ANNOTATION

of dissertation work by Tanabayeva Shynar Baimakhanovna on the topic **«Morphofunctional changes in the liver against the background of portal hypertension and after its correction (experimental study)»**, submitted for the degree of Doctor of Philosophy (PhD) in the specialty 8D10103 – «Medicine»

Relevance of the research topic.

Portal hypertension is a serious complication of cirrhosis, and its sequelae, including ascites, esophageal varices, hepatic encephalopathy, and hepatorenal syndrome, lead to significant morbidity and mortality [Turco L, et al., 2019]. Portal hypertension is defined as a sustained increase in intraluminal pressure in the portal vein and its collaterals with a mean pressure of more than 12 mmHg. Art. An increased portal pressure gradient may result from increased portal blood flow, increased vascular resistance, or a combination of both [Bosch J, et al., 1992].

The etiology of increased portal resistance is usually classified according to anatomical location into prehepatic, intrahepatic, and posthepatic causes [Bloom S, et al., 2015].

Treatment of portal hypertension is applied in three clinical scenarios: prevention of the first variceal bleeding (primary prevention), treatment of an acute bleeding episode, and prevention of variceal bleeding (secondary prevention) [de Franchis R, et al., 2004].

Portal pressure can be reduced by vasoconstrictors, which reduce portal vein inflow, and vasodilators, which reduce intrahepatic resistance [Garcia-Pagan JC, et al., 1990].

Although pharmacological therapy has limited effectiveness for portal hypertension, portosystemic shunt procedures are highly effective in reducing portal pressure. The choice of the optimal option for portocaval shunting remains the subject of ongoing debate, which is associated with the complexity of the problem, in particular the features of the pathogenesis and clinical course of portal hypertension [Tretyakov AA., et al., 2016].

Bypass procedures, although not curative, provide longer-lasting and more effective treatment than drug or endoscopic therapy in preventing recurrent variceal bleeding [Rosemurgy AS, et al., 2003].

Surgical or radiological shunt interventions that divert portal blood into the systemic circulation can achieve even greater reductions in portal pressure [de Franchis R, et al., 2004]. A large number of surgical techniques (shunts and devascularization procedures) have been developed to treat portal hypertension, and many publications support or reject their use [Mercado MA., et al., 2015].

There are 2 types of surgical shunts: central (non-selective) and non-central (selective) [Rosemurgy AS, et al., 2005]. Central shunts decompress the portal system, reducing portal pressure and thus indirectly decompressing the varices by directing their portal flow.

What all nonselective portosystemic shunts have in common is that they eliminate portal perfusion of the liver. This means that brain toxins absorbed in the intestines cannot be eliminated from the body. As a consequence, an increased incidence of postoperative encephalopathy was observed in prospective randomized trials comparing selective and nonselective shunts [Langer, B, et al., 1985].

Off-center shunts selectively decompress gastroesophageal varices without directly affecting portal pressure by decompressing the varices through the short gastric vessels or coronary vein. Also, with selective shunts, the risk of postoperative liver failure is lower compared to non-selective shunts, since portal perfusion of the liver remains unchanged [Klempnaue J, et al., 2001].

There are potential long-term complications, the most significant of these complications include shunt thrombosis, increased surgical risk during subsequent liver transplantation, and the development of portosystemic encephalopathy [Zakaryan NV, et al., 2018].

Hepatic encephalopathy is a special type of brain dysfunction caused by liver failure and/or portal-systemic shunting [Amodio P., 2018]. Encephalopathy is a common problem after traditional bypass surgery in patients with cirrhosis, but is uncommon in patients without liver parenchymal disease [Valayer J, et al., 1998]. Encephalopathy after shunt placement may occur due to decreased hepatopetal blood flow,which leads to functional impairment of the organ and an increase in the flow of toxic substances into the systemic circulation through the liver [Sachdev A, et al., 2003].

Chronic portosystemic encephalopathy, which can develop after bypass surgery and may be refractory to medical treatment, is a devastating clinical problem that impairs life and leads to permanent neurological deficits [Uflacker R, et al., 1987].

In this regard, tissue engineering has shown promise for growing tissue in vitro to replace dysfunctional organs in vivo [Griffith LG, et al., 2002]. However, its use for the regeneration of large functional organs has achieved little clinical success [Dimmeler S, et al., 2014]. One of the main reasons is that these organs have an abundant, well-organized circulatory network that is too complex for current techniques to replicate in engineered tissues [Dorrello NV, et al., 2017].

Poor vascularization has become a critical limitation explaining the poor performance of artificial tissue grafts in the body [Jain RK, et al., 2005].

In this regard, due to the difficulty of performing organ transplantation, “transformation” instead of “transplantation” of an organ may serve as an optimal solution. This transformation can be based on the reconstruction of an existing organ in the body in order to develop the function of another, dysfunctional one.

According to the results of an earlier study, researchers experimentally transformed the spleen into an organ that functions like the liver [Wang L, et al., 2020], however, in this study, the “function” of the liver embedded in the spleen was performed outside the bloodstream, which shows that this model of transformation of the spleen into the liver is somewhat imperfect.

Isolated hepatocyte transplantation is of interest because of its potential role in the treatment of severe liver diseases [Bumgardner GL, et al., 1988].

The spleen is considered the most privileged anatomical site for hepatocyte transplantation [Hillan KJ, et al., 1989], as the spleen can trap a limited but sufficient number of hepatocytes within its sinusoids, providing conditions very similar to the natural cellular microenvironment.

In this regard, intrahepatic hepatocyte transplantation may be beneficial, demonstrating potential therapeutic value in the treatment of liver diseases [Bumgardner GL, et al., 1988].

Thus, our study was aimed at assessing the results of treatment of complicated portal hypertension against the background of portosystemic shunt interventions by using an intracorporeal autoorganic biofilter in an experiment.

The aim of the dissertation research is to provide a morphofunctional assessment of changes in the liver against the background of portal hypertension and after its correction.

Research objectives.

1. To study the factors and pathogenetic mechanisms that contribute to development of complications due to portal hypertension, including post-shunt complications.
2. To develop a model of portal hypertension and study morphological and morphometric changes in the liver, biochemical parameters, portal pressure level, cognitive functions and survival depending on the degree of narrowing of the caudal vena cava in the experiment.
3. Develop a methodology for the formation of intracorporeal autoorganic biofilter by transplanting living liver cells into the spleen parenchyma to ensure functional transformation of the spleen and development of liver function under experimental conditions.
4. Give a comparative assessment of the integral indicators of application portocaval shunt, intracorporeal autoorganic biofilter and their combined use against the background of simulated portal hypertension with varying degrees of narrowing of the caudal vena cava under experimental conditions.

Object and subject of research:

The study was conducted at the Laboratory of Experimental Medicine of the B. Atchabarov Research Institute of Fundamental and Applied Medicine, S.D. Asfendiyarov Kazakh National Medical University NJSC(Almaty, Kazakhstan).

The study used 502 rats obtained from the vivarium of the B. Atchabarov Research Institute of Fundamental and Applied Medicine (Almaty, Kazakhstan) with a standard diet and care.

The research design includes 3 blocks:

Block I – creation of a complicated model of PH by narrowing the lumen of the caudal vena cava, (n - 160);

Block II - creation of a model of an intracorporeal autoorganic biofilter (n - 18);

Block III – the use of portosystemic shunting interventions against the background of a model of complicated PH and a model of an intracorporeal autoorganic biofilter (n - 324);

I block. Modeling of complicated portal hypertension using the method of narrowing the lumen of the caudal vena cava.

Laboratory animals (n = 160) were divided (randomization) into five groups.

Group I: control group (CG): no intervention (n = 20);

Group II: second control group (SG): the chest cavity was opened using a right-sided lateral approach, the caudal vena cava was not narrowed (n = 20);

Group III: intervention group (IG-1): the lumen of the caudal vena cava is narrowed by a plastic ring by 25% in the area located above the diaphragm (n = 40);

Group IV: intervention group (IG-2): the lumen of the caudal vena cava is narrowed by a plastic ring by 50% in the area located above the diaphragm (n = 40);

Group V: intervention group (IG-3): the lumen of the caudal vena cava is narrowed by 75% in the area located above the diaphragm (n = 40).

Model of complicated portal hypertension using the method of narrowing the lumen of the caudal vena cava in an experiment.

Modeling the occlusion of the caudal vena cava in the experiment was carried out according to the previously described method ("Method for modeling the occlusion of the inferior vena cava in the experiment" / Patent No. of the Republic of Kazakhstan for the model: 5796).Depending on the planned model, 25%, 50% and 75% of the degree of narrowing of the inferior vena cava, plastic semicircular rings of the required size were made.

II block. Creation of a model of intracorporeal autoorganic biofilter

The model an intracorporeal autoorganic biofilter was carried out according to the previously described method (Patent of the Republic of Kazakhstan for an invention and a Eurasian patent for an invention "Method for converting spleen parenchyma into tissue that functions as liver parenchyma in an experiment".

III block. The use of portosystemic shunt interventions against the background of a model of complicated PH and a model of an intracorporeal autoorganic biofilter.

The animals animals were randomized into 3 groups:

Group I: In this group, animals underwent modeling of PH with narrowing of the caudal vena cava + a portacaval shunt (PCG) was used (n = 108). Group I was divided into 3 series:

Series 1: modeling of PH was carried out with a narrowing of the caudal vena cava by 25% + a portacaval shunt was used (n=36), (PCG25).

Series 2: modeling of PH was carried out with a narrowing of the caudal vena cava by 50% + a portacaval shunt was used (n=36), (PCG50).

Series 3: modeling of PH was carried out with a narrowing of the caudal vena cava by 75% + a portacaval shunt was used (n=36), (PCG75).

Group II: In this group, animals underwent modeling of PH with narrowing of the caudal vena cava + an intracorporeal autoorganic biofilter was used (n = 108), (IAB).

Series 1: modeling of PH with narrowing of the caudal vena cava by 25% + use of an intracorporeal autoorganic biofilter (n=36), (IAB25).

Series 2: modeling n of PH with narrowing of the caudal vena cava by 50% + use of an intracorporeal autoorganic biofilter (n=36), (IAB50).

Series 3: modeling of PH with narrowing of the caudal vena cava by 75% + use of an intracorporeal autoorganic biofilter (n=36), (IAB75).

Group III: In this group, animals underwent modeling of PH with narrowing of the caudal vena cava + a portacaval shunt was used + an intracorporeal autoorganic biofilter was used (n = 108), (PCG + IAB).

Series 1: modeling of PH with narrowing of the caudal vena cava by 25% + use of portocaval shunt + use of intracorporeal autoorganic biofilter (n=36), (PCG+IAB25).

Series 2: modeling of PH with narrowing of the caudal vena cava by 50% + use of portacaval shunt + use of intracorporeal autoorganic biofilter (n=36), (PCG+IAB50).

Series 3: modeling of PH with narrowing of the caudal vena cava by 75% + use of portocaval shunt + use of intracorporeal autoorganic biofilter (n=36), (PCG+IAB75).

Research methods:

The following research methods were used (depending on the observation period): measurement of body weight of laboratory animals, measurement of body temperature, assessment of pain on the Grimace scale, macroscopy of abdominal organs (liver, spleen), measurement of liver mass, behavioral test, histological assessment, morphometry of abdominal organs, measurement of portal pressure, assessment of animal survival.

To assess changes in liver function, the level of transaminases - ALT, AST, and alkaline phosphatase - was determined in blood samples. Based on the results of ALT and blood AST data, the AST/ALT coefficient was calculated.

Provisions for defense:

1. An experimental model of portal hypertension has been developed and validated, which reproduces the morphofunctional changes in the liver caused by portal hypertension.

2. The mechanisms of structural reorganization of the liver in portal hypertension, including endothelial dysfunction and capillarization of sinusoids, have been identified, which helps to understand the causes of ineffective perfusion of the organ and aggravation of the disease

3. An innovative method has been developed for the formation of an intracorporeal autoorganic biofilter by transplanting living liver cells into the spleen parenchyma, which contributed to the functional transformation of the spleen and the development of liver function, providing an alternative to traditional methods of treating portal hypertension.

4. A comparison was made of the effectiveness of portocaval shunting and intracorporeal autoorganic biofilter, including their combined use, in conditions of experimental portal hypertension.

5. An algorithm has been developed for determining the cognitive functions of rats under conditions of portal hypertension to assess the impact of the disease and the effectiveness of new therapeutic approaches on cognitive abilities.

Results:

An experimental model of portal hypertension leads to parenchymal dystrophy, dilation of central veins, a decrease in the mitotic index and functional cell mass of the liver ($p \leq 0.05$), a decrease in the AST/ALT coefficient in the blood ($p \leq 0.001$), an increase in portal pressure ($p \leq 0.001$), a decrease in cognitive functions ($p \leq 0.05$) and the survival rate ($p \leq 0.01$) of laboratory animals compared with the control group, depending on the degree of narrowing of the caudal vena cava. Transplantation of living liver cells into the spleen parenchyma using the model of formation of an intracorporeal autoorganic biofilter, it demonstrated an increase in the mass of the spleen ($p=0.001$), a decrease in the volume of lymphoid regions, capillaries and an increase in the volume of dense cell clusters due to the formation of hepatocyte clusters on day 60 under experimental conditions. Combined use of a portocaval shunt and intracorporeal autoorganic biofilter did not lead to an increase in reaction delay time in assessing cognitive functions compared with the control group ($p<0.05$), contributed to a decrease in portal pressure in the PCG 25 and PCG 50 groups, respectively ($p<0.05$) and increased survival rates in the PCG 25 and PCG 50 groups, respectively ($p<0.05$), and on day 60, the AST/ALT coefficient in these groups ($p=0.782$ and $p=0.823$) had no significant differences with the control group, compared with the deviations in the PCG 50 and PCG 75 groups ($p\leq0.001$).

Scientific novelty.

1. A modeling method has been developedsuprahepatic hypertension under experimental conditions (Patent of the Republic of Kazakhstan for a utility model No. 5796 dated November 18, 2020. A method for modeling suprahepatic hypertension in an experiment.// Tanabaeva Sh.B., Fakhradiev I.R., Almabaev Y.A., Tanabaev B.D.).

2. A method has been developed for converting spleen parenchyma into tissue that functions as liver parenchyma under experimental conditions(Patent of the Republic of Kazakhstan for invention No.36697 dated 12/04/2024. Method for converting spleen parenchyma into tissue functioning as liver parenchyma in an experiment // Tanabaeva Sh.B., Almabaev Y.A., Fazylov T.R., Fakhradiev I.R.; Application for Eurasian prepatent for invention No. 230396 dated 08/15/2023.A method for converting spleen parenchyma into tissue that functions as liver parenchyma in an experiment.).

3. An algorithm has been developed for determining the cognitive functions of rats by tracking the position of rats in real time in radial maze tests (Prepatent of the Republic of Kazakhstan for a utility model No. 2024/0054.2 dated January 15, 2024. Algorithm for determining the cognitive functions of rats by tracking the

position of rats in real time in radial labyrinth tests // Tanabaeva Sh.B., Almabaev Y.A., Fakhradiev I.R., Kulimbet M.B., Zhumagaliuly A., Fazylov T.R.).

4. New data obtained during an experimental study of morphological changes in the liver and assessment of survival rates depending on the degree of narrowing of the caudal vena cava provide new understanding of the mechanisms of adaptation of liver tissue to changed circulatory conditions, which opens up prospects for the development of new methods for diagnosing and treating portal hypertension.

5. Key factors influencing the development of post-shunt complications in complicated portal hypertension in experiments have been identified and systematized.

Practical significance of the obtained results.

1. Research advances the science of hepatology by obtaining data on the mechanisms of development of portal hypertension and methods of its correction, improving current methods of diagnosis and treatment based on new pathophysiological data.

2. The creation of an experimental model of portal hypertension contributes contribution to improving methods for testing new surgical and therapeutic approaches, allowing more accurate assessment of their effectiveness and safety before clinical trials.

3. Method developed as part of an experimental study the formation of an intracorporeal autoorganic biofilter opens a new page in cell transplantology, providing fundamental knowledge for the development of new surgical and therapeutic treatment approaches.

4. The developed algorithm for measuring cognitive functions can be used to evaluate the effectiveness of new surgical and therapeutic approaches in preclinical studies, facilitating the development and evaluation of new treatments.

Personal contribution of a doctoral candidate.

The dissertation candidate personally, under the guidance of scientific consultants, carried out the experimental part of the study, carried out a thorough analysis, statistical processing of data and interpretation of the results obtained during the study, and made reasonable conclusions.

Conclusions.

1. Endothelial dysfunction, paracrine disorders between activated hepatocytes and sinusoidal endothelial cells, as well as the processes of capillarization and sinusoidal remodeling contribute to the structural reorganization of the liver in portal hypertension, aggravating the disease due to ineffective perfusion of the organ, while portosystemic shunting, despite its effectiveness in the treatment of portal hypertension, leads to significant complications, including hepatic encephalopathy.

2. The developed model of portal hypertension leads to parenchymal degeneration, dilation of the central veins, decrease in the mitotic index and functional cell mass ($p \leq 0.05$) of the liver, decrease in the AST/ALT ratio in the blood ($p \leq 0.001$), increase in portal pressure ($p \leq 0.001$), decrease in cognitive

functions ($p \leq 0.05$) and survival ($p \leq 0.01$) compared with the control group, depending on the degree of narrowing of the caudal vena cava in the experiment.

3. The developed methodology for the formation of intracorporeal autoorganic biofilter showed that transplantation of living liver cells into the spleen parenchyma leads to an increase in the mass of the spleen ($p = 0.001$), a decrease in the volume of lymphoid areas, capillaries and an increase in the volume of dense cell accumulations due to the formation of hepatocyte clusters on day 60 under experimental conditions.

4. Combined use of a portacaval shunt and intracorporeal autoorganic biofilter did not lead to an increase in reaction delay time compared to the control group (149±15 and 157±27 versus 150±19, respectively, $p \leq 0.05$), provided a decrease in portal pressure (6.5±1.2 and 10 .3±3.1 versus 7.2±1.9 and 11.6±1.5 in the PCG 25 and PCG 50 groups, respectively, $p \leq 0.05$) and increased survival (95%CI 28.8-30.1 and 95%CI 29.0-29.9 versus 95%CI 27.9-29.8 and 95%CI 26.7-29.5 in the PCG 25 and PCG 50 groups, respectively, $p \leq 0.05$), and the AST coefficient /ALT on day 60 in these groups ($p=0.782$ and $p=0.823$) do not have significant differences with the control group, which contrasts with significant deviations in the PCG 50 and PCG 75 groups ($p \leq 0.001$).

Approbation of the results of the dissertation.

The main provisions of the dissertation were reported and discussed at the international scientific and practical conference of TSMU named after. Abuali ibni Sino “Achievements and problems of fundamental science and clinical medicine”, Dushanbe, 2020; XIV International Scientific and Practical Conference in Memory of Academician Yu.I. Borodin “Lymphology: from fundamental research to medical technologies”, Novosibirsk, 2021; At the international scientific and practical conference “Ecological genetics and public health: achievements and prospects”, Almaty, 2023.

Approbation of the dissertation work took place at an extended meeting of the Department of Anatomy of KazNMU named after. S.D. Asfendiyarov, at a meeting of the Scientific Committee in the field of “Surgical diseases” (2024).

Implementation of research results into practice.

4 security documents have been developed:

1 patent of the Republic of Kazakhstan for utility model No. 5796 dated November 18, 2020. A method for modeling suprahepatic hypertension in an experiment.

1 patent of the Republic of Kazakhstan for an invention No. 36697 dated 12/04/2024. A method for converting spleen parenchyma into tissue that functions as liver parenchyma in an experiment.

1 Eurasian prepatent for invention No. 230396 dated 08/15/2023. A method for converting spleen parenchyma into tissue that functions as liver parenchyma in an experiment.

1 prepatent of the Republic of Kazakhstan for a utility model No. 2024/0054.2 dated 01/15/2024. An algorithm for determining the cognitive functions of rats by tracking the position of rats in real time in radial maze tests.

Publications.

Based on the results of the study, 7 scientific papers were published: 3 – in journals recommended by the Committee for Quality Assurance in the Field of Education and Science of the Ministry of Education and Science of the Republic of Kazakhstan; 1 – in the magazine, included in the international database Scopus and Web of Science Core Collection (Clarivate Analytics) - Q2; 3 – in materials of international conferences; 4 documents of protection have been developed: 1 patent for an invention of the Republic of Kazakhstan, 1 prepatent of utility model of the Republic of Kazakhstan, and 1 -Eurasian prepatent for an invention.

The scope and structure of the thesis.

The dissertation is presented on 150 pages of typewritten text and consists of a list of abbreviations and symbols, an introduction, a literature review, a description of materials and methods, the results of one's own research, a conclusion, discusssion and a list of references. The work is illustrated with 46 drawings and 26 tables. The bibliographic index includes 222 sources.