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Histological Liver Changes in Experimental Obstructive Cholestasis with Partial Outflow Restoration

Cambios Histológicos Hepáticos en la Colestasis Obstructiva Experimental con Restauración Parcial del Flujo de Salida

Ivan Mamontov[1*], Tamara Tamm[2], Ihor Ivakhno[3], Viacheslav Panasenko[4], Volodymyr Padalko[5], Valentyn Nepomniashchyi[6], Alina Yaroshenko[7]

SUMMARY

Objective: *This work aimed to establish histological changes in the structure and activity of the liver caused by experimental obstructive cholestasis.* **Method:** *Several methodological approaches were used such as experimental, biochemical, and comparison were applied.* **Results:** *It was possible to describe the essence of such a disease as cholestasis and its impact on the liver. At the same time, it was proved that recanalization with incomplete recovery of bile outflow was possible due to the normalization of the total bilirubin (TB) level. Morphological changes in the liver were most pronounced during acute cholangitis. The study examined what processes occur in the body during the recovery of bile flow, including after the complete blockage of such organs as the liver and bile ducts. Consequently, signs that expressed the abnormality of the liver structure of animals were revealed. At each of the stages of the experiment, specific indicators that determined the condition of the studied internal organs of rats according to the levels that correspond or do not correspond to the norm were established.* **Conclusion:** *The obtained conclusions are of high value, as they are an important basis for continuing the research on this topic and can also be used in the practical activities of medical workers.*

Keywords*: Complete obstruction, TB level, bile ducts, recanalization, fibrosis.*

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ORCID: 0000-0003-0059-2715[1]
ORCID:0000-0001-6372-2092[2]
ORCID:0000-0002-5229-0068[3]
ORCID:0000-0002-2803-799[4]
ORCID:0000-0002-2690-8995[5]
ORCID:0000-0001-6262-6795[6]
ORCID:0000-0001-5058-1251[7]

[1]Department of Surgery No. 6, Kharkiv National Medical University, Ukraine, Kharkiv 61022. E-mail: mamontov_iv@ukr.net
[2]Department of Surgery No. 6, Kharkiv National Medical University, Ukraine, Kharkiv 61022. E-mail: tamm.t99@yahoo.com
[3]Department of Pathological Anatomy and Forensic Medical Examination, Kharkiv National Medical University, Ukraine, Kharkiv 61022. E-mail: ihor.ivakhno@ukr.net

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RESUMEN

Objetivo: *El trabajo tuvo como objetivo establecer los cambios histológicos en la estructura y actividad del hígado provocados por la colestasis obstructiva*

[4]Department of Histology, Cytology and Embryology, Kharkiv National Medical University, Ukraine, Kharkiv 61022. E-mail: vchslvpanasenko@gmail.com
[5]Department of General and Clinical Pathology, V.N. Karazin Kharkiv National University, Ukraine, Kharkiv 61022. E-mail: padalkovolodymyr1@gmail.com
[6]Department of Surgery No. 6, Kharkiv National Medical University, Ukraine, Kharkiv 61022. E-mail: val.nepomniashchyi@ukr.net
[7]Department of Therapy No. 2, Kharkiv National Medical University, Ukraine, Kharkiv 61022. E-mail: al.yar@ukr.net
*Corresponding author: Ivan Mamontov
E-mail: mamontov_iv@ukr.net

experimental. **Método:** *Se utilizaron varios enfoques metodológicos, entre ellos el experimental, el bioquímico y el de comparación*. **Resultados:** *Fue posible describir la esencia de una enfermedad como la colestasis y su impacto en el hígado. Al mismo tiempo, se demostró que la recanalización con recuperación incompleta del flujo de bilis era posible debido a la normalización del nivel de bilirrubina total (BT). Los cambios morfológicos en el hígado fueron más pronunciados durante el curso de la colangitis aguda. El estudio examinó qué procesos ocurren en el cuerpo durante la recuperación del flujo biliar, incluso después del bloqueo completo de órganos como el hígado y los conductos biliares. En consecuencia, se revelaron signos que expresaban la anormalidad de la estructura del hígado de los animales. En cada una de las etapas del experimento se establecieron indicadores específicos que determinaron el estado de los órganos internos de las ratas estudiados según los niveles que corresponden o no a la norma*. **Conclusión:** *Las conclusiones obtenidas son de alto valor, ya que son una base importante para continuar la investigación de este tema, y también pueden ser utilizadas en las actividades prácticas de los trabajadores médicos*.

Palabras clave: *Obstrucción completa, nivel de TB, vías biliares, recanalización, fibrosis*.

INTRODUCTION

Obstruction of the extrahepatic biliary tract is a common complication of benign and malignant diseases of the extrahepatic biliary tract and adjacent organs (1). As a result of the development of obstruction, a number of changes occur in the body, which include the formation of biliary hyperplasia, the appearance of fibrosis, or liver failure. Considerable attention has been paid to this question in scientific doctrine, in particular, it has been actively studied by various specialists. Despite this, in connection with the deterioration of the external conditions of the development and vital activities of society, the question of the effect on the liver of the incomplete recovery of the outflow of bile, which is formed to a greater extent by the natural course of the disease or is achieved by therapeutic procedures, has become especially relevant (2-5). Obstructive cholestasis resulting from benign or malignant disease frequently complicates clinical outcomes through adverse hepatic changes. Partial bile flow restoration, often occurring naturally or following interventions, impacts subsequent liver pathology in unclear ways. Elucidating these histological alterations is critically important given the high disease burden, yet previous studies have not comprehensively examined the effects of incomplete outflow recovery. Additional research is urgently needed to fully characterize the cellular and tissue changes associated with partial bile drainage restoration after obstruction.

Based on this, the work aimed to study the histological changes of the liver during its experimental obstruction after partial restoration of outflow. For this purpose, it was experimentally induced cholestasis in male rats, establishing indicators of liver pathology, studying the features of reparation, as well as cellular reactions to interference in their activity, describing the process of development of fibrosis and cirrhosis of the liver and establishing the possible complications of cholestasis.

This experimental model of obstructive cholestasis makes it possible to study in depth the pathomorphosis of the liver, features of reparation, cellular reaction, the development of fibrosis and cirrhosis of the liver, and the types of abnormalities that can develop on its basis (6-9). All these indicators play an important role, not only in the process of the liver, as an internal organ, but also in the entire structure of the body. The most common method of experimental modeling of complete and long-term obstruction of the extrahepatic bile ducts is the ligation of the bile duct with its transection (10-13).

At the same time, the impossibility of patency is also characteristic of a ligated bile duct, especially in the initial stages. However, recanalization occurs later with the restoration of bile outflow (14). Special attention should be paid to this aspect in the context of a possible study of the restoration of bile outflow in case of complete obstruction and the effect of this restoration on histological changes of the liver and common bile duct (CBD).

In this regard, Lazcanoiturburu et al. (15) studied the time frame for liver recovery based on the simulation of cholestasis. The researcher also created experimental conditions, because of which they concluded that after two days the regenerative mechanisms of liver tissue were activated. Streltov et al. (16), and Mejidov et al. (17) demonstrated the influence of biliary

tract decompression on the dynamics of lipoperoxidation processes. It was established that on the 7th day after the experiment, lipid peroxidation was at the highest level in terms of pathogenesis and negative impact on the liver, taking into account cholestasis. In turn, Dzyubanovskyi and Gudyma (18), and Hrabchak and Bedeniuk (19) assessed the effect of bile duct decompression on the rate of bile formation in the body after experimental cholestasis. They established that such dynamics are different, depending on the animal whose liver is being studied. Average – two-week indicators, which are characterized by decompression of the common bile duct. Based on the results it was possible to characterize the methodology of conducting experimental cholestasis, as well as to reveal its influence on changes in the liver structure and outflow restoration processes. However, there are some limitations in the studies regarding the process of incomplete recovery of bile outflow after cholestasis, since they may not have addressed this aspect in detail or considered the important variations in histological changes that may result from incomplete restoration of outflow. Also, previous studies did not consider adequately the mechanisms of cellular response and tissue remodeling in the context of partial restoration of bile outflow. Thus, further research is required to obtain a complete and accurate picture of the histological changes occurring in the liver with incomplete recovery of bile outflow. Such research can be of great importance for improving the understanding of the pathological processes that occur in the liver during obstructive cholestasis. This may have practical application in clinical practice to improve the results of treatment of patients with similar diseases.

This research has a significant value as it delves into a lesser-explored aspect of liver health; with obstructive cholestasis being a common complication of various diseases, the study's focus on the effects of incomplete bile outflow restoration on liver histology is particularly relevant. By comprehensively investigating cellular reactions, fibrosis development, and potential complications, the research enhances our understanding of the complexities surrounding liver pathophysiology. This contribution carries the potential to refine treatment approaches, advance the field's knowledge, and ultimately improve patient outcomes, making it a valuable resource for both researchers and clinicians worldwide.

MATERIALS AND METHODS

The research was organized and conducted based on the Institute of Biology of Karazin Kharkiv National University. In the experiment were used 96 mature male rats, with a body weight of 270-310 g, kept periods of 12 hours of light and dark, with food and tap water *ad libitum*. Two models of cholestasis were used. Surgical procedures were performed in antiseptics conditions; a midline laparotomy was performed under general anesthesia. Complete common bile duct obstruction (CCBDO) was performed by ligation and transection between two sutures (41 animals) (3,13). 39 animals underwent the ligation of the common bile duct without its transaction, which was subsequently accompanied by recanalization and partial bile outflow restoration (PBOR) (11). 10 non-operated and 6 sham-operated animals served as the control.

The animals were removed from the experiment on the 1st, 3rd, 7th, 14th, 21st, 28th, and 35th days of the postoperative (PO) period, the condition of the abdominal organs was assessed, and the diameter of the CBD above the ligature was measured using a caliper and a ruler. The criterion for restoring the passage of bile was its presence in the lumen of the duodenum. The total bilirubin level (TB) in blood serum was determined by a standard biochemical method. Liver samples were stored in a special solution containing formalin (10 %). The Van Gieson method was used for staining histological preparations with hematoxylin and eosin. The degree of fibrosis was assessed (20). Pathological and biochemical examination of the animals that died but were not removed from the experiment was not carried out. The obtained indicators and other numerical data were expressed as mean standard deviation (SD). Student's t-test was used to determine changes between two groups of variables. In the course of working with categorical data, the χ^2 criterion was significant. $P<0.05$.

Normalization of the level of bilirubin while preserving the signs of cholestasis in the liver can be caused by several mechanisms related to the regulation of bile secretion, transport of bile, and processing of bilirubin. Some possible mechanisms are Dilation of the bile ducts; Stimulation of bile secretion; Regulation of bile transport; Reduction of inflammatory processes; Support of liver function and increasing the removal of bilirubin from the blood. These mechanisms can act separately or together, depending on the specific conditions of the disease and the approach to treatment.

The method of comparison in the study involved the comparison of normal indicators with those obtained during the experiment. This tool made it possible to process data that characterized the effect of cholestasis on the liver. In addition, the method of comparison involved the study of such histological changes in different periods, depending on the number of days from which the experiment had passed. It made it possible to compare different states of organisms before and after the formation of experimental conditions.

The work was carried out by the conditions and rules defined in the "European Convention for the Protection of Vertebrate Animals Used for Experimental and Other Scientific Purposes" (1986). The study was approved by the Bioethics Commission of the Kharkiv Medical Academy of Postgraduate Education, No. 3266.

RESULTS

12 of 41 animals (29.3%) with CCBDO died during the experiment: up to PO day 7; 3 animals; between the PO days 15 and 21: 2 rats; between the PO day 22 and 28: 5 rats; between the PO day 29 and the PO day 35: 2 animals. Throughout the experiment, the skin, visible mucous membranes, and internal organs at autopsy had icteric discoloration. Moreover, the liver of the animals was enlarged, and the common bile duct above the site of ligation and transection was dilated and contained transparent bile of varying color intensity. The TB blood level significantly exceeded the control group indices, especially on PO day 1 (Figures 1 and 2).

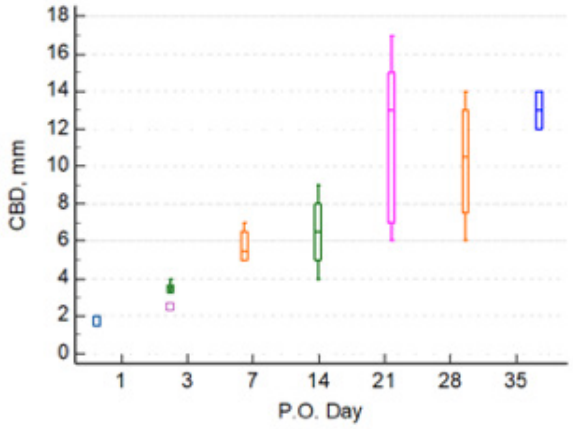

Figure 1. Common bile duct dilatation in CCBDO.

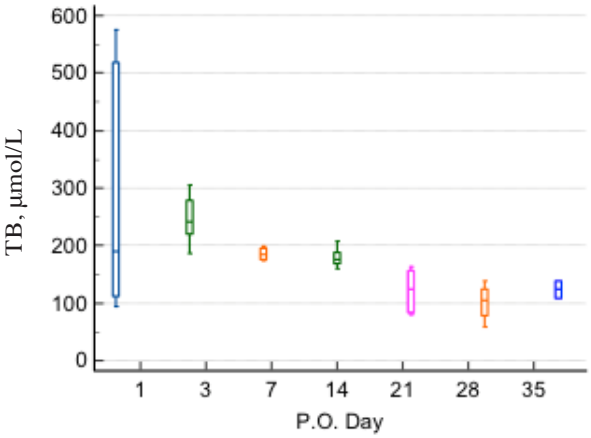

Figure 2. Total bilirubin level in CCBDO. Total bilirubin was expressed in µmol/L

Histological changes of the liver on the PO day 1 are characterized by edema and vascular hyperemia. In addition, they were infiltrated by leukocytes, which provoked an increase in the size and width of the bile ducts. There was no fibrosis. On PO day 3, signs of an acutely developed pathological process remained – edema and hyperemia of vessels with their infiltration by leukocytes. Indicators that show a low level of focal hepatocellular necrosis were obtained. Along with this, there was a proliferation of the bile ducts, moderate infiltration of portal tracts with mononuclear cells, congestion, and vessel

leukocytosis. In one case, fibrosis was absent, in the other 2 cases fibrosis corresponded to grade 1 around the newly formed bile ducts. On PO day 7, the properties of inflammation were less pronounced, including edema, leukocyte infiltration, and hyperemia. Port-portal septa consisting of collagen fibers were established. In addition, such partitions contain new bile ducts. In two cases, the fibrosis corresponded to grade 1, and in another two – to grade 2. On PO day 14, during the expansion of these zones with the phenomena of ductal proliferation, the histoarchitectonics of the liver were rearranged, and functionally active liver parenchyma was observed to shift. In 4 cases, the fibrosis had the grade 4, and 2 animals – grade 5.

On PO day 21, the proliferation processes of cholangiocytes increased. This was expressed in the destruction of the liver lobular structure. As a result, the parenchyma was expressed in the form of islands, which include groups of hepatocytes. The latter lacks a bundle histostructure, because of which they are surrounded by new cholangiocytes. They were characterized by weak fibrosis, which was expressed in the form of a net connected with collagen fibers. Based on the Van-Gieson method for studying connective tissue and fibrous changes in organs in various diseases, it was possible to detect connective tissue fibers on the plane of the partitions. Where these components were distant, the formation of cholangiocytomas of the liver parenchyma occurred. In different cases, the grade of liver fibrosis differed. It was grade 3 three times, grade 4 twice, and only once grade 5 in 1. On PO day 28, mainly due to the progressive proliferation of the bile ducts, hepatocytes, generally, were located in groups and apart from each other, without forming lobules. In all cases, fibrosis corresponded to grade 4. Extreme indicators of the proliferation of salted components were established on PO day 35. Liver fibrosis was grade 4. At the same time, hepatocytes were placed differently, both together (in groups) and singly (without forming lobules).

In the experiment PBOR, 8 animals (20.5%) died: up to the PO day 7 day: 5 animals; no death between the PO day 8 and 15; between the PO day 15 and 21 days: 1 animal; between the PO day 22 and 28:1 rat; between the PO day 29 and 35: 1 animal. During the first 14 days of the experiment, all the animals had icteric skin, visible mucous membranes, and internal organs at autopsy. Rat livers were enlarged, CBD above the ligation site was dilated, and it contained clear bile of varying color intensity. However, on PO day 21, despite the CBD dilatation above the ligature (4.5±2 mm), there were no macroscopic signs of obstructive icterus in 2 of 7 cases. In these cases, the TB level was 5.5±1.5 μmol/L and did not significantly differ from the TB level in the control group ($p>0.05$). TB normal level (3.1±1.5 μmol/L) was also observed in 4 of 5 animals on PO day 35. These animals also had dilated CBD (4.1±0.8 mm) (Figure 3).

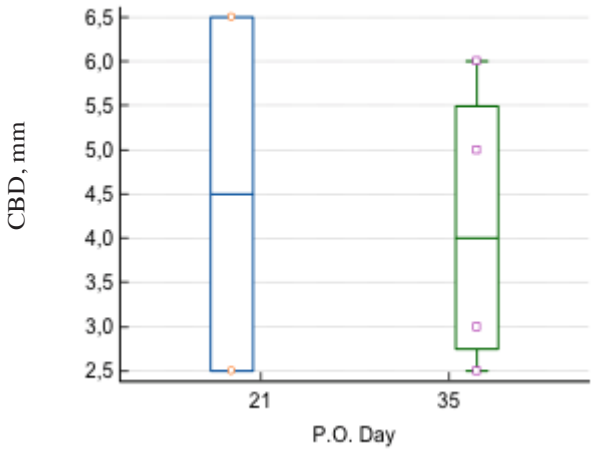

Figure 3. CBD dilatation in PBOR and normal TB level

On the PO days, 7 and 14 animals had bile in the duodenum lumen in 1 of 4, and 2 of 4 cases, respectively. On PO day 21, bile in the duodenum was observed in 4 of 7 animals; on PO day 28 in 2 out of 2 rats; on PO day 35 – in 4 of 5 animals. According to the presence of a normal TB level in several animals on the PO days 21 and 35, it is reasonable to consider the results of the experiment depending on the TB level, which is an important marker of cholestasis. Figures 4 and 5 show the distribution of animals with hyperbilirubinemia depending on the experiment duration, the TB level, and the CBD diameter above the ligature.

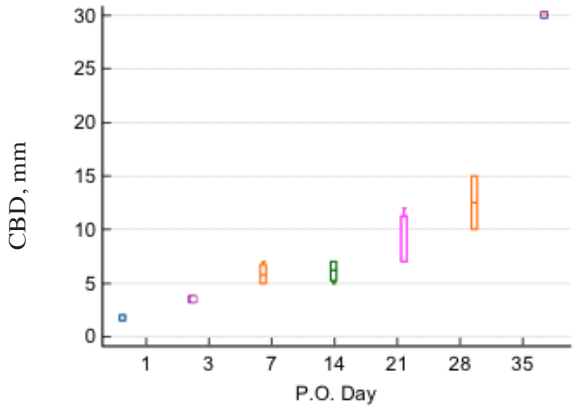

Figure 4. Common bile duct dilatation in PBOR with hyperbilirubinemia.

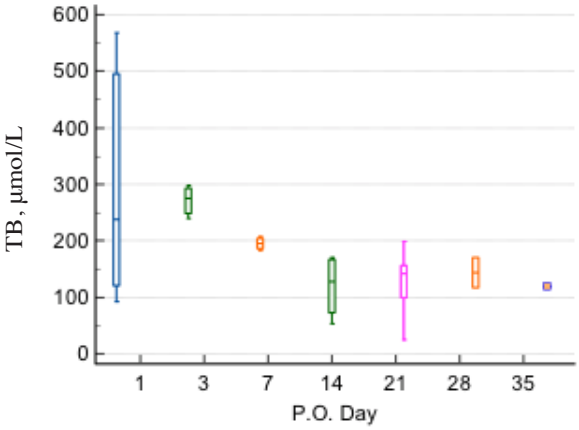

Figure 5. Total bilirubin level in PBOR with hyperbilirubinemia

In the case of ligating the CBD without its transaction on the PO days 1-14, the liver histological picture fully corresponded to CCBDO. This also applies to cases in which bile was found in the duodenum lumen. Liver fibrosis was absent on day 1 in all cases. On PO day 3, it occurred in 2 out of 3 cases and corresponded to grade 1. On PO day 7 all 4 cases had fibrosis of grade 2, and on PO day 14: 3 out of 4 cases had grade 4, and one grade 5. Differences in the liver histostructure with PBOR in comparison with CCBDO were found on PO day 21 and they are associated with the TB level. With an increased level of TB, on PO days 21 and 28, the histological changes in the liver having CCBDO and PBOR were the same and did not depend on the presence of bile in the duodenum. On PO day 21, 4 of 5 cases had liver fibrosis of grades 4, and 1 case grade 5. On PO day 28, only one animal had grade 4 fibrosis. On PO day 35, one animal with PBOR and hyperbilirubinemia in the liver histostructure showed pronounced proliferation of cholangiocytes and oval cells, mainly without glandular lumens. False lobules were found but without significant displacement of the liver parenchyma. In some portal zones, there was scanty mononuclear inflammatory infiltration, and vascular stasis, in the form of a septum and a system of collagen fibers surrounding the newly formed bile ducts, entwining individual cholangiocytes and corresponding to the grade 5. The CBD diameter of this animal was 30 mm, and there was pus bile in its lumen (Figure 6).

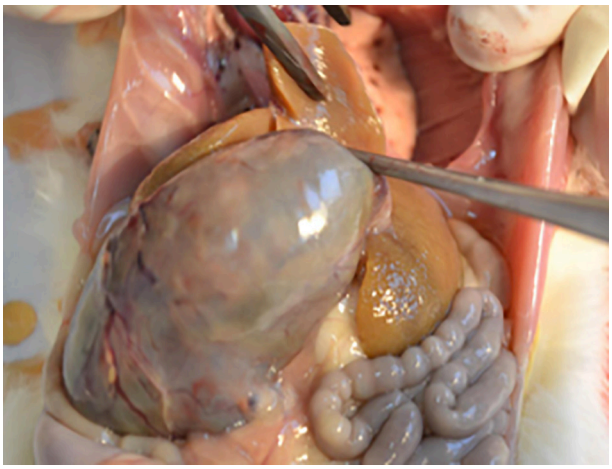

Figure 6. The CBD dilatation of about 30 mm of the animal with PBOR complicated by cholangitis on the PO day 35.

At a normal TB level on PO day 21 (2 animals), the liver histological picture was heterogeneous. In one case, vascular congestion was observed in the liver, a moderate increase in the number of bile ducts due to their proliferation, and a significant expansion of the portal zones due

to predominantly newly formed bile ducts and stromal cells. Grade 2 fibrosis was present in most of the portal zones with rare thin porto-portal septa. In another case, the liver histostructure was close to normal. The bile ducts were slightly dilated, as well as there was a slight mononuclear infiltration with the absence or weak fibrosis of a few portal zones. On the PO day 35 of PBOR, 4 animals with normal TB levels had also variable liver histological pictures. In one case, the liver histostructure was close to normal: a slight expansion of the portal zones due to stromal cells, fibroblasts, cholangiocytes scant infiltration of mononuclear cells (lymphocytes), and vascular congestion. Fibrosis was absent (Figure 7).

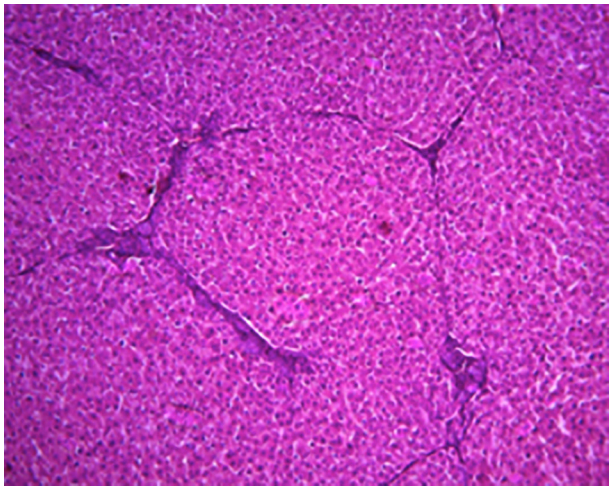

Figure 7. The liver histostructure with PBOR and normal TB level (PO day 35). The lobular structure of the liver is preserved. The portal zones are slightly enlarged and contain the bile ducts. Circular G+E magnifier x100.

The other three cases had enlarged portal zones in the liver due to the stroma, fibroblasts, cholangiocytes, and meager focal infiltration by mononuclear cells and vascular congestion. Fibrosis in one case was grade 1, in another grade 2, and one animal had the grade of fibrosis regarded as 2-3, depending on the field of view of the histological specimen.

DISCUSSION

The study of experimental cholestasis is usually carried out to identify the features of the pathological process development, including the phenomena and substances that initiate further pathological changes in the liver – development of fibrosis and cirrhosis (1,6). The other reasons are evaluating the effectiveness of various medicinal agents in acute and chronic liver pathology (21); studying the natural history of obstructive cholestasis in the aspect of the development of its complications, associated with the hepatobiliary system – cholangitis, liver failure (5,9), or systemic ones (8,12).

There are two main models to study experimental obstructive cholestasis. In one, the bile duct is ligated (22), and in another, the duct is transacted between two ligatures (10-13). The difference between these models is that after a simple ligation, over time, leads to recanalization with the restoration of bile outflow (11,14). This phenomenon was first described in 1823. When the ligated bile duct is transacted, restoration of bile outflow does not occur and long-term complete obstruction of the CBD remains (3). The main task of the treatment of patients with obstruction of CDB is to restore bile outflow. This encouraged to study experimentally the liver changes in partial obstruction of the CBD, and outflow restoration after complete obstruction (23,24).

To study the bile outflow restoration, models with surgical interventions redo could be used. They include the formation of anastomosis between the dilated bile duct and the intestine or the use of bile drainage (25). The disadvantages of these models are the relative complexity and the need to perform reoperation. To study the liver changes in partial bile outflow restoration after complete obstruction of the CBD, it was decided to use the phenomenon of recanalization of the CBD after its ligation. The obtained results were compared with complete obstruction–ligation and transection of the CBD. Different ways of assessing the restoration of the bile passage in the experiment are used. X-ray confirmation of the passage of contrast media to the duodenum is a reliable one but is associated with the relative complexity of implementation (11,26). The

normalization of TB levels also undoubtedly reflects the fact of bile outflow restoration. However, it does not indicate the exact terms of restoration (14,27). In the current study, the presence of bile in the duodenum as the criterion for restoring the bile passage was used. This assessment may be quite subjective, but it compares favorably with its simplicity. In addition, the histological changes of the liver according to the TB level were compared.

According to the obtained data, the bile passage restoration first was noted on PO day 7 – in 1 of 4 animals. On PO day 14 – in 2 of 4 cases, and at the end of the experiment – on PO day 35 – bile in the duodenal lumen was in 4 of 5 cases. This correlates with another study, according to which the partial restoration of bile outflow in ligation of the CBD of rats occurs on the PO days 7-12 (11). It should be noted that in ligating the CBD at the initial stages of the experiment – up to this term – PO day 7 – almost all cases have complete obstruction. This is confirmed by the histology data of the liver, which are identical in both study groups. Moreover, on PO day 14, when recanalization was recorded in half of the cases, the histological changes of the liver also did not differ from histological changes in the case of complete obstruction (28,29). Histological changes of the liver are characterized by signs of acute inflammation and alteration on the PO days 1 and 3, and subsequently, by progressive proliferation of the bile ducts and the development of liver fibrosis up to grades 4-5, with no significant differences in the degree of fibrosis between the study groups. The blood TB levels up to 14 days also did not differ statistically in both study groups. The absence of significant differences on the PO day 14 (180±7 for CCBDO and 119.5±28 μmol/L for BOPR, $p>0.05$), probably is associated with the small number of animals – 6 and 4, respectively (28).

Thus, up to 14 days, despite the partial restoration of bile outflow, the morphological picture of the liver and CBD are identical to complete obstruction, despite a tendency for the TB level to decrease. Later, in animals with PBOR and persistence of hyperbilirubinemia, there were no differences in the liver histological picture and the TB value ($p>0.05$) in comparison with CCBDO. In the liver, there was a proliferation of bile ducts, which predicted the replacement of hepatocytes, as well as the appearance of fibrosis. As a result, there was a loss of normal histostructure, which included false elements. The degree of fibrosis does not depend on the model. The CBD wall of both groups on the PO days 21 and 28 had subacute inflammation (a type of inflammation that falls between acute and chronic inflammation in terms of its duration and intensity) with decreasing acute inflammation. However, on the PO day 35, the only animal with PBOR and hyperbilirubinemia showed the presence of acute cholangitis. As mentioned before, the normalization of the TB level in animals with PBOR was recorded on the PO days 21 and 35. In these cases, the liver histological picture varied from almost normal to cholestatic changes with grade 3 fibrosis.

With complete bile duct obstruction (CSBDO), already by day 7, liver fibrosis of grades 1-2 is observed. By day 14, fibrosis reaches grades 4-5. Therefore, in CSBDO decompression in the first 7 days is necessary to prevent significant fibrosis. In partial obstruction (PBOR) with hyperbilirubinemia, fibrosis develops similarly to CSBDO, so early decompression is also needed. With PBOR with normal bilirubin, on days 21-35, either no fibrosis or only insignificant grade 1-2 fibrosis is seen. This suggests partial obstruction with preserved biliary function can last up to 3-4 weeks without significant fibrosis. Therefore, the optimal timing of decompression to prevent liver fibrosis is with complete obstruction, within the first 7 days; with partial obstruction with hyperbilirubinemia, also within the first 7 days; and with partial obstruction with normal bilirubin, delayed decompression up to 3-4 weeks is possible (30-32).

Abshagen et al. (33) in their comprehensive study, use a murine model of bile duct ligation to conduct an in-depth study of temporal changes in biochemical, histological, and transcriptional parameters during obstructive cholestasis. Analysis of more than 6 000 data points over time allows us to characterize the different phases of the disease, from early damage to progression. Parameter correlation statistics reveal coordinated responses, relate transcript dynamics to pathological processes, and identify potential biomarkers for disease staging. Careful multilevel profiling provides key insights into the complex pathophysiological processes in

obstructive cholestasis, significantly improving the understanding of this disease. Ultimately, the obtained data lay an important basis for the development of improved diagnostic and therapeutic strategies to overcome the significant clinical burden of cholestatic liver diseases.

Yokoda and Rodriguez (34) demonstrated that comprehensively summarizes current knowledge about the pathogenesis of cholestatic liver diseases. This highlights the central role of cholangiocytes in the response to injury and how sustained inflammatory signaling and genetic/epigenetic dysregulation can contribute to chronic ductular reaction and fibrosis (35-37). The authors detail key mechanisms including bile acid toxicity, mitochondrial dysfunction, immunogenetic factors, and matrix remodeling. They also discuss promising preclinical developments targeting these pathogenic pathways, as well as recent clinical trials of new treatments for specific cholestatic diseases. Overall, their study provides a holistic overview of the complex pathophysiology underlying cholestatic disorders and highlights the need for further research to improve diagnostic and therapeutic strategies.

A recent review by Aller et al. (38) also described three key inflammatory phenotypes that are expressed in the liver interstitium during obstructive cholestasis. They proposed an ischemia/reperfusion phenotype early on mediated by oxidative stress. This is followed by a leukocytic phenotype with activation of liver macrophages and infiltration of immune cells (39). Lastly, an angiogenic phenotype emerges characterized by cholangiocyte proliferation and peribiliary plexus development. Our findings align with this proposed model of successive inflammatory phases in the cholestatic liver interstitium. The ischemia/reperfusion phenotype we observed may explain the edema and oxidative damage in the early stages after bile duct ligation. The later prominence of immune cells and enzymes is consistent with the acquisition of a leukocytic phenotype. Finally, our observation of matrix changes and arterializations agrees with the concept of a late angiogenic phenotype. Further research is needed to fully validate this stepwise model of inflammation in obstructive cholestasis. However, our results lend support to the hypothesis that distinct inflammatory phenotypes are expressed sequentially during cholestatic liver injury.

CONCLUSIONS

Histological changes occurring in the structure and functions of the liver were determined. The source of such changes is the experimental development of obstructive cholestasis. The essence of the above-mentioned disease and its direct effect on the liver was revealed. The fact that recanalization with incomplete restoration of bile outflow accompanied by normalization of TB was substantiated. As for the structure and activity of the liver, these components were most affected in the case of acute cholangitis.

When ligation of the CBD in rats, its recanalization occurs approximately on the PO day 7-14. By the PO day 35 normalization of the TB level occurs in 4 out of 5 surviving animals ($p<0.01$). Recanalization with incomplete restoration of bile outflow, which does not lead to normalization of the TB level, is accompanied by histological changes of the liver, the same as in complete cholestasis. An exception is acute cholangitis, in which the morphological changes of the liver are more pronounced.

It is possible to assert the presence of characteristics that indicate the presence of abnormalities in the structure of the liver of male rats, obtained as a result of a comparison at each step of the experiment of the state of the rats' organs and indicators that are the norm.

In purpose to obtain more in-depth knowledge of the processes occurring in the liver and bile ducts in the restoration of the bile outflow after its complete obstruction, further research is needed, including using the morphometric method.

Comparative assessment of the effectiveness of various methods of surgical decompression could provide useful data. Morphometric studies to obtain quantitative data on histological changes would reduce subjectivity. Therefore, this direction requires further study taking into account the above limitations and optimization of the methodology to obtain more thorough data.

It is also necessary to pay attention to the limitations of this study and directions for

further research. A small number of animals in the experimental groups reduces the statistical significance of the results. The lack of a control group of intact animals for comparison of histological changes is another limitation. The subjectivity of assessing the presence of bile in the duodenum as a criterion for restoring bile flow is also a limitation. The effect of sex and age of animals on the results was not studied. To increase statistical significance, the number of animals in experimental groups should be increased. Adding a control group of intact animals would improve the study. More objective methods of assessment of recovery of bile flow should be used.

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