Biotechnological Techniques to Improve Regenerative Abilities of Liver Tissue in Experimental Cholestasis

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Abstract. The study examined the effect of plant hepatoprotectors obtained in the process of biotechnological techniques and thermal effects on liver tissue to prevent the destruction of cell membranes and stimulate regenerative processes in hepatocytes in experimental models (12 dogs). All animals underwent complex conservative therapy, including a plant hepatoprotector with the code name GTPS-4, containing a purified dry extract from the fruits of milk thistle, dry extract from tansy, St. John's wort, turmeric, birch leaves and immortelle flowers. Conservative therapy was combined with thermal effects on the liver tissue to enhance reparative regeneration. The combination of herbal preparations and contact coagulation techniques gave the best result, and the optimal combination of herbal preparations in the form of collection was also proposed. The structural and functional state of the liver was assessed according to the results of morphological studies. The performed complex therapy significantly improves the condition of the liver tissues, its regenerative abilities and normalizes homeostasis indicators. The inclusion of plant hepatoprotectors in complex conservative therapy is a promising direction in the development of biotechnology in hepatology.

1. Introduction

The most severe complication of many diseases of the hepatopancreatoduodenal zone is a violation of the outflow of bile with the development of cholestatic hepatitis. The main reasons for the development of obstructive cholestasis are cholelithiasis and choledocholithiasis in 45–55% of cases, tumors of the hepatobiliary region in 35–45% of cases, as well as purulent and sclerosing cholangitis, cicatricial strictures, pancreatitis, and pancreatic cysts in 8–11% of cases [1, 2].

Violation of the outflow of bile entails an increase in pressure in the lumen of the bile ducts above the level of obstruction. An increase in intraluminal pressure leads to the penetration of bile and its components through the stretched walls of the bile ducts into the Disse space, affects hepatocytes and passes through the sinusoids into the bloodstream. The time factor is of great importance in this case, the longer the accumulation of bile in the ducts lasts, the more the pressure in the hepatic ducts increases and the liver cells are exposed to more toxic effects, and endotoxicosis also increases. As a result of all the processes described, significant structural damage occurs in hepatocytes, because of which violations of numerous functions of the liver develop, which is expressed in the form of liver failure and is the cause of high mortality from 14 to 44% [3, 4].

During their life, liver cells can divide 1–2 times, if there are no special stimulating effects. Many researchers have noticed that with trauma of the liver tissue or the loss of a part of an organ, the recovery
processes and the regenerative capacity of the cells of the liver parenchyma are enhanced, which manifests itself in the multiplication of cells by division, the formation of highly specialized cells, their movement, as well as the restoration and reorganization of the structure of the connective tissue, the phenomena of neoangiogenesis during the transformation capillary network.

The nature of structural changes in the liver parenchyma is largely due to the severity and duration of cholelithiasis. Morphological lesions are manifested by a combination of protein and fatty degeneration of hepatocytes, the presence of necrotic changes inside the liver lobule, uneven inflammatory infiltration of the portal and intralobular stroma by macrophages, single lymphocytes and neutrophils, the development of portal and perisinusoidal sclerosis [5, 6, 7].

These pathological changes continue to progress after the elimination of the etiological factor. In 40–60% of patients, clinical symptoms are usually not expressed with already existing structural abnormalities of the liver, and the data of biochemical studies do not correlate with the severity of morphological changes. Aggravation of disorganization of liver structures leads to chronicity of the process and further enhances morphofunctional disorders of the hepatobiliary system, patients are at risk of developing cirrhosis of the liver and its complications. An effective way to restore liver function and liver tissue structure is to stimulate proliferative processes. An alternative to the cellular multiplication of hepatocytes during the growth of the liver is hypertrophy of hepatocytes and the formation of new cellular elements, which is a component of a single restorative reaction of the liver [8, 9].

It was found that even after the loss of 70% of the liver parenchyma, it is possible to restore its volume and function due to mitotic division of the remaining structural elements. An important point for the restoration of the disturbed morphofunctional state of the liver is the suppression of the vicious circle of various pathophysiological mechanisms leading to the chronicization of the pathological process and its transition to cirrhotic. An effective solution to this issue is the development of new methods for stimulating the reparative regeneration of liver tissue, both because of complex conservative treatment and surgical methods of correction [10, 11].

Functionally active plant compounds with effects that reduce inflammation, inhibit oxidative reactions, stimulate the immune system, destroy microbial agents, and improve bile duct peristalsis and bile outflow have successfully proven themselves as methods of complex conservative stimulation of liver regeneration. Components of herbal preparations with a pronounced antioxidant effect, for example, flavonoids, play a dominant role in influencing actively the links of pathogenesis. Plant polyphenols can stop or significantly reduce the oxidative effect of free radicals, help protect cell membranes from destruction, ensure the preservation of intracellular structures, prevent the damaging effects of excess histamine release [12]. When analyzing the action of herbal preparations, the combination of various active compounds and the mutual influence of phytore preparations, synergism or antagonism must be considered. Also, when choosing a hepatoprotector, the necessary requirements for such drugs are considered. They must penetrate well through the intestinal villus into the vascular bed, primarily pass through the liver and participate in the entire physiological complex of biochemical processes in the liver tissues, prevent the formation or neutralize toxic compounds, reduce the inflammatory response, help reduce the formation of excess connective tissue fibers and improve regenerative processes in the liver. Plant hepatoprotectors are distinguished by the properties of correction and normalization of metabolic processes in the body, inhibit lipid peroxidation, have antihypoxic activity, can combine with toxins and prevent their interaction with other components, reduce their toxicity or form their inactive analogs, and also contribute to the formation of nucleotides. Infusions of medicinal plants contain various glycosides, polysaccharides, steroids, antioxidant enzymes, vitamins, polyunsaturated acids, polyphenols, which explains their inhibitory effect on oxidation. The combination of active components of various plants is characterized by significant variability, therefore, it is necessary to use specially selected combinations of collections of plant raw materials, characterized by a unique set of positive qualities of hepatoprotectors and capable of effectively stimulating liver regeneration to obtain the most optimal therapeutic effect. Such an integrated approach opens up great opportunities for the creation of new combined herbal preparations for the treatment of cholestasis [11, 12].
It is possible to achieve the normalization of the histoarchitectonics of the liver tissue with the stimulation of the regenerative processes of the liver by means of its partial microresection, however, it was noted that this operation is associated with a certain risk of complications and deaths. Resection is contraindicated in chronic persistent hepatitis, any stage of primary biliary cirrhosis, hepatocellular chronic active hepatitis, and cirrhosis of any genesis. Unlike resection, electrocoagulation of the liver (2–3 volts, 7 amperes) is simple and effective to use, and is less aggressive than liver resection. When using electrocoagulation of 23–25% of the liver surface, a decrease in the degree of fibrosis is achieved.

The use of one method aimed at liver regeneration is often ineffective; therefore, several authors [12, 13] suggest using a combination of methods. Given the relevance of the issue, it was decided to conduct an experimental study to determine the most optimal combination of methods that stimulate the regeneration of liver tissue using biotechnological techniques.

2. Purpose
To improve the results of the treatment of cholestasis by stimulating the regeneration of liver tissue by the method of combined therapy with hepatoprotectors of plant origin and electrocoagulation of the liver parenchyma.

3. Materials and methods
An experimental model of long-term cholestasis was formed in 12 unbred dogs, whose weight ranged from 7 to 20 kg. The stages of the experiment and monitoring of the state of the animals were carried out in accordance with the “Rules for conducting research using experimental animals” of the Ministry of Health of the Russian Federation. All dogs were in the same comfortable environment for keeping and caring, during the study they adhered to the same diet and composition. The animals underwent removal of the gallbladder by laparoscopic access and ligation of the common bile duct under general anesthesia.

Subsequently, the animals were divided into two groups:

I group – control group: 6 dogs with experimental occlusion of the common bile duct lasting 21 days, which underwent bypass choledochoduodenostomy (CDA) on day 22.

II group – 6 dogs with experimental occlusion of the common bile duct lasting 21 days, for whom choledochoduodenostomy was developed on the 22nd above the duodenum in combination with electrocoagulation of liver tissue to enhance regenerative abilities, in the postoperative period the animals underwent complex conservative therapy with hepatoprotectors of plant origin.

A fragment of liver tissue was removed in both experimental groups for the purpose of histological control, this manipulation was carried out before modeling biliary occlusion, 21 days after ligation of the common bile duct during surgery to form choledochoduodenostomy and 30 days after experimental obstructive jaundice.

Tissue was taken from three different parts of the organ, considering segmental division, with a volume of 1 cm3; pieces of a triangular shape were excised, with the apex facing the periphery of the segment and the base directed in depth like an isosceles triangle. A thorough stop of bleeding was carried out by coagulation and suturing. Subsequently, the preparation of histological preparations was carried out in a standard way with staining with hematoxylin-eosin and according to Van Gieson.

At the first stage of the experiment, the animals of both groups under combined endotracheal general anesthesia with artificial ventilation of the lungs, through the laparoscopic access, underwent cholecystectomy and ligation of the common bile duct in the supraduodenal part. All animals underwent liver biopsy at the first stage of the experiment to study the initial state of the liver. When analyzing the histological picture of liver tissue preparations taken before the creation of occlusion of the common bile duct in both groups, a normal structure of the liver parenchyma was revealed with the preservation of the structure of the hepatic tracts.

At stage II, dogs of the first group (control group) underwent supraduodenal choledochoduodenostomy, liver biopsy was performed. Hepatoregenerative intervention was not performed. In animals of the second group (main group), at stage II, along with CDA, liver tissue was
coagulated on the upper surface in alternating order with a 40 W ball electrode, the duration of exposure at each point was 5 seconds.

At stage III (30 days after CDA application), all dogs underwent liver biopsy using the same technique, and the animal passed to another stage of the experiment.

Experimental animals that underwent an operation to create a model of biliary occlusion, on the first day and the days following the surgery, for the purpose of detoxification, were infused with intravenous administration of 5% glucose solution, in an amount of 50 ml/kg of animal weight per day, with the addition of a solution of trental 2.0, vitamin C – 5% -10.0, vitamins of group B (Vit B1 – 1.0 p/m, Vit B6 – 1.0 i/m every other day), vitamin E 100 mg p/m, for the prevention of development purulent complications, cholangitis, all experimental animals underwent antibiotic therapy (gentamicin solution 80 mg/day/m). Thanks to the developed methods of postoperative management of experimental animals, we were able to observe changes in the liver after 21 days of obstructive jaundice and to carry out further stages of research, such as the implementation of hepatoregenerative interventions on the liver, subjected to the destructive effects of hyperbilirubinemia. The animals of the second main group were daily fed with a decoction of the hepatoprotective collection GTPS-4, containing a purified dry extract from the fruits of milk thistle, dry extract from tansy, St. John's wort, turmeric, birch leaves and immortelle flowers.

4. Results

After 21 days after the modeling of cholestasis, at the second stage of the experiment during the surgical intervention, pronounced external changes in the liver were recorded in all animals: large sizes of the organ are noted, the parenchyma is saturated with bile and has an olive tint, the consistency is compacted, the anterior lower edge is thickened, smoothed. As a result of examination of the common bile duct, its expansion was found to 10 mm at a rate of 2 mm. As a rule, the adhesion process in the abdominal cavity was absent or was expressed slightly.

A liver biopsy was performed prior to supraduodenal choledochoduodenanastomosis (SDCDA). SDCDA was applied with a single-row continuous suture with atraumatic absorbable suture Vicryl 4-0 to eliminate obstructive jaundice. After that, the operation in the animals of the first group was completed, and in the second group, electrocoagulation of the liver was carried out to stimulate the regenerative processes.

A pronounced balloon and fine-grained dystrophy of hepatocytes, dilated bile capillaries, expansion of the portal tracts, intralobular stagnation of bile, bile lakes, inflammatory changes in the walls of the bile ducts, violation of the architectonics of the hepatic beams, signs of cholestatic hepatitis were found in all animals after 21 days of mechanical occlusion and cholestasis because of the analysis of the histological picture of the liver tissue (figure 1). The described structural changes characterize deep morphological and functional disorders in the liver.

When examining the histological picture of the liver tissue 30 days after the experimental occlusion, it was found that in the animals of the first control group, only a small part of the pathological changes, such as the expansion of the bile capillaries and portal tracts, underwent reverse development. Thus, only processes of an acute nature, depending on an increase in pressure in the biliary system, and disappearing after its decompression, have been eliminated. And more serious structural abnormalities in the liver, such as dystrophy of hepatocytes after a prolonged biliary occlusion, not only did not disappear after the imposition of CDA, but also progressed, further aggravating pathological changes in the liver architectonics (figure 2).
Figure 1. Histological specimen of the liver tissue of a dog with experimental cholestasis lasting 21 days. Staining with hematoxylin-eosin and according to Van Gieson mag. x600.

Figure 2. Histological specimen of the liver tissue of the dog of the I control group after elimination of jaundice on the 30th day. Staining with hematoxylin-eosin and according to Van Gieson mag. x150.
Figure 3. Histological specimen of liver tissue in a dog of group II after elimination of jaundice and hepatoregenerative intervention on day 30. Staining with hematoxylin-eosin and according to Van Gieson mag. x150.

Table 1. Morphometric parameters of liver tissue in dogs.

| Elements of the stroma and parenchyma of the liver | Collagen fibers (%) | Hepatocytes (%) | Lobule of the liver (%) | Glycogen (%) | Fat inclusions (%) |
|---------------------------------------------------|---------------------|-----------------|-------------------------|--------------|-------------------|
| Cholestasis lasting 22 days                        | 12.1 ± 0.3          | 40.2 ± 1.2      | 24.1 ± 0.4              | 75.02 ± 2.21 | 2.39 ± 0.19       |
| I group                                           |                     |                 |                         |              |                   |
| Condition after decompression day 30              | 20.1 ± 0.3          | 51.3 ± 1.2      | 31.2 ± 0.4              | 76.02 ± 2.21 | 3.29 ± 0.19       |
| II group                                          |                     |                 |                         |              |                   |
| Condition after decompression and regeneration stimulation day 30 | 4.5 ± 0.4*         | 69.2 ± 1.3*     | 57.5 ± 0.3*             | 83.03 ± 1.13 | 1.74 ± 0.14       |

* When compared with the control group p < 0.05
In the second main group of animals, where the application of CDA was supplemented with hepatoregenerative intervention, the histology of liver tissue was also studied 30 days after the combined use of biliodecompressive and hepatoregenerative intervention. At the same time, it was found that insignificant phenomena of hepatocyte dystrophy persisted, the remaining pathological changes in the liver were eliminated (figure 3).

After laparoscopic electrocoagulation of the liver, a significant decrease in the volume of collagen fibers was noted, an increase in the number of hepatocytes with signs of hypertrophy was noted. During the study of histological preparations, an increase in the accumulation of glycogen was revealed in comparison with the first control group. The histological study of liver preparations in dogs of the second main group confirmed the activation of regenerative processes in the liver, and the clinical picture also improved. The greatest changes during the study were found on the 30th day after the experimental exposure. A significant decrease in the number of stromal collagen fibers was revealed in comparison with the control group. At the same time, the volume of hepatocytes increased, a high content of glycogen in their hyaloplasm was revealed in comparison with the first group of animals. A decrease in the volume of fatty infiltration of liver cells was also noted, the parameters of the volume and ratio of acini remained unchanged, the most pronounced regeneration of cellular elements was noted, with a decrease in the volume of collagen fibers and the severity of fatty infiltration. The number of hepatocytes with the content of glycogen in them increased (table 1).

Thus, it can be considered experimentally confirmed that with a combination of the operative restoration of bile flow, reduction of hypertension in the biliary tract, electrocoagulation of liver tissue and the use of plant hepatoprotective charges in the postoperative period, the regenerative abilities of the liver are significantly improved, its architectonics and structural and functional state are restored.

5. Conclusion
1. As a result of the experiment, it was found that the only restoration of the outflow of bile and a decrease in pressure in the bile ducts does not contribute to the elimination of severe morphological and functional changes in the liver.
2. An integrated approach to treatment, including the elimination of occlusion of the biliary tract, stimulation of liver tissue regeneration by electrocoagulation and the use of plant hepatoprotective preparations contributes to the reverse development of pathological disorders in the liver, which dictates the need for their use in the clinic.
3. The revealed positive effect of the proposed combination of hepatoprotective herbal collection in comparison with individual preparations makes it possible to use it as a promising agent for stimulating liver tissue regeneration, use it in the treatment and prevention of liver diseases.

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