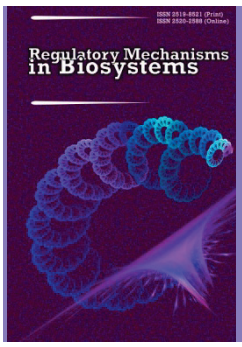

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Predictors of liver fibrosis during ligation of the common bile duct in rats

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Determination of predictors of liver fibrosis during ligation of the common bile duct in experimental animals demonstrates the dynamics of the development of biochemical, immunological, and morphological markers of cholestatic disease, which is subsequently accompanied by the formation of liver cirrhosis. An increase in the degree of obstructive jaundice caused pronounced negative changes in the clinical picture of the disease and morphostructural changes of the liver, which, depending on the time of obstruction, became irreversible. In the conditions of a chronic experiment on rats with the aim of identifying patterns of development of obstructive jaundice, assessing the processes of liver damage and developing treatment directions, modeling of obstructive jaundice was performed with further study of the clinical picture and behavioral reactions, biochemical, immunological, morphological data depending on the duration of obstruction of the biliary tract. During the study, three stages of disease development were established: initial (up to 7 days), compensatory (8–15 days) and decompensation stage (from 16 days after ligation of the common bile duct). Hyperbilirubinemia and accumulation of bile acids, an increase in the content of medium-weight molecules with the activation of lipoperoxidation and endotoxemia occurred against the background of strengthening the processes of connective tissue organization. The assessment of the effect of cytokines made it possible to establish a reliable negative correlation between IL-6, TNF-α and IL-10, which led to the strengthening of cytotoxic reactions. Against the background of an imbalance of the cytokine chain under the conditions of the experiment, obstruction of the biliary tract from the first days of ligation was accompanied by the expression of the profibrogenic cytokine TGF-β1 with the activity of fibrotic processes in the liver parenchyma, while a reliable relationship was established between an increase in the concentration of TGF-β1 and fibrosis ($r = 0.445$; $P = 0.041$). When assessing the histostructure of rat livers, after 20 days, developing cirrhosis was detected in 25.0% of the animals. Micronodular cirrhosis with loss of the beam structure of the liver parenchyma and fibrosis of the lobes was reproduced in 33.3% of the animals. Against the background of cholestatic hepatitis, secondary obstructive biliary cirrhosis of the liver was reproduced in 58.3% of the animals. Ligation of the common bile duct in rats causes inflammatory, necrobiotic, dystrophic changes in the liver, which, against the background of increasing endogenous intoxication, increased lipoperoxidation and cholestatic phenomena, stimulate a cascade of processes of excessive organization of connective tissue, the final effect of which is the formation of liver cirrhosis. Prospects for further research are the development of processes of influence on the mechanisms of liver regeneration and slowing down of fibrotic reactions. Additional studies are needed to determine changes in liver tissue in the posticteric period and factors affecting the healing of cholestatic liver damage and regeneration of hepatocytes.

Keywords: obstructive jaundice; experimental cholestasis; cytokines; fibrosis; liver cirrhosis; purulent cholangitis.

Introduction

Obstructive jaundice is a serious, life-threatening condition that can lead to death from sepsis and multiple organ failure (Oguz et al., 2018). Cholestatic liver diseases, which develop on the background of extrahepatic bile duct obstruction, progress to more severe forms with increased hepatobiliary damage, cholangitis, and, ultimately, liver fibrosis and cirrhosis (Van Campenhout et al., 2019). If left untreated, chronic liver fibrosis progresses to hepatocellular carcinoma and liver cirrhosis characterized by ascites, jaundice, hepatic hypertension and hepatic encephalopathy (Schuppan & Afdhal, 2008; Ginès et al., 2021). Liver cirrhosis represents the end-stage of chronic liver disease, and liver fibrosis is regarded as the point of deterioration of liver architecture (Terai & Tsuchiya, 2017; Terai & Sakamaki, 2021; Novo et al., 2020).

Liver fibrosis is initiated by damaged hepatocytes and infiltrated macrophages, which secrete profibrogenic cytokines such as transforming growth factor-β (TGFβ), platelet-derived growth factor (PDGF), connective tissue growth factor (CTGF), and vascular endothelial growth factor (VEG). An important role in hepatocytes in liver fibrosis is played by TGFβ (Pellicoro et al., 2014; Akkız et al., 2024; Wang et al., 2024), which affects the activation of stellate cells of the liver, plays a vital role in the pathological process of liver fibrosis (Yang et al., 2020). Hepatic stellate cells, located between hepatocytes and endothelial cells, proliferate and are activated by these profibrogenic cytokines to produce extracellular matrix components such as collagen and fibronectin (Ma et al., 2022).

An experimental model widely used to investigate the pathophysiology of biliary cirrhosis and potentially new treatments is common bile duct ligation in rats (Van Campenhout et al., 2019; Ozozan et al., 2020). Rats are widely used in experimental research due to their small size, low cost, high viability and short reproductive cycle (Bulte et al., 2014; Kiani et al., 2022). In experimental studies, rats are used to create models of biliary obstruction. When building a biliary tract obstruction model, the common bile duct is not cut, but only ligated (Gong & Li, 2008; Lv et al., 2018). Ligation of the common bile duct causes different stages of cholestatic liver disease, which are cholestasis, which is subsequently accompanied by inflammation and finally fibrosis and cirrhosis of the liver (Jorge et al., 2001; Daneze et al., 2011; Moraes et al., 2020). The advantage of this approach is that in a short time, in contrast to clinical conditions, it is possible to reproduce various stages of the development of the disease and clarify the terms and mechanisms of the pathology (Lv et al., 2018). But,

despite the large number of studies that investigated the state of the hepatocyte in obstructive jaundice, there is no single view on the degree of depth of disturbances in its functioning. Some researchers in their works indicate that hepatocyte function is not affected in the early stages of obstructive jaundice (Salas-Silva et al., 2021; Donato et al., 2022). Others hold the point of view that changes occur from the first day of jaundice. Subsequently, as a result of complete obturation, within 24–48 hours, necrosis of individual hepatocytes, swelling of the central lobes, and a decrease in their glycogen content are observed in the liver (Zhou et al., 2003; Ishiwatari et al., 2024). Most of the changes in the expression of hepatic transport proteins (both in hepatocytes and cholangiocytes) are thought to be changes aimed at minimizing cellular damage caused by retained, potentially toxic components of the biliary tract, such as bile salts and bilirubin (Boyer, 2007; Thakkar et al., 2017; Paulusma et al., 2022).

Understanding the pathophysiological changes of obstructive jaundice remains a challenge for planning current and future treatment (Pavlidis & Pavlidis, 2018; Kutovyi et al., 2018). Additional studies are needed to determine changes in liver tissue in the posticteric period and factors affecting the healing of cholestatic liver damage and regeneration of hepatocytes. Therefore, the objective of our study was to assess the effect of bile duct ligation in rats on the expression of predictors of liver fibrosis in the progression of obstructive jaundice.

Materials and methods

The deontological aspects of the research were resolved within the existing international conventions and legislation of Ukraine, principles of bioethics. The selection of animals for the experiment, the research protocols and the removal of animals from the experiment were approved by the local Bioethics Committee (protocol No. 3 dated 03.20.23) of Oles Honchar Dnipro National University (Dnipro, Ukraine). The housing, nutrition, care of the animals and their removal from the experiment were carried out in accordance with the principles laid down in the "European Convention for the Protection of Vertebrate Animals Used for Experimental or Other Scientific Purposes" (Strasbourg, France, March 18, 1986, ETS No. 123), the Charter of the Ukrainian Association for Bioethics and GLP norms (1992) in accordance with the requirements and norms of ISN C8R (2002), in the Law of Ukraine "On the Protection of Animals from Cruelty" (Kyiv, 02.21.2006, No. 3447-IV) and Regulations on Ethics of the Ministry of Health of Ukraine dated November 1, 2000, No. 281. The animals were provided with generally accepted sanitary and hygienic conditions with a standard diet (Zapadnjuk et al., 1983): they were fed the standard feed "Purina dedent chow" (fat – 20.6%, protein – 32.4%, carbohydrates – 47.0%) and sufficient water. The Bioethics Commission did not detect any moral and ethical violations during the experimental studies.

The research was carried out under the conditions of a chronic experiment on 80 rats of the Wistar line, aged 22–23 weeks, with an average weight of 224.3 ± 4.9 g. In order to identify the patterns of development of mechanical jaundice of non-neoplastic origin, evaluate the processes of liver damage, and develop directions for treatment, modeling of obstructive cholestasis was performed according to the experimental method of K. Yorganci (Yorganci et al., 2004). The control group, without ligation of the common bile duct, consisted of 12 (15.0%) rats from their total number. To establish the stages of development of experimental obturation cholestasis, material was collected on the 1st, 5–7th, 15th, 16–18th and 30th days of the experiment. After a 5-day quarantine, surgical intervention was performed under anesthesia with the help of calypsol (0.005 mg/kg), which was potentiated by the introduction of diphenhydramine (0.03 mL/kg, intramuscularly). After treatment of the operative field in aseptic conditions, a laparotomy was performed with subsequent ligation of the common bile duct with a double ligature. The abdominal cavity was sutured in layers. Under the conditions of the experiment, depending on the term of cholestasis, under calypsol anesthesia (0.005 mg/kg), rats were guillotine-decapitated with blood sampling and segmental liver biopsy. Blood and tissue samples were collected for evaluation of biochemical markers of cholestasis and histopathological studies.

Biochemical methods for the study of cholestasis markers (Pieters et al., 2021): the level of alanine aminotransferase (ALT), total bilirubin (TBIL), total cholesterol content, β-lipoproteins, x-lipoprotein, hexosamines, bile acids, oxyproline protein-bound in serum was analyzed using Spectra MAX (Molecular Devices, LLC, San Jose, USA, 2022) and commercial kits from BioVision (Milpitas Blvd, Milpitas, USA) (Lee et al., 2022). Serum levels were assayed using commercial kits according to the manufacturer's instructions. The content of TGF-1 in the blood serum of rats was determined by the immunoenzymatic method with a DRG test kit (DRG, International Inc., Germany). The principle of the ELISA method is based on the quantitative determination of the antigen directly by its layer-by-layer "sandwich" binding with antibodies specific to it. The study was conducted in accordance with the instructions for the test set. Quantitative determination of the concentration of IL-6, IL-10, TNF-α, TGF-1 in the blood serum was performed using ELISA using test systems (Roche, Mannheim, Germany) according to the manufacturer's recommendations.

Histological methods of liver research (Arjmand et al., 2020). Biopsies were fixed in Bouin's fluid overnight. After dehydration in a series of alcohols of increasing strength, the pieces of tissue were passed through chloroform and embedded in paraffin. Thin 5 μm histological sections were obtained on a rotary microtome. After deparaffinization in xylene and alcohol, the sections were stained with hematoxylin and eosin according to the Mallory-Slinchenko method. The histotopographic features of the organization of the parenchyma of the organ, its acinar structure, the state of the portal tracts (lymphoplasmacytic infiltration, the state of small bile ducts, arterioles and venules), the presence and intensity of protein and fatty dystrophy, and variants of fibrosis were taken into account when assessing the state of the liver.

Samples were compared using Tukey's test. Data in tables are presented as mean ± standard deviation (x ± SD). The data were quantitative continuous variables with normal data distribution, therefore the Pearson correlation coefficient (r) was calculated with P-value corresponding to 95% confidence interval (Petrie & Sabin, 2020).

Results

In the first 7 days after ligation of the common bile duct, the rats developed external signs of obstructive jaundice: jaundiced staining of the auricles, rich yellow color of urine. Compared to the control group, they reacted poorly to noise stimuli, their fur was disheveled, lost its shine, and some of them became aggressive. Along with the change in behavioral reactions during the modeling of obstructive jaundice, biochemical changes in the blood serum of rats were also noted. In these terms, the content of bilirubin increased by 28.24 times due to the direct fraction compared to the indicators in the intact group of animals, with a corresponding increase in alanine aminotransferase by 9.31 times and alkaline phosphatase by 8.42 times ($P < 0.05$) (Table 1).

Table 1

Characteristics of the markers of bile outflow in the blood serum of rats depending on the terms of the obstruction (x ± SD, n = 60, the duration of the experiment is 30 days)

Laboratory indicator	Control (n = 12)	1st day after ligation (n = 9)	7th days after ligation (n = 9)	15th day after ligation (n = 9)	18th day after ligation (n = 12)	30th day after ligation (n = 9)
Bilirubin (total), μmol/L	9.26 ± 0.16^{a}	116.65 ± 12.41^{b}	141.12 ± 12.74^{c}	117.44 ± 15.13^{d}	172.03 ± 12.74^{e}	233.06 ± 16.42^{f}
Bilirubin (conjugated), μmol/L	5.12 ± 0.23^{a}	79.43 ± 9.16^{b}	84.01 ± 7.43^{d}	46.03 ± 6.45^{d}	86.36 ± 9.21^{e}	157.44 ± 14.81^{f}
Level of alanine aminotransferase, mmol/L	0.63 ± 0.04^{a}	4.37 ± 0.22^{b}	5.64 ± 0.35^{c}	3.51 ± 0.12^{d}	5.25 ± 0.42^{e}	1.13 ± 0.26^{f}
Alkaline phosphatase, mmol/L	1.34 ± 0.08^{a}	12.54 ± 2.51^{b}	10.97 ± 0.98^{d}	6.65 ± 0.72^{d}	8.37 ± 0.64^{e}	9.22 ± 0.71^{f}

Note: different letters in a row indicate samples that are significantly ($P < 0.05$) different from one another according to Tukey's Honest Significant Difference Test.

The analysis of the content of β-lipoproteins allowed us to establish their excessive formation already at the initial stage of the development of cholestasis (Table 2). At the same time, the level of β-lipoproteins was correlated with the content of bile acids (r = 0.476; P = 0.054). The content of x-lipoprotein in comparison with control indicators on the 7th day after bile duct ligation in rats was increased by 7.5 times to 93.04 ± 10.73 units, (P < 0.01). An increase in the level of abnormal x-lipoprotein in animals confirmed its involvement in the development of intrahepatic cholestasis, as evidenced by the direct relationship of its content with the concentration of bile acids (r = 0.673; P = 0.001) and with gamma-glutamyl transferase (r = 0.544; P = 0.005) The content of bile acids from the first day differed significantly (P < 0.05) from the control numbers and on the 7th day was equal to 0.72 ± 0.09 mmol/L. Bilirubinemia and the accumulation of bile acids reflected the retention variant of the syndrome of endogenous intoxication, among the components of which are recognized substances of medium and low molecular weight (PCiNMM) and products of lipid peroxidation. These processes took place against the background of endotoxemia, expressed on the 3rd–7th day sof ligation due to an increase in the content of medium-mass molecules (MMM) by 2.17 times to 1605.45 ± 126.97 mg/mL compared to the control (P < 0.011). This also applied to the fractional composition of medium-mass molecules, where an increase in MMM -λ-210 was observed – by 2.21; MMM -λ-254 – by 2.53 and MMM -λ-280 – by 2.92 times (P < 0.013). Since these fractions consist of the most toxic hydrophobic substances that have a high affinity for biological structures, and are in the plasma almost completely in a bound state in the form of complexes with albumin or low-density lipoproteins, it should be assumed that the increase in their level is associated with with the activation of free radical oxidation processes. In addition, the growth of this fraction indicates the active involvement of not only proteins, but also nucleic acids in catabolic processes. Therefore, the increase in lipoperoxidation products led to an increase in endogenous intoxication. Thus, in the 3rd-7th days after ligation, the content of malondialdehyde increased by 3.12 times to 11.26 ± 1.44 nmol/L, (P < 0.014).

Impaired lipid metabolism, in particular, the metabolism and transport of cholesterol and β-lipoproteins, was also determined in the animals. It was established that their concentration gradually increased as the severity of cholestasis increased. Thus, in the 3rd-7th days after ligation, X- and β-lipoproteins increased by 7.49 and 7.47 times, respectively, to 93.04 ± 10.73 mmol/L and to 28.03 ± 1.52 units (P < 0.001). During this period of cholestasis, 15.0% of animals included in the experiment died.

Table 2

Characteristics of changes in the biochemical parameters of the blood serum of rats depending on the terms of obstruction (x ± SD, n = 60, duration of the experiment – 30 days)

Indicator	Control (n = 12)	1st day after ligation (n = 9)	7th days after ligation (n = 9)	15th day after ligation (n = 9)	18th day after ligation (n = 12)	30th day after ligation (n = 9)
Malondialdehyde, mmol/mL	3.62 ± 0.13^{a}	6.79 ± 0.72^{b}	11.26 ± 1.44^{c}	6.04 ± 0.48^{d}	7.33 ± 0.82^{e}	13.26 ± 1.21^{f}
X-lipoproteins, unit	12.4 ± 2.2^{a}	40.0 ± 5.2^{b}	93.0 ± 10.7^{c}	36.7 ± 2.6^{d}	46.0 ± 1.4^{e}	112.0 ± 9.1^{f}
Hexosamines, mmol/L	8.93 ± 0.54^{a}	9.04 ± 0.65^{a}	9.12 ± 0.37^{a}	9.69 ± 0.71^{a}	10.03 ± 0.84^{b}	12.67 ± 0.73^{c}
Oxyproline protein-bound, μmol/L	237 ± 21^{a}	241 ± 23^{a}	240 ± 17^{a}	305 ± 22^{b}	428 ± 45^{c}	631 ± 26^{d}
Gamma-glutamyl Transferase, μkat/L	0.23 ± 0.017^{a}	0.37 ± 0.06^{a}	0.51 ± 0.08^{b}	0.38 ± 0.04^{a}	0.49 ± 0.09^{a}	0.62 ± 0.081^{c}
Medium mass molecules (MMM), mg/L	781 ± 49^{a}	955 ± 79^{b}	1605 ± 127^{c}	1228 ± 109^{d}	1431 ± 114^{e}	1723 ± 94^{f}
MMM-λ-210, unit	0.3923 ± 0.018^{a}	$0.536 \pm 0{,}04^{b}$	0.8472 ± 0.123^{c}	0.6119 ± 0.012^{d}	0.6433 ± 0.084^{e}	0.9357 ± 0.114^{f}
MMM-λ-254, unit	0.0305 ± 0.004^{a}	0.0427 ± 0.005^{b}	0.0758 ± 0.018^{c}	0.0386 ± 0.004^{d}	0.0392 ± 0.005^{e}	0.0941 ± 0.001^{f}
MMM-λ-280, unit	0.0418 ± 0.002^{a}	0.055 ± 0.007^{b}	0.1192 ± 0.016^{c}	0.0551 ± 0.007^{d}	0.0617 ± 0.007^{e}	0.1372 ± 0.013^{f}
Bile acids, mmol/L	0.27 ± 0.03^{a}	0.48 ± 0.03^{b}	0.72 ± 0.09^{c}	0.31 ± 0.04^{a}	0.34 ± 0.03^{a}	0.96 ± 0.03^{d}
Cholesterol, mmol/L	1.98 ± 0.16^{a}	2.81 ± 0.34^{b}	5.27 ± 0.69^{c}	2.82 ± 0.31^{d}	4.31 ± 0.53^{e}	7.12 ± 0.54^{f}
β-lipoproteins, unit	3.75 ± 0.25^{a}	14.53 ± 0.75^{b}	28.03 ± 1.52^{c}	11.02 ± 0.91^{d}	14.02 ± 0.96^{e}	32.56 ± 1.97^{f}

Note: different letters in a row indicate samples that are significantly (P < 0.05) different from one another according to Tukey's Honest Significant Difference (HSD) Test; MSM – medium mass molecules, MSM-λ-210, MSM-λ-254, MSM-λ-280 – molecules with the corresponding average mass.

During the 8–15th days of cholestasis, the condition of the animals stabilized. While preserving the external signs of jaundice, they responded more adequately to noise stimuli, their fur regained its former luster, and their aggressiveness decreased. During this period, there was almost no increase in the studied indicators. According to Table 1 compensatory processes against the background of a decrease in the concentration of bilirubin, alanine aminotransferase and alkaline phosphatase were characterized by the improvement of other biochemical markers of subhepatic cholestasis. Therefore, the content of x-lipoproteins decreased, but remained elevated by 3.01 times (P < 0.05). The content of bile acids exceeded the control indicators by 1.15 times and was 0.31 ± 0.04 mmol/L, the content of malondialdehyde remained elevated at almost the same level as on the 7th day of ligation and was 6.04 ± 0.48 nmol/mL. The intensity of connective tissue organization processes by the content of protein-bound oxyproline slightly increased and amounted to 305.28 ± 22.43 μmol/L. During this period, 7.5% of the experimental animals died.

In the next period of development of the pathological process, the content of bilirubin in the blood continued to increase steadily and reliably (P < 0.05). From the 16th day of cholestasis, the condition of the animals worsened again: the external signs of jaundice intensified, adynamia increased, and the reaction to noise stimuli practically disappeared. The strengthening of the processes of cholestasis and intoxication in animals with long-term ligation of the common bile duct was expressed in the increase of all the investigated indicators (Tables 1 and 2). In these terms, one should also note a significant increase in the content of hexosamines to 10.03 ± 0.84 mmol/L and oxyproline protein-bound to 428.04 ± 45.26 μmol/L compared to the control, which indicated an increase in the processes of connective tissue organization.

The content of x-lipoproteins, compared to control indicators, increased by 4.85 and 9.12 times on the 30th day of ligation (P < 0.05). The content of bile acids exceeded the control indicators by 1.34 times and was equal to 0.96 ± 0.03 mmol/L. The level of oxyproline protein-bound compared to the control increased by 1.91 times and amounted to 631.01 ± 26.18 μmol/L. The increase in the average mass of molecules from the 18th day to 1431.38 ± 114.05 mg/mL reached the maximum concentration by the 30th day and was 1723.15 ± 93.91 mg/mL, significantly different from normal values (P < 0.001). The growth of MMM was correlated with the increase of lipoperoxidation products according to malondialdehyde indicators, which were 7.33 ± 0.82 nmol/mL (r = 0.516; P < 0.012), which significantly deepened the intoxication processes. An increase in the level of bilirubin on the 30th day to 233.06 ± 16.42 μmol/L was accompanied by a decrease in the concentration of alanine aminotransferase to 1.13 ± 0.26 mmol/L, which indicated the depletion of cell mass and manifested the nature of an unfavorable course. During this period, 25.0% of the experimental animals died.

The impact of pro- and anti-inflammatory cytokines was evaluated in conditions of obstructive jaundice and cholestatic liver damage. The results of studies depending on the terms of ligation of the common bile duct are presented in Table 3. According to the obtained data (Table 3), the activation of pro-inflammatory cytokines from the 2nd–3rd days after ligation can contribute to the further progression of inflammation. A high level of pro-inflammatory cytokines IL-6, TNF-α and a decrease in the activity of the anti-inflammatory mediator IL-10 determine the activity of the process. At the same time, excessive activation of pro-inflammatory cytokines IL-6, TNF-α leads to increased cytotoxic reactions. Thus, the level of IL-10 on the 30th day after ligation significantly (P < 0.05) decreases compared to the control by 2.25 times, while IL-6 and TNF-α increase by 2.01 and 18.13 times, respectively (P < 0.001). A reliable negative correlation was established between IL-6 and TNF-α and IL-10 (r = –0.492; P = 0.022). Against the background of an imbalance of the

cytokine chain, from the 3rd day after ligation, the expression of the profibrogenic factor TGF-β1 increased by 1.64 times, then gradually increased by 2.18 times on the 18th day ($P < 0.05$) and by 2.51 times on the 30th day after ligation ($P < 0.05$), which demonstrates an active reaction of the profibrogenic cytokine TGF-β1, to increase cholestasis and the duration of biliary tract obstruction, which is subsequently accompanied by the activity of fibrotic processes in the liver parenchyma with increased synthesis of extracellular matrix proteins.

Table 3

The level of cytokines in the blood serum of rats depending on the terms of obstruction (x ± SD, n = 42, the duration of the experiment is 30 days)

Indicator	Control (n = 12)	3th day after ligation (n = 9)	18th day after ligation (n = 12)	30th day after ligation (n = 9)
IL-6, pg/mL	9.72 ± 1.24^{a}	13.03 ± 1.61^{b}	33.63 ± 6.84^{c}	19.31 ± 2.04^{d}
IL-10, pg/mL	28.64 ± 1.83^{a}	41.35 ± 3.17^{b}	22.51 ± 1.43^{c}	13.27 ± 1.02^{d}
TNF-α, pg/mL	2.26 ± 0.31^{a}	11.64 ± 0.92^{b}	33.34 ± 4.14^{c}	39.98 ± 2.98^{d}
TGF-β1, pg/mL	8525.51 ± 218.56^{a}	14237.04 ± 312.08^{b}	18052.06 ± 208.41^{c}	$21741.03 \pm 106,14^{d}$

Note: different letters in a row indicate samples that are significantly ($P < 0.05$) different from one another according to Tukey's HSD Test; IL-6, IL-10 – interleukins, TNF-α – tumor necrosis factor, TGF-1 – transforming growth factor.

The assessment of the features of the histostructure of the liver of rats when creating a model of subhepatic cholestasis showed that already a day after ligation of the common hepatic duct in 11.3% of rats there was full blood typical for the liver, minor signs of protein dystrophy of the parenchyma without a significant difference in the changes in the structure of the liver by 2 the 3rd day of ligation of the common bile duct (Fig. 1a). A significant increase in the activity of NADPH-diaphorase was established, which indicated the activation of metabolic processes in the organ. 5 days after ligation of the common bile duct of the liver, acute necrobiosis of the organ was observed in 66.7% of the animals, which was manifested by neutrophilic infiltration of the liver parenchyma. The lesion areas covered the portal and periportal zones of the liver lobes (Fig. 1b).

On the 7th day of ligation, single mitoses of hepatocytes in the liver beams and cells in a state of eosinophilic degeneration were observed in the parenchyma. Proliferation of Kupffer cells was found in sinusoids (Fig. 1c, 1d). Marked protein dystrophy of the liver was observed in 22.2% of rats. Therefore, already in the early stages of obstructive cholestasis, significant liver damage was observed. Simultaneously with the destructive processes, regeneration processes of liver parenchyma and bile ducts were observed. Violation of bile flow in 33.3% of rats led to the development of destructive purulent cholangitis of the interlobular and septal bile ducts (Fig. 1e).

From the 16th day, manifestations of reactive inflammation were observed with the accumulation of neutrophil leukocytes in the portal tracts and parenchyma of the liver. Protein dystrophy of the liver with proliferation of Kupffer cells in the sinusoids predominated. Bridge-like fibrosis septated the liver parenchyma. Acidophilic degeneration of hepatocytes involved single hepatocytes. The study of correlations made it possible to establish a reliable relationship between chronic inflammation and protein dystrophy ($r = 0.553$; $P = 0.035$), between the degree of cholestasis and fibrosis ($r = 0.671$; $P = 0.008$).

16–18 days after ligation of the common bile duct, 83.3% of the examined rats showed a pattern of granular protein dystrophy of the liver. Active hepatitis was found in 41.7% of animals 16–18 days after ligation of the common bile duct (Fig. 1f). At the same time, the portal and periportal zone of the liver parenchyma were infiltrated by lymphocytes, plasma cells, and leukocytes. After 20 days, developing cirrhosis was detected in 25.0% of animals. The study of correlations in experimental animals made it possible to establish a reliable relationship between the activity of chronic inflammation and cholestasis ($r = 0.867$; $P < 0.001$), between the activity of inflammation and necrosis ($r = 0.672$; $P = 0.026$). Micronodular cirrhosis with loss of the beam structure of the liver parenchyma and fibrosis of the lobes was reproduced in 33.3% of rats (Fig. 1i).

So, in the experiment, in 58.3% of cases, secondary obstructive biliary cirrhosis of the liver was reproduced against the background of cholestatic hepatitis, which was manifested by the appearance of necrobiotic zones in the liver parenchyma, eosinophilic degeneration of hepatocytes, purulent cholangitis, dystrophy, fibrosis of the portal tracts and liver parenchyma, proliferation of sinusoidal macrophages, Kupffer cells.

Discussion

The analysis of the obtained data indicates that an increase in the degree of cholestasis in experimental animals causes pronounced negative changes in the clinical picture of the disease and morphostructural changes of the liver, which, depending on the time of obstruction, became irreversible, which complements other research (Long et al., 2015; Roy & Lambert, 2017). The widespread effect of obstructive jaundice affected the biliary tree, liver cells, liver function and caused systemic complications (Liu et al., 2023).

Cholestasis phenomena were confirmed by a significant ($P < 0.05$) increase in the content of bilirubin, bile acids, alkaline phosphatase, and X-lipoproteins, the level of which increased as the severity of cholestasis increased, with increased activity of intracellular cholestasis, processes of connective tissue organization, products of lipoperoxidation, and endotoxemia ($P < 0.001$) Already at the initial stage of cholestasis development, the level of β-lipoproteins correlated with the content of bile acids ($r = 0.476$; $P = 0.054$). The content of X-lipoproteins in comparison with the control indicators on the 7th day after ligation of the bile ducts in rats increased by 7.52 times ($P < 0.01$), which can be interpreted as an acute period of cholestatic liver damage (Turk et al., 2016). Analysis of changes in the liver and the processes of fibrosis coincided with the processes of connective tissue organization. Negative correlation of protein-bound oxyproline with cholesterol, which is a hydrophobic endotoxin ($r = –0.864$; $P = 0.001$) and X-LP ($r = –0.878$; $P = 0.051$) in animals from the first days of ligation indicates a causal association of the development of fibrosis with the syndrome of endogenous intoxication already at the initial stage of the development of cholestasis. The evaluation of the impact of the cytokine regulation link allowed us to establish a reliable negative correlation between IL-6, TNF-α and IL-10 ($r = –0.492$; $P = 0.022$), which led to the strengthening of cytotoxic reactions. Against the background of the imbalance of the cytokine link, up to 30 days after ligation ($P < 0.05$), the concentration of TGF-β1, which is a cytokine with several biological functions and which is mainly involved in the stimulation of fibrosis and the treatment of inflammation, increased by 2.51 times (Krzemień et al., 2016). A reliable relationship was established between the increase in the concentration of TGF-β1 and fibrosis ($r = 0.445$; $P = 0.041$), while transforming growth factor beta (TGF-β) and resident stellate cells of the liver are the main mediators of its damage (Geh et al., 2021; Salas-Silva et al., 2019), and stellate cell activation and hepatocellular necrosis and proliferation of bile duct epithelium occur as a result of cholestatic liver damage (Turk et al., 2016). The study of correlations made it possible to establish a reliable relationship between chronic inflammation and protein dystrophy ($r = 0.556$; $P = 0.035$), between the degree of cholestasis and fibrosis ($r = 0.671$; $P = 0.008$). After 20 days, developing cirrhosis was detected in 25.0% of the animals. A reliable relationship was established between the activity of chronic inflammation and cholestasis ($r = 0.863$; $P = 0.001$), between the activity of inflammation and necrosis ($r = 0.672$; $P = 0.026$), between cholestasis and fibrosis ($r = 0.604$; $P = 0.041$). Three stages of disease development are established: initial (up to 7 days), compensatory (8-15 days) and decompensation stage (from 16 days after ligation of the common bile duct).

Evaluation of the features of the histostructure of the rat liver when creating a model of subhepatic cholestasis showed that ligation of the common bile duct is accompanied after 7 days by acute necrobiosis of the liver with leukocyte infiltration of the parenchyma, portal tracts, destructive damage to the bile ducts, eosinophilic degeneration of hepatocytes, proliferation of hepatocytes and Kupffer cells (Odisseos et al., 2020).

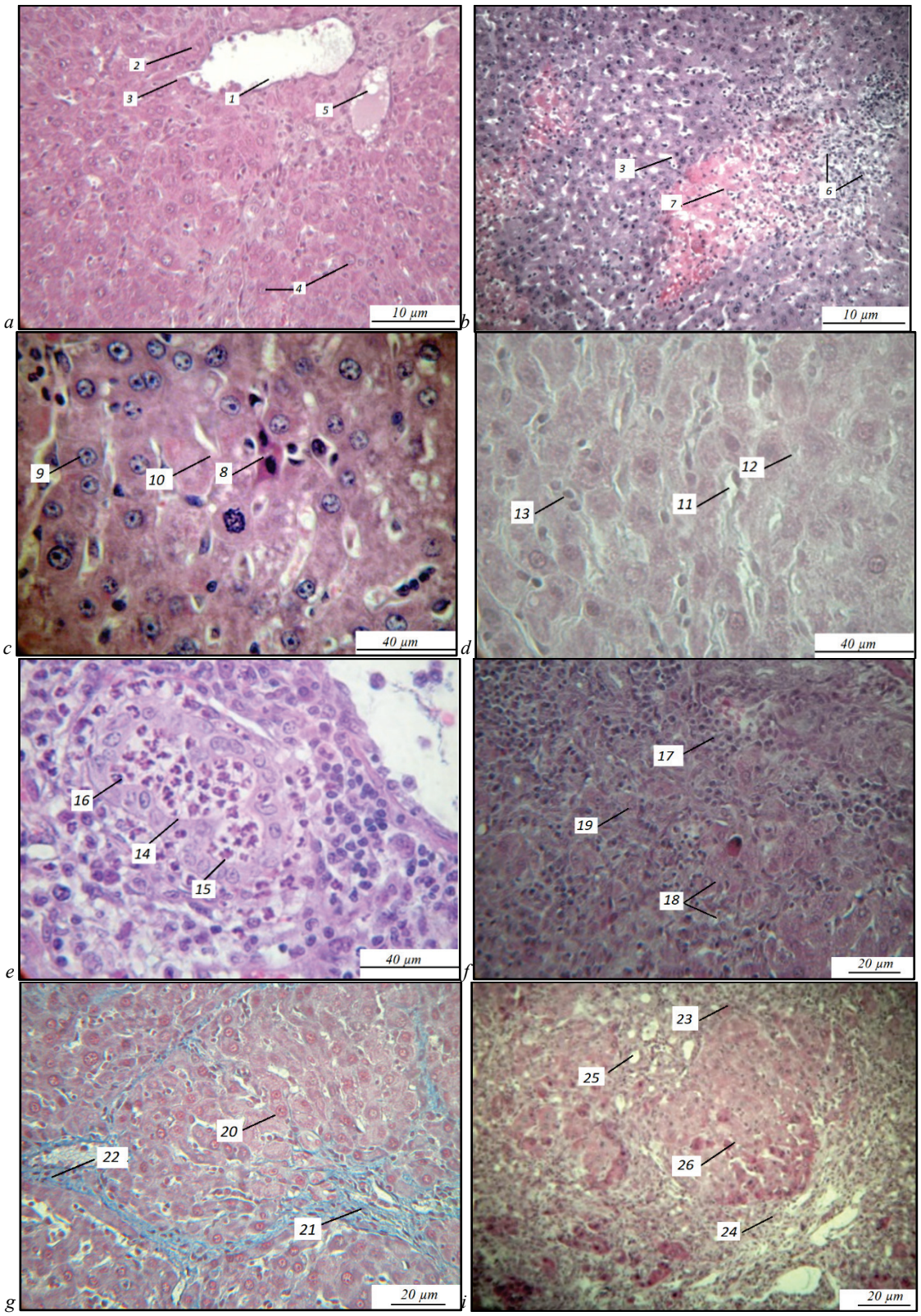

Fig. 1. Histostructure of the liver after ligation of the common bile duct: *a* – 1st day (preserving the normal structure of the liver parenchyma); *b* – 5th day (neutrophil infiltration of the parenchyma with acute necrobiosis); *c* – 7th day (appearance of cells with eosinophilic degeneration and cells in a state of mitotic division in the liver parenchyma); *d* – 7th day (proliferation of Kupffer cells); *e* – destructive purulent cholangitis of the bile duct of the portal zone; *f* – 16th day after ligation, phenomena of active hepatitis; *g* – 18th day after ligation (signs of developing cirrhosis); *i* – 20th day after ligation, micronodular liver cirrhosis; *1* – central vein; *2* – hepatocytes in the liver bundles; *3* – sinusoid; *4* – single hepatocytes in a state of protein dystrophy; *5* – lipocytes; *6* – neutrophilic infiltration of the parenchyma with acute necrobiosis; *7* – regeneration node; *8* – appearance of cells with eosinophilic degeneration; *9* – cells in a state of mitotic division in the liver parenchyma; *10* – sinusoidal capillary; *11* – proliferation of Kupffer cells; *12* – hepatic lobe; *13* – hepatocyte; *14* – loss of particle structure; *15* – islands of false lobes; *16* – separated groups of hepatocytes; *17* – focal interlobular necrosis; *18* – hydropic dystrophy of hepatocytes; *19* – loss of particle structure; *20* – protein (granular) dystrophy of the liver; *21* – fibrous septation of liver parenchyma, signs of developing cirrhosis; *23* – interlobular vein; *24* – lymphohistiocytic infiltrate; *25* – fibrous septation; *26* – large droplet fatty dystrophy; *27* – regeneration zone; staining with hematoxylin and eosin

Therefore, the data in which early proliferation of bile duct epithelium begins with jaundice that persists for 48 hours or more is consistent with our research (Turk et al., 2016). Micronodular cirrhosis with loss of the beam structure of the liver parenchyma and fibrosis of the lobes was reproduced in 33.3% of rats. In general, secondary obstructive biliary cirrhosis of the liver against the background of cholestatic hepatitis was reproduced in 58.3% of animals.

Conclusion

During ligation of the common bile duct in rats, a model of cholestatic liver damage was developed with an assessment of the expression of biochemical, immunological, and morphological predictors of the fibrotic process depending on the terms of the obstruction. Against the background of changes in behavioral reactions during the modeling of obstructive jaundice, hyperbilirubinemia and accumulation of bile acids and, subsequently, an increase in the content of hexosamines and oxyproline were noted, which indicated an increase in the processes of connective tissue organization. Violation of the formation and outflow of bile acids negatively affected the structural and functional state of the liver. Against the background of the imbalance of the cytokine link of immunity and the strengthening of cytotoxic reactions, obstruction of the bile ducts from the first days of ligation was accompanied by the expression of the profibrogenic cytokine TGF-β1 and led to organic changes in the liver that steadily progressed. And, as a result, in 58.3% of animals against the background of cholestatic hepatitis, reproduction of secondary obstructive biliary cirrhosis of the liver appeared, while already in the early stages of obstructive jaundice significant damage to the organ was observed. Based on the obtained clinical, biochemical and morphological data, it is possible to state that ligation of the common bile duct in rats causes inflammatory, necrobiotic, dystrophic changes in the liver, which, against the background of growing endogenous intoxication, increased lipoperoxidation and cholestatic phenomena, stimulate a cascade of processes of excessive organization of connective tissue, with the final effect which is the formation of liver cirrhosis.

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