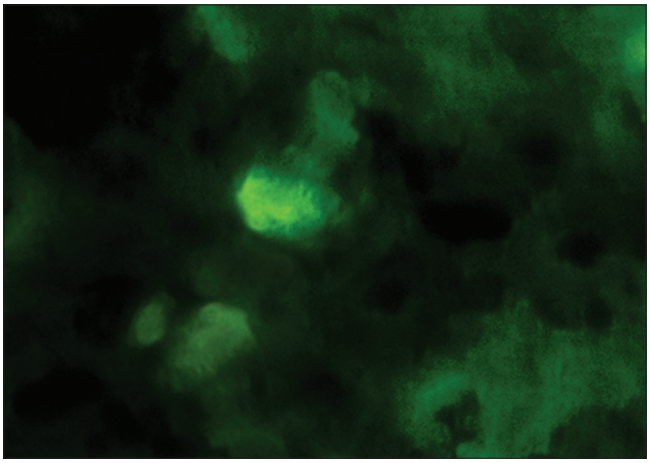

Figure 3: The liver of an acute postnatal hypoxia group rat on day 35 of the experiment. Excessive diffuse expression of fibronectin in the interstitium of the liver. Coon's indirect method with MAB to fibronectin ×600.

collagen fibers and no elastic fibers. Microscopically, hemodynamic changes were detected in the liver in the form of vascular plethora, the presence of diapedetic hemorrhages, and signs of edema, the severity of which on day 1 of the experiment was maximum in the CAH group, minimum in the APH group, and was decreasing with increasing experimental time.

With the increase of the experimental time in the liver stroma of rats of C, APH, and CAH groups, regular age thickening of bunches of connective tissue fibers and a decrease in distance between them were noted. Interestingly, in the APH group at days 14 and 35, and in the CAH group at days 1, 14, and 35, the above changes were more pronounced, increasing with rising experimental time, which, in our view, is due not only to age changes but also to the action of such a damaging factor as hypoxia, which is known to stimulate the activity of fibroblastic cells, resulting in an excessive amount of connective tissue fibers [11].

Found during the histological examination of microsamples, more pronounced sclerotic changes in the CAH group rat's liver compared to the AHP group were caused by the fact that the CAH group had a more aggressive damaging factor, represented by CAH characterized by long-lasting effect and determined by the development of chronic placental insufficiency. The latter has been described by many scientists in pregnant women diagnosed with hypertension [12]. In the APH group, the offspring's were affected by APH, which, in our view, is a kind of a stressor that leads to the activation of fibroblastic diferon cells, which on day 14 of the experiment is manifested by excessive production of fibrous connective tissue.

While analyzing the percentage ratio of parenchymal, stromal, and vascular components and stromal-parenchymal coefficient, the indicators of which are shown in Table 1, all the groups demonstrated a significant ($p < 0.05$) increase in the above-mentioned morphometric index with age. On days 14 and 35 of

the experiment, the stromal-parenchymal coefficient in the APH group compared to Group C was significantly ($p > 0.05$) different, and it was considerably ($p < 0.05$) higher in the CAH group at all stages of the experiment. The stromal-parenchymal coefficient of the liver in the CAH group notably ($p < 0.05$) exceeded the one in the AHP group at all terms of the experiment.

Thus, the obtained data of stromal-parenchymal coefficient and the results of histological examination in group CAH compared to Group C confirm the development of sclerotic changes in the liver. Similar changes were found by the morphological study of the liver of full-term stillbirths from mothers with Stage II hypertension [13]. Although the stromal-parenchymal coefficient of the liver did not differ significantly on day 1 of the experiment in group APH, compared with Group C, the results of microscopic examination also documented the development of sclerotic changes from day 14 of the experiment, which as mentioned above was manifested by more distinctive thickening of the bundles of connective tissue fibers and decreasing the distance between them, by the presence of FoV, where only collagen fibers were determined among the connective tissue fibers.

Analyzing the data in Table 2, it was found that on day 1 of the experiment in APH group compared with Group C indicators of the optical luminescence density of both collagens did not differ significantly ($p > 0.05$), and in group CAH compared to Group C were considerably higher ($p < 0.05$), which indicated the development of sclerotic changes. However, on day 14 and especially on day 35 of the experiment, the indicators of the optical luminescence density of collagen Types I and III in the groups APH and CAH were significantly higher ($p < 0.05$) than the corresponding indicators of Group C. In group APH, sclerotic changes on days 14 and 35 of the experiment were more pronounced compared to group APH (Figure 2).

Analyzing the processes of collagen formation on day 1 of the experiment in Group C, a significant ($p < 0.05$) predominance of Type III collagen over Type I collagen on the 1st, 14th, and 35th day of the experiment was revealed. In group APH, a significant ($p < 0.05$) predominance of Type III collagen over Type I collagen was detected on day 1 of the experiment, and a significant ($p < 0.05$) predominance of Type I collagen over Type III collagen was detected on days 14 and 35 of the experiment. In group CAH, Type I collagen significantly (<0.05) prevailed over Type III collagen on days 1 and 14 of the experiment. It is known that in the process of maturation of connective tissue, immature Type III collagen is first produced, which then turns into mature Type I collagen [14]. The revealed features of collagen formation in the liver at day 1 also indicate the development of sclerotic changes in group CAH. On day 35 of the experiment in the group of CAH, Type III collagen significantly ($p < 0.05$) dominated over Type I collagen.

With increasing experimental time, all groups demonstrated a significant ($p < 0.05$) increase in the optical luminescence density of collagen Types I and III, which in Group C is related to age changes, whereas in groups APH and CAH are caused by both age-related changes and excessive activation of collagen formation processes as a result of damaging factor.

One of the main adhesive glycoproteins of connective tissue is fibronectin, which provides cell fixation in the intercellular space via interaction with membrane receptors, binds to collagen fibrils, and enhances the processes of phagocytosis as a "molecular glue." The role of fibronectin in the development of liver cirrhosis has been proven [15].

On day 1 of the experiment, the optical luminescence density of fibronectin in Groups C and APH did not differ significantly ($p > 0.05$), and in the group CAH was significantly higher ($p < 0.05$) compared to Group C and group CAH. However, on days 14 and 35 of the experiment, the optical luminescence density of fibronectin was significantly ($p < 0.05$) higher in groups APH and CAH compared with Group C, and in group CAH, this figure was significantly ($p < 0.05$) higher compared to group CAH for both day 14 and day 35.

Our analysis of fibronectin expression also shows the development of sclerotic changes in the liver of rats in group APH from day 14, and in group CAH from day 1, the severity of which increases with age and reaches its maximum in modeling CAH compared with APH.

Conclusion

1. CAH caused by hypertension in mothers leads to an increase and growth of stromal-parenchymal coefficient of offspring's liver from day 1 to day 35. This is caused by the development of sclerotic changes, manifested by thickening of bundles of connective tissue, decreasing space between them, the presence of only collagen fibers among connective tissue fibers, the activation of collagen formation processes with predominance of mature collagen of Type I over immature collagen of Type III on day 1 and day 14 of the experiment and predominance of collagen of Type III over collagen of Type I on day 35 of the experiment, the increase of fibronectin expression.
2. APH on day 1 of the experiment does not affect the stromal-parenchymal coefficient of the offspring's liver, but from day 14, it leads to the development of sclerotic changes in this organ, which increase to day 35, less pronounced compared to sclerotic changes in CAH, and characterized by thickening of bundles of connective tissue with the following decreasing space between them,

the distinctive dominance collagen fibers over elastic areas with nearly total absence of elastic fibers, increase of fibronectin expression, the activation of collagen formation with Type I collagen prevalence over Type III on day 14 and day 35 of the experiment.

The relevance of the publication to scientific research works

The study was carried out within the frame of scientific research of the Department of Human Anatomy and Physiology at Kharkiv V.N. Karazin National University «The detection of maternal pathology influence on the development of fetus and newborn», stare registration № 0121U108326.

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