GEORGIAN BIOMEDICAL NEWS

ISSN (Online): 2720-8796 ISSN (Print): 2720-7994 VOLUME 1. ISSUE 2. APRIL-JUNE 2023

Morphological Changes of the Portal Triad During Experimental Cholangitis

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DOI: 10.52340/GBMN.2023.01.01.25

ABSTRACT

Background: Despite various surgical options, searching for the most effective treatment strategy for cholangitis continues. In light of this, investigations on the interaction between the vascular and biliary components of the portal triad during cholangitis are undoubtedly significant.
Objectives: The present study aimed to investigate vascular and biliary structures of the portal triad during experimental cholangitis conditions.
Methods: 25 rat models were used for experimental cholangitis. Morphological changes of portal triad structures were studied by histological (Hematoxylin and Eosin staining, H&E), immunohistochemical (Pan Cytokeratin AE1/AE3 staining), and histochemical (Masson's Trichrome staining) methods. On preparations stained with H&E and Cytokeratin, histomorphometry of the hepatic bile ducts, their lumen, and the cells of the gallbladder mucosa was performed.
Results: The portal triad was infected entirely, with an epicenter in the peribiliary tissue. Bile ducts were dilated, and their walls were significantly thickened and infiltrated. Thrombosis of the portal veins with damaged arteries and bile ducts was revealed. In addition, the luminal mucus layer was completely damaged.
Conclusions: During cholangitis, pathological processes in the liver develop rapidly due to an inflammatory response in sinusoids caused by bile contamination and high concentration of endotoxins.
Keywords: Bile ducts; cholangitis; liver; portal triad.

BACKGROUND

Cholangitis, or acute inflammation of the bile ducts, was first described by Jean-Martin Charcot in 1877, and in 1903 a link between suppurative cholangitis and bile duct obstruction was first noted by Refers. One of the main etiologic factors for developing acute cholangitis is the presence of stones in the common bile duct. Other causes may include post-traumatic structures of the bile ducts, tumors of the biliary system and pancreaticoduodenal regions, and parasitic invasion.[1,2]

If the permeability of the biliary tract is compromised, bacteria grow in bile, and with complete blockage, the number of microorganisms in bile approaches that of feces. Without surgery, acute purulent cholangitis is fatal.[3]

A group of researchers found an association between mechanical jaundice, acute cholangitis, and biliary sepsis in an experimental cholangitis model in Wistar rats triggered by E. Coli contamination of the common bile duct. Biliary sepsis is distinct from acute cholangitis and requires a special approach for diagnosis and treatment.[4,5]

Based on the research, the authors found that ligation of the common bile duct (without infection) causes mechanical jaundice without any signs of acute cholangitis. Furthermore, ligation of the common bile duct, with contamination, leads to the development of acute cholangitis on the seventh day, when focal damage of the mucous membrane of the ducts begins.[6-8] Based on the findings of the complex pathomorphological studies of the common bile duct, the authors concluded that, in addition to the two known factors (cholestasis and infection), a third factor - damage to the mucous membrane of the bile ducts - is required for the development of acute cholangitis.[9-12]

The changes that occur in the liver during experimental cholangitis are relatively well studied, at least in determining the degree of damage to the hepatocytes. In contrast, data on the morphological changes of the structures portal triad during experimental cholangitis is minimal.

The present study aimed to evaluate the morphological changes in the portal triad in the case of experimental cholangitis.

METHODS

The experiment was performed on Wistar rats weighing 200-250 g. After laparotomy and distal ligation of the common bile duct, a microbial suspension of the hemolytic strain E. Coli N195 (1.105 CFU) (colony-forming unit) was injected directly above the ligature at 1 ml/kg body weight. Rats were sacrificed under ether anesthesia on days 3, 6, and 12 after administration of the microbial suspension.

We used hematoxylin and eosin (H&E) staining to examine liver tissue and monoclonal antibodies AE1/AE3

and Ki-67 to examine bile duct epithelial cells. Masson's method has been used to differentiate liver connective tissue.

Morphometric analysis of preparations stained with hematoxyline-eosin and cytokeratin was used to determine the volume of the bile ducts, their lumen, and cells of the bile mucosa.

For statistical analysis, the T-test and three-way analysis of variance (ANOVA) were utilized.

RESULTS

On the third day after surgery, the common bile duct was slightly enlarged when opening the abdominal cavity macroscopically. There were no noticeable changes in other organs.

The proliferation of bile ducts, dilatation of the lumen with pyknosis of the epithelium, and shedding were seen in hematoxylin and eosin-stained samples. The portal triad was found to have an overabundance of eosinophils. Furthermore, nonobstructive blood microthrombi were seen in the lumen of the portal vein branch compressed by the bile duct. The portal triad syndrome was associated with hepatocyte enlargement and a tendency of Disse spaces to enlarge.

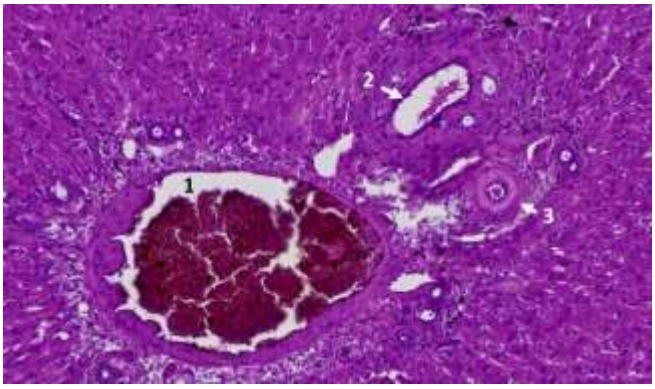
The bile duct lumen was enlarged on the sixth day, and their walls were infiltrated and thickened in the preparations. Signs of inflammation in the portal tract were prominent. The portal vein lumen was coated with shaped elements, some containing bacterial colonies. Furthermore, hepatocyte growth was seen surrounding the portal tract against necrobiosis.

On the 12th day, the portal complex was thoroughly infiltrated, with the peribiliary tissue appearing to be the epicenter. The bile duct was dilated, and its wall was thickened and infiltrated, as was the peribiliary tissue. Thrombosis of portal veins was observed with damage to arteries and bile ducts. The mucous layer in the lumen was shredded entirely off. Fibrosis with dense collagen fibers was seen around the bile duct (Fig.1).

As a result of the translocation of infection on some preparations, purulent masses arising from hemolytic effects were observed in the lumen of the portal vein, along with the masses of peeled debris from the endothelium. Thrombosis and phlebitis of the portal and hepatic veins were well expressed. The central veins were dilated and thrombosed. The lobe was swollen and was in a state of necrobiosis (Fig.2 and Fig.3).

The portal vein collapsed due to the pressure of the dilated bile ducts in the portal tract, and its small remnant and the capillary branches of the next row that originate from it were presented. Necrotic zones are observed in the area of the portal tract, in its center and periphery. The lumen of the bile duct was wholly desquamated. A thrombotic cluster of different shape elements with bacterial invasion is manifested in the preserved branch of the portal vein, which was narrowed due to fibrosis (Fig.4).

FIGURE 1. Experimental cholangitis on the 12th day after surgery. H&E stain, 10x40.



Explanations: 1-Portal vein; 2-Bile duct, epitheliolytic shredding off, thrombosed lumen; 3-Artery wall hyalinization, thrombosed lumen.

FIGURE 2. Thrombosis of the portal tract and hepatic veins (marked with an arrow) in the case of experimental cholangitis on the 12th day after surgery. H&E stain, 10x5

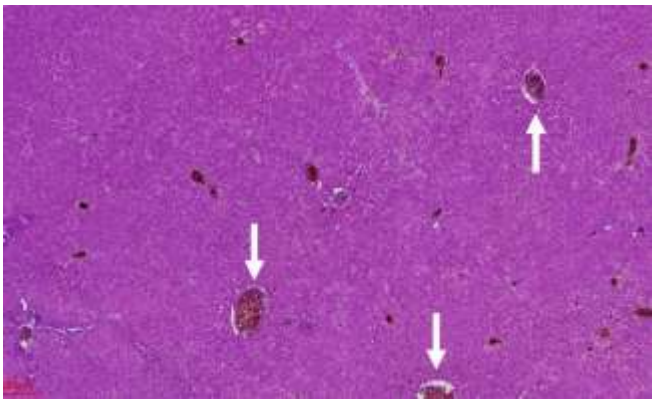
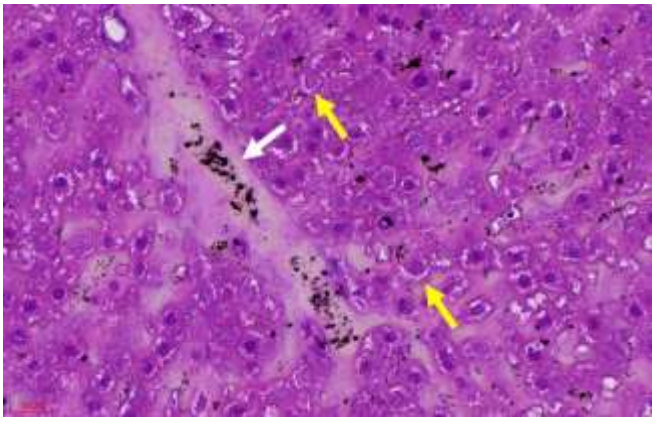


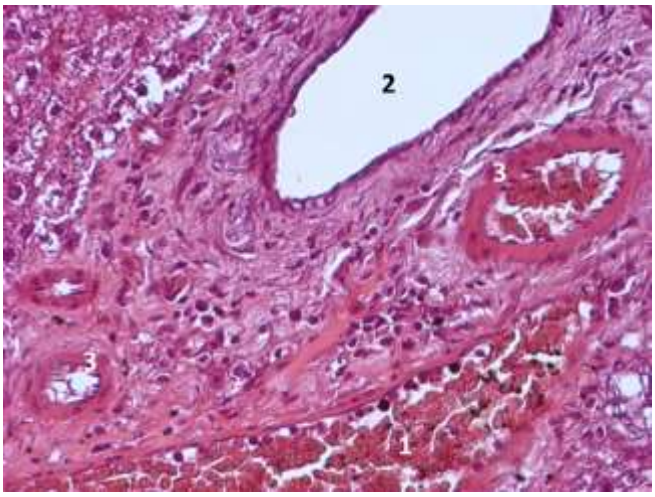
FIGURE 3. Bacterial colonization, hepatocyte lesions and plasma coagulation in the case of experimental cholangitis on the 12th day after surgery. H&E stain, 10x40



Explanations: The white arrow shows bacterial colonies in subsegmental veins. The yellow arrows show hepatocyte lesions of various degrees with perinuclear edema and coagulation of plasma in the vein and adjacent sinusoidal part.

Georgian Biomedical News
ISSN (Online): 2720-8796 ISSN (Print): 2720-7994

FIGURE 4. Periductular fibrosis in the case of experimental cholangitis on the 12th day after surgery. H&E stain, 10x40



Explanations: 1-portal vein; 2-periductular fibrosis; 3-arteries.

The rat bile duct volume, lumen, and mucous membrane cells were also investigated in materials taken from liver tissue after surgery (Tab.1). Different shapes of epithelial layers of the bile, i.e., luminal, circular (closed), and non-circular (open) (with flakes of epithelial cells), were detected.

TABLE 1. Morphometric analysis of bile duct proliferation in the background of experimental cholangitis at different time points

Time	Bile duct volume	Bile duct lumen volume	Epitheliocyte dolume	Cells to bile duct ratio
Control	22,84±8,15	7,56±3,44	2,39±0,46	6,21±0,98
Day 3	75,24±11,85	10,15±0,70	10,07±2,55	6,46±0,47
Day 6	58,86±18,73	12,74±3,12	5,35±1,72	8,62±0,47
Day 12	55,35±14,03	14,55±3,14	1,45±0,25	28,13± 0,13

Note: The data are average and refer to circulatory bile ducts only. The volume is expressed as 10-5mm^2. The difference between the control and experimental groups is reliable (p<0.05).

The ratio of epithelial cells to the bile duct was calculated by subtracting the volume of the lumen of the bile duct from the volume of the bile duct and then dividing by the volume of the epitheliocytes.[13-15]

Our findings reveal that bile duct proliferation is restricted during experimental cholangitis because bacteria in the bile duct lumen cause loss and necrosis of epithelial cells.

DISCUSSION

The increased pressure in the ducts is an essential factor in the etiology of cholangitis. Bacterial translocation from rat bile ducts into the central vein has been demonstrated in studies using corrosive preparations of the ducts.[16-19] A cholangiovenous shunt was forming as the pressure increased. Bacteria invaded the liver sinusoids from the bile ducts through the Disse space. Therefore, endotoxins and microbiological substances could enter the Kupffer cells without resistance.[17,20]

On the sixth day of the experimental cholangitis, significant distention of sinusoids, dilation of bile ducts, excessive pus formation in bile ducts, and hypertrophy of the epithelium and focal necrotic regions were observed in the preparations.

The consequences of cholangitis on the liver should be comparable to those of sepsis. Activation of Kupffer cells is the triggering mechanism during sepsis.[10] The only difference is that purulent bile enters the sinusoid during cholangitis under pressure through the space of Disse. Some mechanisms of hepatocyte injury have been identified and validated. It has been demonstrated that the inflammatory response causes self-harm through hypoxia, microthrombi, cell destruction, necrosis, or apoptosis.[21,22] Kupffer cells contribute to the absorption of toxic chemicals but also damage the hepatocyte membranes.

CONCLUSIONS

Cholangitis causes a rapid inflammatory response due to bacterial contamination and a high concentration of endotoxins in the sinusoids. In addition, bile duct proliferation is restricted because microorganisms in the duct lumen promote the shedding and necrosis of epitheliocytes.

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AKNOWLEDGEMENTS

We would like to thank our colleagues as well as the entire laboratory team of the Morphological Institute at Ivane Javakhishvili Tbilisi State University (TSU).

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ISSN (Online): 2720-8796 ISSN (Print): 2720-7994

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Received 26 Apr 2023

Accepted 6 May 2023