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MORPHOLOGICAL SIGNS OF THE HEPATIC FUNCTION DECOMPENSATION WITH EXPERIMENTAL COMPLETE OBSTRUCTION OF THE EXTRAHEPATIC BILE DUCTS

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The complete obstruction of the extrahepatic bile ducts (COEHBD) in the experiment is accompanied by changes in the liver, which can display its function decompensation, which causes the death of animals. The purpose of the project was to study the liver morphological changes in experimental COEHBD, depending on the obstruction duration and the mortality associated with it. The simulation of COEHBD was performed on 36 rats by ligation and transection of the choledoch. Animals were sacrificed on the 3rd, 7th, 14th, 21st, 28th and 35th days. Histological and morphometric liver studies were carried out. Pathological changes in the liver were progressive at the peak of lethality (7 out of 11 dead animals) within the last two weeks of the experiment and were characterized by: proliferation of the bile ducts, proliferation and hyperplasia of hepatocytes, the growing number of sinusoidal cells, fibrosis with a complete loss of the normal liver histoarchitectonics and replacing of its parenchyma with proliferating. bile ducts. The morphological signs preceding the liver function decompensation, which is accompanied by the mortality peak with COEHBD, are: the maximum value of the portal zones density index and the sinusoid- hepatocytes number, the nuclear-cytoplasmic hepatocytes ratio reduction after the previous maximum, which accordingly reflects the maximum proliferative activity of the bile ducts and the activity of sinusoidal cells against the background of the hepatocytes proliferative capacity reduction.

Key words: experimental cholestasis, complete obstruction of the bile ducts, liver morphology, morphometry.

The study is a fragment of the research project "Features of the tissue inflammatory response depending on the kind of etiology and the disease localization" (2016-2021), state registration No. 0117U000511.

Biliary obstruction is a frequent complication of both tumor and non-tumor diseases of extrahepatic bile ducts. Its main clinical manifestation is jaundice, which laboratory sign is the blood serum hyperbilirubinemia. The prolonged obstruction of the extrahepatic bile ducts inevitably leads to the liver failure development [2].

The most common method of biliary obstruction experimental modeling is the bile ducts ligation. To reproduce a complete and prolonged obstruction, ligation must be supported by the bile duct transection between the ligatures [1, 9]. In rats and other animals, ligation of the common bile duct without its transection is accompanied by recanalization and restoration of bile outflow [10].

Morphological changes in the liver with biliary obstruction are characterized by the development of alteration and acute inflammation, bile ducts proliferation, fibrosis and, ultimately, the liver cirrhosis development [5].

It should be assumed that under conditions of the complete extrahepatic bile ducts obstruction (COEHBD), within certain terms, histological changes in the liver will reflect the compensatory abilities breakdown of both the liver and the body as a whole, which, in its turn, leads to the death of animals.

The purpose of the work was to study the morphological changes in the liver with experimental COEHBD, depending on the obstruction duration and the associated mortality.

Materials and methods. In the experiment, male rats weighing 270-310 g, kept in the standard laboratory conditions, were used.

The operation was performed under antiseptic conditions, with general anesthesia. After laparotomy, the choledoch ligation and its transaction between the two ligatures were performed (36 animals) [11]. The total of 10 non-operated animals served as a control.

Animals were sacrificed on the 3rd, 7th, 14th, 21st, 28th and 35th days. The content of serum bilirubin was determined by the standard method. The liver was sampled and fixed in 10% formalin solution. Histologic specimens made by the standard method were stained with hematoxylin and eosin and according to the Van Gieson method. Morphometric analysis was performed. The software for morphometric studies was ImageJ.

Proliferation of the bile ducts was quantified by the bile ducts number (BDN) in the portal zones and the portal zones density index (PZDI) — the calculated ratio of the portal zones volume to the total tissue volume [6]. In order to assess the sinusoidal cells response, their number was assessed in a given field of view, which was taken to be 5.6×10^4 μm², and the sinusoid-hepatocytes number (SHN) was also recorded as the number of sinusoids per 1000 hepatocytes. For the quantitative assessment of hypertrophy, the

hepatocytes area (HA) and the nuclear-cytoplasmic ratio (NCR) were taken into account - the ratio of the hepatocytes nucleus and cytoplasm area. The degree of fibrosis was assessed according to Ishak, et al. [3].

The experiment was carried out in compliance with the requirements and provisions of the "European Convention for the Protection of Vertebrate Animals used for Experimental and Other Scientific Purposes", 1986.

Results and their discussion. Of the 36 animals with COEHBD, 11 (30.5%) rats died: within the terms of up to 7 days of the experiment - 2 animals; from the 8th to the 14th day of the experiment there were no deaths; from the 15th to the 21st day 2 rats died; from the 22nd to the 28th day - 5 animals; from the 29th to 35th day - 2 animals.

The total blood bilirubin level and micromorphometric indices of the liver are presented in table 1. The total bilirubin level in all periods of the experiment was significantly exceeded the norm, the maximum value of the index was recorded on the 3rd day. The histologic specimens study revealed that 3 days after the COEHBD, the blood vessels edema and congestion were observed with their leukocyte infiltration. In all cases, there were focal hepatocellular necroses. Simultaneously, proliferation of the bile ducts and hepatocytes was observed.

On the 7th day, the severity of acute inflammation signs - edema, vascular congestion and their leukocyte infiltration - was reduced. However, the number of sinusoidal cells was significantly increased compared to the three days lasting COEHBD (see table 1). Rare porto-portal septa occurred, mainly due to the newly formed bile ducts and the connective tissue scaffold of fine collagen fibers.

Table 1

Blood bilirubin level and micromorphometric parameters of the liver with COEHBD in various periods of the experiment

Experimental period	Control, n=10	3rd day, n=3	7th day, n=4	14th day, n=6	21st day, n=6	28th day, n=4	35th day, n=2
Total blood bilirubin, μmol/l	8±6	247*±20	185±10	180±7	122±16	102±17	124±16
PZDI, cm^3/cm^3	0.01 ±0.005	0.063 ±0.01*	0.07 6±0.02	0.15 ±0.04*	0.27 ±0.08*	0.34 ±0.07	0,39 ±0
BDN, abs.	1±0.1	3.6±0.2*	12.2±2,8*	>20	>20	>20	>20
Fibrosis, degree	0	1	1-2	4-5	3-4	3-4	4
SHN , units per 1000	374±92	506±58*	697±118*	820±106	1134±194*	953±135	888±135
HA, μm^2	180±50	177±24	160±48	164±22	166±27	206*±27	199±28
NCR ,μm^2/ μm^2	0.21±0.05	0.43±0.02	0.45±0.08	0.53±0.03	0.44±0.03	0.32±0.01	0.34±0.01

Note: *- a statistically significant difference ($p < 0.05$) between the indicator of a certain experimental time period and the same indicator of the previous time period.

On the 14th day of COEHBD, the cholangiocytes proliferation continued to grow with signs of fibrosis and the liver histoarchitecture reorganization was observed. Newly formed bile ducts, as a rule, did not form lumens; within the cellular composition of the extended portal zones were cholangiocytes and their predecessors, oval cells. Formation of multiple porto-portal septa due to cholangiocytes proliferation and fibrosis was observed. The changes spread beyond the portal zones and septa into the parenchyma of the lobules, forming roughness of their contours, separation of hepatocytes, formation of false lobules. There were focal hepatocellular necroses. The continuation of the tendency to the increased number of sinusoidal cells was recorded.

On the 21st day of the experiment, cholangiocytes proliferation processes are growing, the correct lobular structure of the liver is completely lost, and the preserved parenchyma is represented by the islets of false lobes, or by groups of hepatocytes that have lost their beam structure and are surrounded by newly formed cholangiocytes with various degrees of differentiation - from oval cells to mature ones. Newly formed cholangiocytes and bile ducts, as a rule, are accompanied with weak fibrosis in the form of a tender network of thin collagen fibers. However, Van Gieson staining showed a distinct presence of connective tissue fibers only in the areas of the preexisting septa and their absence in the liver parenchyma replacement areas with newly formed cholangiocytes.

At the end of 28 days there is a further increase in the cholangiocytes and oval liver cells proliferation, which occupy an even larger area of the section (table 1).Frequently, hepatocytes are arranged in groups separated from each other, without forming false lobules. Fibrosis is a delicate network of collagen fibers in septa without penetrating into the periphery, into the zones of cholangiocytes proliferation. Rare focal hepatocellular necroses are observed.

On the 35th day, the proliferation of cholangiocytes and oval cells is extremely pronounced (fig. 1) and the microscopic picture is similar to that observed with the 28-days COEHBD. Considering the morphometric parameters, a slight increase in PZDI, the absolute number of sinusoidal cells and the SHN can be observed (table 1).

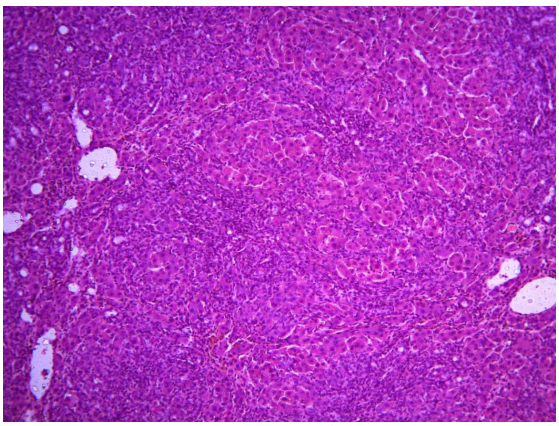

Fig. 1. Liver on the 35th day of the experiment. Disorders of the liver histoarchitecture, significant replacement of functionally active parenchyma with cholangiocytes and oval cells can be seen. Hepatocytes are located in separated groups.

The complete obstruction of the extrahepatic bile ducts (COEHBD) is accompanied by significant mortality: 11 (30.5%) out of 36 animals died. During the first two weeks of the experiment, 2 rats died on the 2nd and the 3rd days. Mortality in such an early period may be due to the acute pathology, or a consequence of technical errors in the operation. During the 3rd week of the experiment, the lethality was not significant - 2 rats died. The peak of mortality falls on the last 2 weeks of the experiment - from 22 to 35 days - 7 animals died. It should be noted that out of 8 rats planned to be sacrificed on the 35th day, only two had remained by this date. On a provisional basis, this permits to conclude that the mortality rate for COEHBD to the 35th day of the experiment amounts to 75% (6 out of 8 animals).

At all stages of the experiment, the animals had obvious macroscopic signs of cholestasis, the bilirubin level was also high without significant dynamics, which indicates the irreversibility of cholestasis, i.e., the absence of the common bile duct's recanalization. As in other studies [5], these data confirm the reliability of the model with ligation and transection of the common bile duct in the simulation of complete and prolonged obstruction of the extrahepatic bile ducts. The model with choledoch ligation without its transection, frequently used in research, is inadequate to display the processes occurring in the liver with COEHBD, because already on the 7th-12th days, recanalization of the common bile duct and restoration of bile outflow occurs [1, 4, 12], which is accompanied by a significant blood bilirubin content reduction after 14 days of the experiment [4, 12].

The revealed histopathological changes in the liver with COEHBD correspond to the results of other studies [2, 8] and are characterized by the signs of acute inflammation and alteration, most pronounced at the beginning of the experiment, progressive bile ducts proliferation, with replacement of functionally active parenchyma, fibrosis, and, ultimately, gross violation of the liver histoarchitecture.

These processes are accompanied by the response of sinusoidal cells, which include Kupffer cells, Ito cells, endothelial cells and others that are involved into the inflammatory response development in the liver, collagen synthesis, collagenase-induced reactions, have a growth stimulating effect on hepatocytes [8]. The reflection of these effects is the observed increase in the SHN with the maximum value on the 21st day of the experiment and the subsequent slight decrease in this index.

One of the main histopathological manifestations of the obstructive cholestasis is the bile ducts proliferation; micromorphometric indices reflecting this process are the BDN and PZDI. Throughout the whole experiment, their growth has been observed (table 1). The maximum growth rate of PZDI, in comparison with the previous terms, was fixed on the 3rd (compared to the control, $p < 0.001$) and on the 21st day (compared to 14 days, $p < 0.01$).

Under these conditions, a progressive replacement of the hepatocytes volume with proliferating bile ducts occurs, fibrosis develops and a complete loss of the normal histostructure results in the false lobules formed, and on the 28th and 35th days in the arrangement of hepatocytes into separate groups, even without the formation of false lobules. At the same time, micromorphometric indices of HA and NCR reflect compensatory-adaptive processes of proliferation and hyperplasia of hepatocytes [7].

A sign of the hepatocytes proliferation activation is a growth of the NCR index, accompanied by a HA reduction, which is observed up to 14 days, not reaching a statistical value in comparison with the previous terms. Such changes are a consequence of the increased functional load on hepatocytes. On the 21st day, a reliable decrease in the area of their nuclei and NCR (compared to the 14th day, $p < 0.05$) is observed, which is displayed by a reduced proliferation of hepatocytes (table 1). The increased HA observed on the 28th day is a sign of the hepatocytes hyperplasia, which further, along with the continued displacement of functionally active parenchyma, reduction of HA and NCR, indicates the exhaustion of compensatory-adaptive processes in the liver cells on the 35th day.

Thus, in the course of the experiment, against the background of a gradual increasing changes in the morphological picture, there are two peaks of reliable morphological differences compared to the nearest previous period – on the 3rd and the 21st days.

Changes on the 3rd day display the beginning of the pathological process and are reliably different from the norm.

The second peak, on the 21st day, was characterized by a reliable difference in the main morphometric parameters of the liver: PZDI, SHN, NCR in comparison with the 14th day. The twenty first day is the peak of the PZDI growth, which indicates the greatest proliferative activity of the bile ducts, after which this figure continues growing, but not so significantly. Also at this period, the highest value of the SHN is recorded, an index that indicates the maximum intensity of both the response and the sinusoidal cells' stimulating effects under the pathological process conditions.

After a previous growth, from the 21st day there occurs the hepatocyte NCR reduction, which indicates inhibition of the hepatocytes proliferative activity. All this indicates the breakdown of the organ's compensatory capabilities and the liver functioning decompensation, which coincides with the significant lethality of animals after the 21st day - on the 4th and the 5th week of the experiment.

The performed study of the liver morphological changes, must apparently be compared to the functional changes, which requires further studies.

Conclusions

1. Experimental complete obstruction of the extrahepatic bile ducts lasting for 35 days is accompanied by significant mortality (75%), with its peak within the last two weeks of the experiment, from the 22nd to the 35th day, 7 out of the 11 dead animals.

2. Experimental complete obstruction of the extrahepatic bile ducts is accompanied by the pronounced morphological changes of the liver: bile ducts proliferation, proliferation and hyperplasia of hepatocytes, increase of the sinusoidal cells number, fibrosis, with complete loss of the normal liver parenchyma histoarchitecture and its replacement by proliferating bile ducts.

3. The morphological signs preceding the liver function decompensation, which is accompanied by a peak of mortality, are the maximum value of PZDI and SHN and the NCR reduction after the previous maximum, which respectively reflects the maximum proliferative activity of the bile ducts and the activity of the sinusoidal cells against the background of the reduced proliferative capacity of hepatocytes.

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Реферати

МОРФОЛОГІЧНІ ОЗНАКИ ДЕКОМПЕНСАЦІЇ ПЕЧІНКОВОЇ ФУНКЦІЇ ПРИ ЕКСПЕРИМЕНТАЛЬНІЙ ПОВНІЙ ОБСТРУКЦІЇ ПОЗАПЕЧІНКОВИХ ЖОВЧНИХ ШЛЯХІВ

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Повна обструкція позапечінкових жовчних шляхів (ПОПЖШ) в експерименті супроводжується змінами печінки, які можуть відображати декомпенсацію її функції, що призводить до загибелі тварин. Мета дослідження: вивчення морфологічних змін печінки при експериментальній ПОПЖШ в залежності від тривалості обструкції і пов'язаної з нею летальності.

МОРФОЛОГИЧЕСКИЕ ПРИЗНАКИ ДЕКОМПЕНСАЦИИ ФУНКЦИИ ПЕЧЕНИ ПРИ ЭКСПЕРИМЕНТАЛЬНОЙ ПОЛНОЙ ОБСТРУКЦИИ ВНЕПЕЧЕНОЧНЫХ ЖЕЛЧНЫХ ПУТЕЙ

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Полная обструкция внепеченочных желчных путей (ПОВЖШ) в эксперименте сопровождается изменениями печени, которые могут отображать декомпенсацию ее функции, что приводит к гибели животных. Цель исследования: изучение морфологических изменений печени при экспериментальной ПОВЖШ в зависимости от продолжительности обструкции и связанной с ней

Моделювання ПОПЖШ проводилося на 36 щурах шляхом перев'язки і перетину загальної жовчної протоки. Тварин виводили з експерименту на 3, 7, 14, 21, 28 і 35 добу. Проводили гістологічне та морфометричне дослідження печінки. Патологічні зміни печінки носили наростаючий характер з піком летальності (7 з 11 загиблих тварин) на останні два тижні експерименту і характеризувалися: проліферацією жовчних проток, проліферацією і гіперплазією гепатоцитів, збільшенням кількості синусоїдальних клітин, фіброзуванням з повною втратою нормальної гістоархітектоніки печінки і заміщенням її паренхіми проліферуючими жовчними протоками. Морфологічними ознаками, які передують декомпенсації функції печінки, що супроводжується піком летальності при ПОПЖШ, є максимальні значення об'ємної щільності портальних зон і синусоїдно-гепатацитарного числа, зниження після попереднього максимуму ядерно-цитоплазматичного відношення гепатоцитів, що відповідно відображає максимальну проліферативну активність жовчних протоків і активність синусоїдальних клітин на тлі зниження проліферативної здатності гепатоцитів.

Ключові слова: експериментальний холестаз, повна обструкція жовчних шляхів, морфологія печінки, морфометрія.

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Моделирование ПОВЖШ проводилось на 36 крысах путем перевязки и пересечения общего желчного протока. Животных выводили из эксперимента на 3, 7, 14, 21, 28 и 35 сутки. Проводили гистологическое и морфометрическое исследования печени. Патологические изменения печени носили нарастающий характер с пиком летальности (7 из 11 погибших животных) последние две недели эксперимента и характеризовались: пролиферацией желчных протоков, пролиферацией и гиперплазией гепатоцитов, увеличением количества синусоидальных клеток, фиброзированием с полной потерей нормальной гистоархитектоники печени и замещением ее паренхимы пролиферирующими желчными протоками. Морфологическими признаками, которые предшествуют декомпенсации функции печени, сопровождающейся пиком летальности при ПОВЖШ, являются максимальные значения объемной плотности портальных зон и синусоидально-гепатацитарного числа, снижение после предыдущего максимума ядерно-цитоплазматического отношения гепатоцитов, соответственно отражает максимальную пролиферативную активность желчных протоков и активность синусоидальных клеток на фоне снижения пролиферативной способности гепатоцитов.

Ключевые слова: экспериментальный холестаз, полная обструкция желчных путей, морфология печени, морфометрия.

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ВПЛИВ НИЗЬКИХ ДОЗ КАДМІЮ ЦИТРАТУ НА КАРДІОГЕНЕЗ ЕМБРІОНІВ ЩУРІВ

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Кадмій - це один з небагатьох токсичних важких металів, фізіологічна функція якого в організмі людини на теперішній не встановлена. Cd проявляє свій токсичний вплив при дуже низьких концентраціях і має гострий і хронічний вплив на стан здоров'я. Метою дослідження було експериментальне визначення впливу низьких доз кадмію цитрату на морфометричні показники серця ембріона, передсердно-шлуночкові клапани та коронарні судини при внутрішньошлунковому введенні вагітним самицям щура. Доза цитрату кадмію складала 1,0 мг/кг маси тіла. Експериментально визначено, що вплив цитрату кадмію в зазначеній дозі призводить до зменшення обсягу ендокардіальних подушок передсердно-шлуночкового каналу та зниження кардіофетального індексу у ембріонів щура. В той же час спостерігається збільшення діаметру вінцевих артерій на 11,3%.

Ключові слова: кардіогенез, ембріон щура, ендокардіальні подушки серця, цитрат кадмію.

Робота є фрагментом НДР «Морфофункціональний стан органів і тканин експериментальних тварин та людини в онтогенезі в нормі та під впливом зовнішніх і внутрішніх чинників», № державної реєстрації 0117U003181.

Не зважаючи на комплексний підхід у вирішенні питань забруднення навколишнього середовища, зазначена проблема на теперішній момент залишається актуальною. Серед полютантів біосфери, що представляють найбільшу зацікавленість для різних установ контролю її якості, метали (особливо важкі, тобто такі, що мають атомну вагу більше 40) відносяться до групи найважливіших. Більш за все це пов'язано з біологічною активністю та властивостями багатьох з них. Вплив металів на організм людини і тварин, їх фізіологічна дія є різними і залежить від виду металу, типу з'єднання, в якому він знаходиться в оточуючому середовищі, а також його концентрації. Серед важких металів одні є вкрай необхідними для життя людини і інших живих організмів і належать до так званих біогенних елементів. Інші мають протилежну дію і, при надходженні до живого організму, викликають його отруєння або загибель. Ці метали відносять до класу ксенобіотиків, тобто чужих для живих організмів. Спеціалістами з захисту навколишнього середовища серед металів-токсикантів виділена пріоритетна група. До неї належать кадмій, мідь,