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Research Article

Motor Activity of the Biliary Tract in Primary Sclerosing Cholangitis in the Clinic and Experiment

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Abstract

Aim: To characterize violations of the motor function of the biliary system in PSC in the clinic and experiment; to reveal the morphological correspondence of the studied pathology model.

Materials and Methods: Under observation were 20 patients with PSC in whom the motor function of the biliary system in the clinic was studied electromyographically. The frequency and amplitude of slow waves and spikes, the power of phase and tonic contractions were recorded on the EMG curve. PLC was simulated in 28 rats when 0.1 ml of picrylsulfonic acid was retrogradely injected into the common bile duct.

Results: Electromyography of the common bile duct revealed an increase in the tone of circular muscles, a proportional increase in the power of phase and tonic contractions of the gallbladder with a decrease in the amplitude characteristics of spike activity. When simulating PSC, inhibition of EMG of the common bile duct and the detection of fibrosis of the type "bulbous husk" were noted.

Findings: A decrease in the propulsive activity of the common bile duct in patients was found. The PSC model corresponds to the characteristics of this disease in the clinic.

Keywords: *Primary Sclerosing Cholangitis; Model; Electromyography*

Introduction

Primary sclerosing cholangitis (PSC) (stenosing cholangitis, family large-focal fibrosclerosis) is a chronic cholestatic liver disease of unknown etiology, characterized by non - purulent destructive inflammation, obliterating sclerosis and segmental dilation of the intra - and/or extra - hepatic bile ducts [1].

Prevalence

The true prevalence of PS due to diagnostic difficulties is difficult to establish [2]. PSC is most often diagnosed in the fourth decade of life, and men are twice as likely as women. In the United States, the annual incidence of PSC is approximately 0.5 - 1.3 per 100,000 people, and the prevalence is estimated at 5 - 16. 2 per 100,000 people, which is approximately 30,000 cases in the United States [3]. Outside the United States, the incidence and prevalence of PSC are comparable in Canada [4] and Northern Europe [5]; in North America and Europe, these rates are increasing [6].

In 70 - 80% of patients with PSC, it is combined with chronic inflammatory bowel diseases: more often with ulcerative colitis-UC (60 - 70%), less often with Crohn's disease - BC (10 - 13%), mainly with its forms such as granulomatous colitis and ileocolitis. However, patients with UC PSC are diagnosed in 6% of cases. Isolated PSC is observed in 25 - 30% of patients [1].

Etiology and pathogenesis

The movement of bile along the biliary tract is due to at least two factors: the pressure gradient in different parts of the biliary system, and the difference in intra-cavity pressure in the bladder and duodenum. There is a complex regulation of the functions of this entire system, thanks to which there is a certain consistency in the activity of the liver, gall bladder, common bile duct, pancreas and duodenum. This ensures the timely flow of bile into the intestines during digestion and its accumulation in the gall bladder on an empty stomach. In the digestive phase, the increase in bladder pressure occurs rhythmically in the intervals between the waves of the duodenal peristalsis, the contractions of the bladder correspond to the relaxation of the sphincter apparatus.

The functioning of this complex system is controlled by a number of neurohumoral factors that ensure consistency of actions of all components of the biliary apparatus, which, on the one hand, indicates its high adaptability to constantly changing conditions of digestion, and on the other - allows the development of dysfunction in violation of at least one of the numerous links of regulation.

One of the leading places in ensuring the normal functioning of the biliary system belongs to the autonomic nervous regulation. It is believed that moderate irritation of the vagus nerve causes coordinated activity of the bladder and sphincters, and overexposure - spastic contraction of all these organs, leading to a delay in the evacuation of bile. Irritation (due to various reasons) of the sympathetic nerve leads to relaxation of the gallbladder.

Humoral factors that selectively affect various components of the biliary system are also important: for example, acetylcholine and various cholinomimetics (pilocarpine, proserine) increase the motor activity of the biliary tract. Epinephrine, norepinephrine and hydrocortisone cause Oddi sphincter contraction; α-adrenomimetics stimulate extrahepatic bile tract motility and blockers eliminate this effect. Prostaglandins of group E contribute to the reduction of the gallbladder, and enkephalins and angiotensin inhibit its motor activity.

The most important role in regulating the functions of the biliary system is played by intestinal peptide hormones that have a balanced effect on the tone of the gall bladder mainly: cholecystokinin and gastrin, released during a meal, stimulate its motility and contribute to the release of bile into the duodenum, secretin and glucagon have the same effect. In contrast, neurotensin, vasointestinal hormone, and pancreatic peptide reduce the contractile activity of the gallbladder.

Diagnosis of PSC

Ultrasound is the first, but not the main, method in the range of instrumental methods for diagnosing PSC.

Magnetic resonance cholangiopancreatography. When the common bile and liver ducts are involved in the process, the expansion is determined by the type of diverticular protrusion, with uneven contours, deformation, and multiple zones of narrowing. MRCP is the "gold standard" of diagnosis of PSC.

Multi-slice computed tomography (MSCT), in contrast with MRCP is the method of choice for diagnosis of PSC. Nevertheless, MSCT with contrast serves as one of the methods of differential diagnosis of PSC and bile duct tumor lesion (first of all, Klatskin's tumor).

Endoscopic retrograde cholangiopancreatography (ERCP) is considered as an additional clarifying method of diagnosis in cases where the results of mrcpg and liver biopsy can not be accurately diagnosed or when their implementation is contraindicated. A complex of various invasive and non-invasive methods of radiation diagnostics in patients with PSC allow obtaining reliable information about the state

of the bile ducts, which in the future is of great importance for choosing the optimal tactics of therapeutic and surgical treatment (level of evidence II) [2].

Often, patients with PSC have a clear relationship between the time of pain in the right hypochondrium and various psychogenic factors. Thus, in 82% of people suffering from dyskinesia, especially hyperkinetic forms, during clinical and psychological examination, changes in the psychological status were revealed with a distinct tendency to develop anxiety and depressive disorders. There is a point of view that any form of neurocirculatory dystonia is almost always accompanied by a violation of the motility of the components of the biliary system.

Dyskinesia can occur under the influence of pathological impulses coming from other parts of the gastrointestinal tract and genitourinary system (the so-called "conflict of impulses").

As can be seen from the above data, the pathogenesis of biliary dyskinesia is extremely complex and multifaceted, it is caused by a violation of the innervation of the gall bladder, the action of various humoral factors, intestinal hormones, a violation of the psychological status and various endocrine dysfunctions.

PSC is characterized by a genetic predisposition. The Association of the disease with haplotypes HLA-A1, -B8, -DR3, -DR2, -DR6-DRw52a, etc. is established. various environmental factors are considered as triggers of the disease. It is assumed that the main role in the development of damage to the bile ducts is played by a violation of the bile acid transport systems, which leads to an increase in intra-flow pressure, rupture of the ducts and damage to the liver parenchyma by bile components [7,8]. In PSC in 65 - 87% of patients, antibodies to cytoplasmic antigens of neutrophils with perinuclear type of immunofluorescence (pANCA) are detected in the blood serum. The antigens to which pANCA is produced are currently unknown. The possible significance of catalase, an enzyme involved in the cleavage of hydrogen peroxide to form water and an oxygen molecule, as well as α - enolase, an enzyme involved in glycolysis reactions, in the pathogenesis of the disease, is discussed. In addition, antinuclear antibodies (ANA) (8 - 77%) and smooth muscle antibodies (ASMA) can be detected in patients with PSC) (0 - 83%) [9,10]. Histological changes specific to PSC should be considered sclerosing and obliteration of the intra-and extrahepatic bile ducts by strands of connective tissue of the "bulbous husk" type. PSC differs from primary biliary cirrhosis (PBC) not only in the nature, but also in the level of bile duct damage: in PBC, small intrahepatic and septal bile ducts are affected, while in PSC, any part of the biliary tract may be affected.

Numerous animal models have been developed to study PSC, but given the uncertainties regarding its etiopathogenesis, it is not surprising that none of the models fully match PSC. For example, the mdr2 knockout mouse (ABCB4) [11], the most widely studied PSC model, shows biochemical [12], histological [12] and cholangiographic signs of human PSC [13], but there is no predominance of PSC in males, and there is no Association of PSC with IBD [12]. Thus, there is no consensus on the optimal model, which hinders the development of new treatment methods [14].

Aim of the Study

The goal is to characterize violations of the motor function of the biliary system in PSC in the clinic and experiment; to identify the morphological correspondence of the model of the studied pathology.

Materials and Methods

20 patients with PSC from the Department of Hepatology of the Central research Institute of gastroenterology (senior researcher Fedotova T. N.) were under observation. Upon admission, patients reported itching in 39.8%, pain in the right hypochondrium in 40%, violation of the color, periodicity and consistency of the stool in 60.2% of cases. Objectively, patients showed signs of chronic hepatitis

with possible transformation into cirrhosis in 20.1% of cases, signs of chronic cholecystitis in 60.5% of cases, chronic pancreatitis and pancreatic cysts in 40.2%, narrowing of the common hepatic duct - according to ultrasound research, and ulcerative colitis in 15.5% of cases - according to endoscopic research. According to a biochemical study, an increase in the level of alkaline phosphatase was noted in all observed patients and in 10% of cases - the level of bilirubin.

Morphologically, there were signs of cholestasis, periductal fibrosis with the formation of fibrous rings of the type of bulbous husk, compression of the lumen of the duct to complete obliteration, proliferation of ductules, cholangiectasis, round-cell infiltration around the bile ducts.

The motor function of the biliary system in the clinic was studied electromyographically by applying bipolar platinum electrodes with a diameter of 0.5 - 0.6 mm^2 in the area of projection of the studied organs (the common bile duct and gall bladder, as well as the stomach and duodenum) on the anterior abdominal wall. On the EMG curve, the frequency and amplitude of slow waves and spikes, the power of phase and tonic contractions were recorded using the Conan-M hardware and software complex with a bandwidth from 0.01 Hz to 10 kHz and a noise level less than 1 - 5 mV, with a sensitivity of 96% and a specificity of 95% [15,16].

Primary sclerosing cholangitis was modeled on 28 Wistar rats weighing 200 - 220g. 0.1 ml of picrylsulfonic acid was retrograde injected into the common bile duct in a 1:1 ratio with 96% ethyl alcohol [17]. Motor function of the common bile duct was evaluated by the electromotor activity of smooth muscles. Electromyography (EMG) was performed using the same technique as in the clinic using the Conan-m hardware and software complex. EMG was performed before the experiment, immediately after the introduction of picrylsulfonic acid and on the 15^{th} day of PSC modeling. The EMG curve was used to estimate the frequency-amplitude characteristics of slow waves and the power of tonic contraction.

Morphological examination of the liver and intrahepatic bile ducts was also performed on the 15^{th} day of PSC. Tissue samples were fixed in 9% formalin, dehydrated in alcohols of increasing concentration, histological sections were prepared and stained with methylene blue.

Statistics

Statistical processing of the obtained data was performed using the Statistica-17 software package. All quantitative data that are subject to the normal distribution are presented as M ± m. the Student's criterion (t) was used for processing the obtained data, followed by determining the level of confidence of the differences (p) and the criterion χ^2. Differences between the mean values were considered significant at $p < 0.05$.

Results and Discussion

Research on patients

Electromyographically, the frequency of slow waves of the common bile duct in patients with PSC was 10.7 ± 1.2 per minute (an increase of 18.9% $p < 0.05$) and the amplitude was 0.15 ± 0.04 mV (an increase of 50.2% $p < 0.05$). The power of tonic contractions was 1.605 ± 0.125 (an increase of 78.3% $p < 0.04$). The frequency of spikes was 3.3 ± 0.007 (an increase of 230% $p < 0.01$), the amplitude of spikes was 0.04 ± 0.005 mV (a decrease of 59.8% $p < 0.05$) and the power of phase contractions was 0.132 ± 0.012 (an increase of 32% $p < 0.05$). Thus, the electromyography of the common bile duct revealed an increase in the tone of the circular muscles, which determines a decrease in its propulsive activity.

The frequency of slow waves of the gallbladder was: - 8.3 ± 0.8 V min (an increase of 8.75% $p < 0.05$), the amplitude-0.09 ± 0.001 mV (a decrease of 10% $p < 0.05$). The power of tonic contractions was 0.747 ± 0.0612 (an increase of 56% $p < 0.05$). The spike frequency was 3.6 ± 0.41 (an increase of 260% $p < 0.01$), and the spike amplitude was 0.04 ± 0.007 mV (a decrease of 60.1% $p < 0.05$). The power of phase reductions was 0.144 ± 0.012 (an increase of 44% $p < 0.05$). That is, there is a proportional increase in the power of phase and tonic contractions of the gallbladder.

In addition to the study of the motor function of the biliary tract, the motor function of the upper digestive tract was studied. The frequency of slow EMG waves of the stomach in patients with PSC was 9.4 ± 0.7 per minute (an increase of 72.7% $p < 0.05$), the amplitude was 0.12 ± 0.08 mV (a decrease of 20% $p < 0.01$), the power of tonic contractions was 1.128 ± 0.15 (an increase. 36.7% $p < 0.05$); the frequency of spikes was 3.7 ± 0.3 (an increase of 269.5% $p < 0.01$), the amplitude was 0.02 ± 0.008 per minute (a decrease of 79.9% $p < 0.05$), the power of phase contractions was 0.074 ± 0.0003 (a decrease. by 26% $p < 0.05$). Thus, gastric motility in PSC is characterized by an average increase in the power of the phase and tonic components of contractions.

Electromyographically the frequency of slow waves of the duodenum is reduced in frequency to 14.2 ± 0.83 in min (a reduction of 29% $p < 0.05$), amplitude - 0,12 ± 0,03 mV (an increase of 20% $p < 0.05$), the power of the tonic contractions amounted to 1,704 ± 0,185 (a decrease of 22.5%, $p < 0.05$); the rate of spikes increased to 3.0 ± 0,07 (increase 199,8%, $p < 0.01$), amplitude - 0,12 ± 0,003 min (increasing by 20% $p < 0.05$); the power of the tonic contractions was 0.36 ± 0,04 (an increase of 260%, $p < 0.01$). Thus, the propulsive activity of the duodenum is changed by reducing the power of tonic contractions of the longitudinal muscle layer and increasing the spastic activity of the circular muscles.

Animal research

The frequency of EMG of the common bile duct in intact control group rats was 21.0 ± 4.6 V min, and the amplitude was 0.78 ± 0.3 mV. The power of tonic contractions was 16.38 ± 1.51. Administration of picrylsulfonic acid to rats in the experimental group resulted in an increase in the frequency response of EMG by (44.3%, $p < 0.05$) and a decrease in the amplitude response by 82% ($p < 0.05$). The power of tonic contractions is reduced by 70.9% ($p < 0.05$). EMG of the common bile duct on day 15 of the model of primary sclerosing cholangitis showed a progressive decrease in the amplitude-frequency characteristics of slow-wave activity and a decrease in the power of tonic contractions by 252.8% ($p < 0.01$) of the common bile duct (Table 1).

EMG indicator	EMG models	EMG models, 15 days	Control
The frequency of slow waves	30,3 ± 4,9	28,0 ± 3,1	21,0 ± 4,6
Amplitude of slow waves	0,14 ± 0,01	0,1 ± 0,03	0,78 ± 0,09
Power of tonic contractions	4,24 ± 0,35	2,801 ± 0,17	16,38 ± 1,51
p	P < 0,05		

Table 1: *Electromotor activity of the common bile duct in an experiment under various conditions.*

Morphological examination of intrahepatic bile ducts, conducted on day 15 of the PSC model, revealed a sharp fullness of liver tissue (Figure 1). The expanded sinusoids contained numerous shaped blood elements. Small hemorrhages were observed in different parts of the hepatic parenchyma. Vacuole dystrophy of hepatocytes was detected. Intrahepatic bile ducts were not detected in all the studied fields of vision (Figure 1).

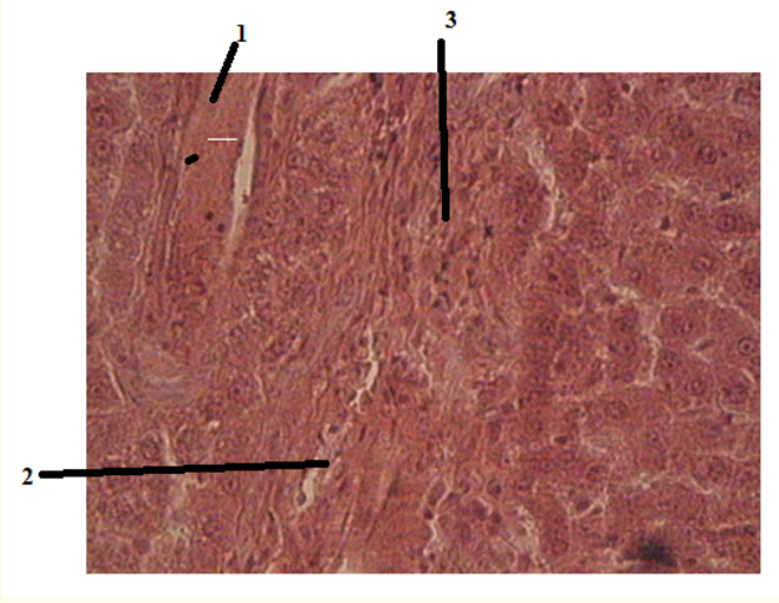

Figure 1: *Liver in primary sclerosing cholangitis in the experiment. 1-Full-blooded liver tissue, 2-Expanded sinusoids with shaped blood elements, 3-Fibrosis of the liver tissue in the form of "bulbous husk" Staining with hematoxylin and eosin. UV x 250.*

The results obtained indicate that the functional state of the common bile duct when modeling primary sclerosing cholangitis (PSC) was characterized by sclerosis of the intrahepatic bile ducts and inhibition of EMG of the common bile duct. Morphologically, intrahepatic bile ducts were not detected on the 15th day of PSC, and connective tissue fibers like "bulbous husks" are formed around the obliterating bile ducts. The resulting model corresponds to the electrophysiological and morphological characteristics of PSC. The detected decrease in the power of contractions of the smooth muscles of the ducts is due to the replacement of smooth muscles with connective tissue.

Conclusion

The study showed that patients with PSC have hypomotor biliary dyskinesia. Against this background, there is an increased propulsive activity of the stomach and a delay in the food content in the duodenum due to spasm of the duodenum circular muscles. Reduced motor function of the common bile duct and gall bladder contributes to the development of hypomotor small bowel dyskinesia due to reduced bile intake, which contributes to the development of excessive bacterial growth syndrome and further progression of hypomotor small bowel dyskinesia.

The presented model of PSC corresponds to the characteristics of this disease in the clinic, which allows it to be used for testing new methods of treatment. The possibility and effectiveness of using the electromyography method for the diagnosis of early stages of PSC is shown.

Bibliography

1. Podymova SD. "Diseases of the liver: A guide for doctors". Edition. 5th, Moscow: Medical Information Agency LLC (2018): 984.

2. Vinnitskaya EV., *et al.* "Current issues in the diagnosis and treatment of primary sclerosing cholangitis (based on the Russian consensus on the diagnosis and treatment of primary sclerosing cholangitis". 91.2 (2019): 9-15.

3. Toy E., *et al.* "The prevalence, incidence and natural history of primary sclerosing cholangitis in an ethnically diverse population". *BMC Gastroenterology* 11 (2011): 83.

4. Kaplan GG., *et al.* "The burden of large and small duct primary sclerosing cholangitis in adults and children: a population-based analysis". *The American Journal of Gastroenterology* 102 (2007): 1042-1049.

5. Boonstra K., *et al.* "Population-based epidemiology, malignancy risk, and outcome of primary sclerosing cholangitis". *Hepatology* 58 (2013): 2045-2055.

6. Molodecky NA., *et al.* "Incidence of primary sclerosing cholangitis: a systematic review and meta-analysis". *Hepatology* 53 (2011): 1590-1599.

7. Ivashkin VT., *et al.* "Autoimmune diseases of the digestive system". *Vestnik of the Russian Academy of Medical Sciences* 70.2 (2015): 139-151.

8. Karlsen TH and Boberg KM. "Update on primary sclerosing cholangitis". *Journal of Hepatology* 59.3 (2013): 571-582.

9. Hart PA., *et al*. "Long term outcomes of autoimmune pancreatitis: a multicentre, international analysis". *Gut* 62.12 (2013): 1771-1776.

10. Rong G., *et al*. "Incidence and Risk Factors for Hepatocellular Carcinoma in Primary Biliary Cirrhosis". *Clinical Reviews in Allergy and Immunology* 48.2 (2015): 132-141.

11. Smit JJ., *et al.* "Homozygous disruption of the murine mdr2 P-glycoprotein gene leads to a complete absence of phospholipid from bile and to liver disease". *Cell* 75 (1993): 451e462.

12. Fickert P., *et al.* "Regurgitation of bile acids from leaky bile ducts causes sclerosing cholangitis in Mdr2 (Abcb4) knockout mice". *Gastroenterology* 127 (2004): 261e27.

13. Tabibian JH., *et al.* "Micro-computed tomography and nuclear magnetic resonance imaging for noninvasive, live-mouse cholangiography". *Laboratory Investigation* 93 (2013): 733e743.

14. Pollheimer MJ., *et al.* "Will we ever model PSC? - "it's hard to bea PSC model!". *Clin Res Hepatol Gastroenterol* 35 (2011): 792-804.

15. Lychkova AE. "Coordination of myoelectrical activity of the small and large intestine". *Experimental Clinical Gastroenterology* (2012): 59-61.

16. Lychkova AE and Puzikov AM. "Electrical activity of the digestive tract and its enteral correction". *Experimental Clinical Gastroenterology* 120.8 (2015): 25-29.

17. Lychkova AE and Puzikov AM. "The method of modeling primary sclerosing cholangitis RF patent № 2516737".

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