Ukrainian Scientific Medical Youth Journal
Issue 2 (139), 2023

ISSN 2786-6661
eISSN 2786-667X

MEDICINE / МЕДИЦИНА

UDC: 616.149-008.331.1:616.36]-092.9
https://doi.org/10.32345/USMYJ.2(139).2023.17-24

Received: December 02, 2022
Accepted: April 13, 2023

A model of prehepatic portal hypertension in rats

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Abstract: *during the study, the methodology of the prehepatic portal hypertension (PPH) development model in Wistar rats was set, using partial portal vein ligation (PPVL). It was followed by the analysis of model stability and its prolongation over time using the indicator of the portal vein constriction rate (PVCR) and the portohepatic perfusion (PHP) dependence on it. It was found that in 4-week-old rats with a body weight of 99.6 ± 2.0 g there was no mortality in PPVL at 53.45%; the reduction of PHP was critical at 69.13% of PPVL; in a group of 6-week-old rats with a body weight of 155 ± 3.5 g, where the median PPVL was 58.67 (95% CI 56.3-59.82) after PPVL, there was no mortality. At the same time, a delay in the physical development of rats was noted in comparison with the control subgroup and the subgroup with false ligation. Parenchymal and vessel changes were registered on serial ultrasound examination.*

Keywords: hypertension, laparotomy, ligation, rats, body weight.

Introduction

Prehepatic portal hypertension (PPH) is an increase of hydrostatic pressure within a portal vein (PV) system, which results from liver blood inflow impairment. As a consequence, portohepatic perfusion (PHP) decreases and variceal bleedings from the enlarged esophageal veins (EEV) develop. Various animal models were created to study the processes that take place in PH. The most common modeling technique is partial portal vein ligation (PPVL) in rats. The first attempts to induce portal hypertension (PH) in animals belong to Neuhof H. (1913), who tried to limit the PV blood inflow in dogs but failed. Later Reynell P.C. (1952) narrowed PV in rats, reaching 50% lethality in his study group of animals. Most of the rats which survived developed PPH. Between 1973-1975 it was shown that the diameter of the stenotic zone and rat's body mass or age were critical in the analysis of the survival rate (Myking & Halvorsen 1973,1975). Besides dogs and rats, PPH models created by means of PV constriction were described in rhesus monkeys (Laufman et al., 1960), pigs (Jensen et al., 1987), rabbits (Jensen, Dybdahl, & Juhl, 1986), and mice (Cheever & Warren, 1963). Further research and PPH animal models systematized the understanding of each model benefits and disadvantages. Thus, dogs, pigs, or rabbits may be effective for esophageal varices studies, while rats, due to their smaller physical size, develop PPH predictably with lower lethality, which is confirmed by

ISSN 2786-6661
eISSN 2786-667X

the studies of Wen et al. (2009) and Rodrigues et al. (2014). PH model in rats became widespread in PH studies for sterile conditions are not necessary for its creation and there is no need in special postoperative care. Wistar and Lewis rats are equally suitable for PPH modeling. Within a few days after PV narrowing liver function tests show a slight decrease in total plasma protein and a slight increase in transaminases and alkaline phosphatase. Liver function tests return to normal in 2 weeks after surgery (Rozga et al., 1985). The mass of the liver in rats after the narrowing of the PV is lower compared liver mass in a group of animals with false ligation (Halvorsen & Myking, 1979). Also, the model of PPH in rats, obtained by PPVL, is used to determine changes in visceral blood circulation and the pathophysiology of hemodynamic changes that occur after ligation (Abraldes, Pasarín & García-Pagán, 2006; Wang, Kuang & Chen, 1994). However, according to literature sources, mentioned studies were performed approximately 2–4 weeks after PPVL, and results beyond this period are difficult to be found. It is also to be mentioned that in most studies adult rats were used. It is assumed that the increased pressure in the PV system can last for a longer period of time and result into a chronic PPH. The purpose of this study is to analyze the existing PH animal models, and in a search for new data to create a stable and time-prolonged PPH model by means of PPVL in experiment, using the indicator of the degree of PV narrowing (DPVN), which will allow to find the relationship between the critical reduction of PHP on one hand and result into PPH development on the other, with further observation and description of changes that occur after the model creation.

In the literature and different works clear data on the rat model lasting 3-6 months were not found, so the main differences of our study are the young age of the animals, the duration of the disease and the stability of the condition at the end of the observation, achieved due to partial ligation of the portal vein. which would make it possible to obtain a long duration of the disease and, accordingly, the opportunity to analyze the changes in the liver that occur at the time of withdrawal of the animals from the study.

The final goal and the investigated processes can be completely different but our technique allows obtaining rats with portal hypertension with 3-6 months or even more after ligation.

Aim

To study and to create the stable prolonged PPH model with all its clinical manifestations and achieve significant relationship between portal vein stenosis and mortality after ligation surgery in young rats.

Materials and methods

The experiment was performed in several stages. Available full-text archives of biomedical and life sciences journal literature were studied on appropriate subject initially, and then the study conception was created. Clear data or models on animals of such a young age were not found, therefore the selection and search of age groups at the first stage was carried out experimentally, including the use of such a catheter, in which there was no absolute lethality during the operation.

Male Wistar rats (n = 40) were included into the study. In the first study group (n = 10) rats were 4 weeks old, and in the second study group (n = 30) – 6 weeks old (Table1). Body mass of rats from group 1 was 99,6 ± 2,018 g. The main and the target division of rats was in the group 2: they were divided into subgroups, where the PPVL was performed with stenosis formation (n = 15), and the ligation without obstruction, so called sham surgery with false ligation, (n = 10), and the group of control (n = 5), with body mass of 155 ± 3,5, 155,7 ± 2,9 and 158,2 ± 3,5 g. correspondingly; Multiple comparisons test showed no difference in rats body mass between groups (p = 0,870). The selection of the optimal catheter for narrowing the PV was carried out experimentally: it was found that at 24G there is a critical narrowing of the PV which leads to acute irreversible decrease of PHP and death so then we used only 22G catheter. Constriction rate after PPVL was calculated using the following formula: $(1 - \pi r^2/\pi R^2) \times 100\%$, where r – is the catheter radius, and R – vessel radius (Rodrigues et al. 2014). In our work we showed it as degree of PV narrowing (DPVN).

All work with experimental animals was carried out in compliance with the Law «On the Protection of Animals from Cruelty Treatment» of Ukraine, the «European Convention for the Protection of Vertebrate Animals Used for Experimen-

ISSN 2786-6661
eISSN 2786-667X

Table 1. Characteristics of animals in the study groups

Total number of Wistar rats (n = 40)					
	Group 1 (n = 10)		Group 2 (n = 30)		
Subgroup	–		PPVL (n = 15)	False ligation (n = 10)	Control (n = 5)
Age	**4 weeks**		**6 weeks**		
Body mass, g	99,6 ± 2,0		155 ± 3,5	155,7 ± 2,9	158,2 ± 3,5
Catheter	22G (n = 2)	24G (n = 4)	22G	–	–
Mortality	0	4	0	0	0

tal and Other Scientific Purposes», principles and norms of biological safety.

The Committee on Clinical Investigation of Bogomolets National Medical University approved this study (Protocol №141 27.01.2021). All the studies were conducted according to implemented guidelines in consideration of GCP-ICH and Declaration of Helsinki. The represented results of the work are a part of scientific study №0122U001363 which is funded by the Health ministry of Ukraine from the state budget.

Prior to the modeling surgery and after it, all the rats from experimental groups were kept in cells with similar conditions: light intensity, microclimate, food and water supply. All the animals were weighted and measured Before the surgical intervention. Ketamine was administered intraperitoneally for general anesthesia at a dose of 50 µg/kg. PPVL was performed by applying a synthetic ligature to the PV main brunch. After abdominal skin epilation, the surgical field was three times desinfected with povidone iodine solution. The median laparotomy was performed – an incision was made in the skin, subcutaneous tissue, muscles, and peritoneum, after which the intestinal loops were brought out and to the right. The anatomy of the liver was studied after the liver was retracted upward to visualize the splenomesenteric confluence (SMC) which is formed by splenic vein (SV) and superior mesenteric vein (SMV) (Fig. 1).

After a thin dissector used to mobilize the PV around its circumference in the most accessible part, which is its middle third usually, to pass carefully a 4/0 monofilament threadbehind it. Then a 24G or 22G (with plastic tubes diameters 0.7 and 0.9 mm respectively) polyurethane catheter was installed parallel to the vein, and a knot was formed around both PV and catheter. Finally a catheter was carefully removed, and a zone of stenosis was obtained, since the diameter of the PV exceeded the diameter of the catheter (Fig. 2).

When the catheter was removed the effectiveness of obstruction in the formed stenosis zone was assessed in case SV and SMV swelling and intes-

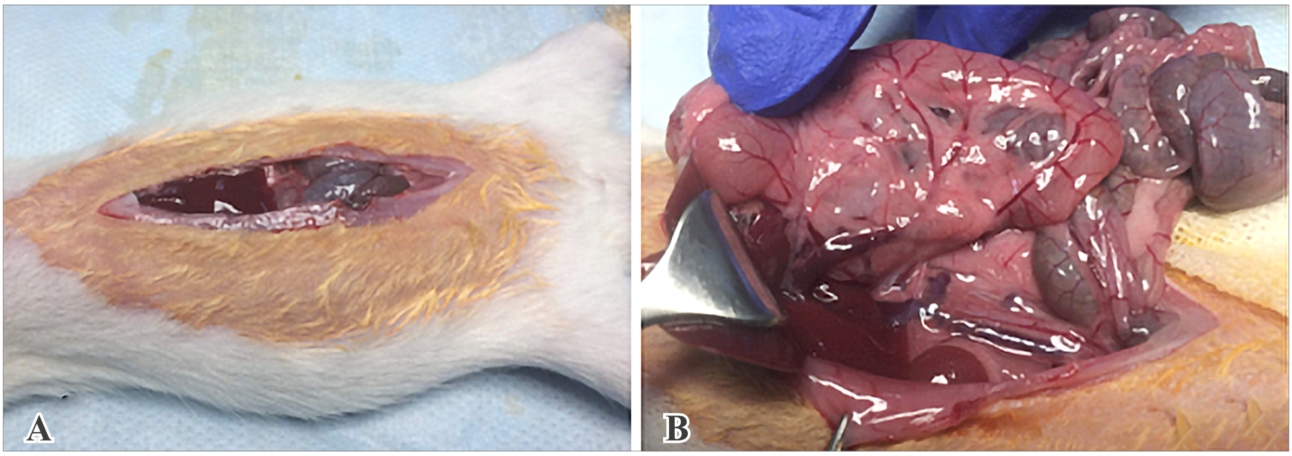

Figure 1. Surgical incision and access to the organs of the abdominal cavity (A) and vessels after visceral rotation to the left (B): 1 – splenic vein, 2 – superior mesenteric vein, 3 – splenomesenteric confluence, 4 – portal vein

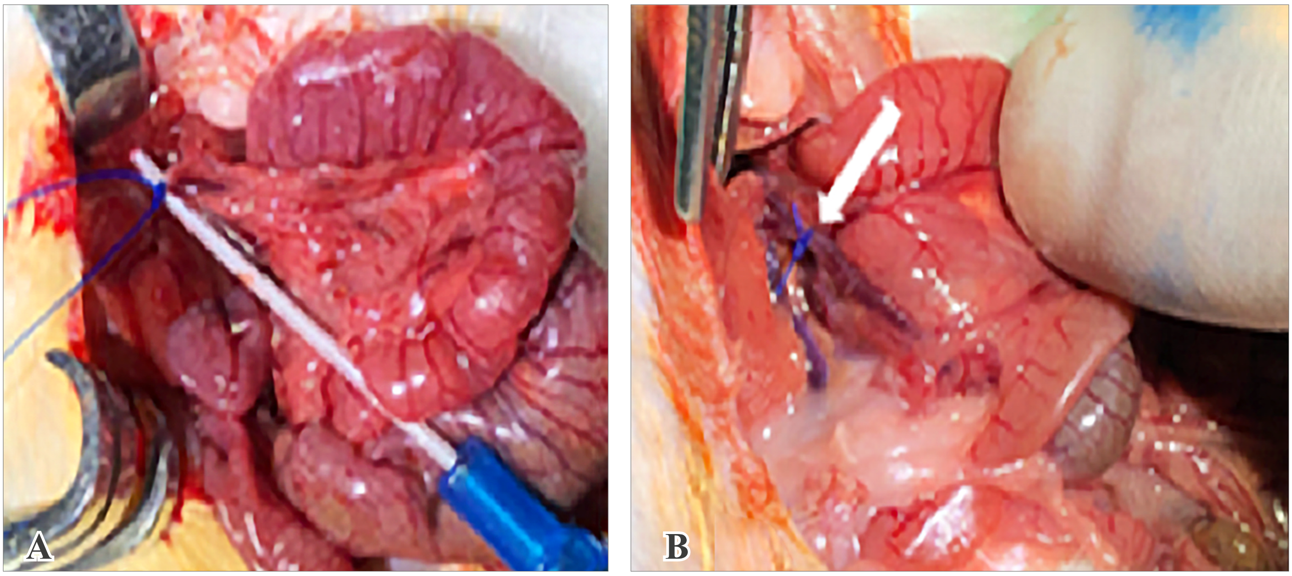

Figure 2. PPH modeling by means of PPVL in rats. A – PV ligation on the 22G catheter, B – overall look after PPVL, partial narrowing of PV main trunk is formed (white arrow)

tinal hyperemia appeared. Layered closure of the wound was performed with a 4/0 polyester thread, the postoperative wound was desinfected with chlorhexidine. In the false ligation subgroup, a catheter was not used and tightening of the thread was not carried out: the knot was freely located on the vessel. All the other stages were performed in a similar technique (Fig. 3).

We did not use any antibiotics after surgery but abdominal cavity was cleaned with normal saline and chlorhexidine solution during operation.

Surgical interventions were performed under optical magnification. The duration of each surgery was from 7 to 9 minutes, after which the rats were returned to their usual conditions. The results of ligation were evaluated by observing the rats behavior, measuring their body mass, and the ultrasound examination of the abdominal cavity, during which the following parameters were compared: the density of the liver parenchyma, collaterals development in the porta hepatis, and the presence of splenomegaly.

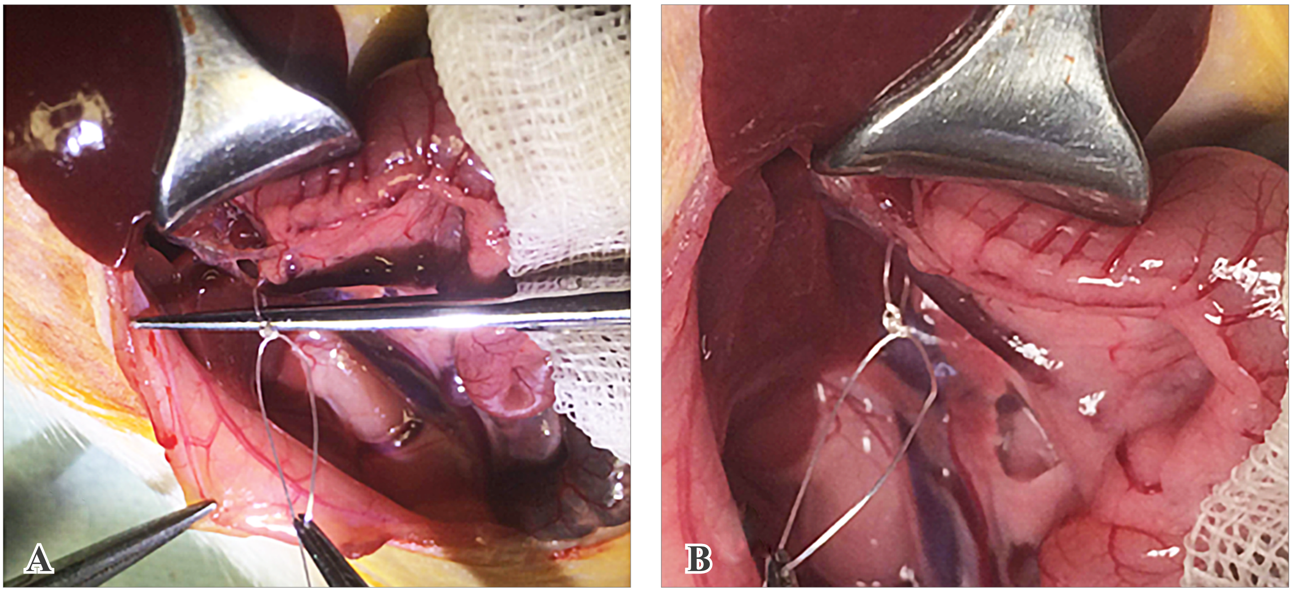

Figure 3. False PV ligation in rats (sham procedure). A – passing the thread and forming of the false loop with the knot, B - overall look of the free thread loop on the main PV branch

Ketamine sedation was used for following ultrasound examination.

Philips HD 11 was used to perform ultrasound examination. PV diameter was measured by means of digital caliper of 0.02 mm division. Statistical analysis was performed using IBM SPSS for Windows version 24.0 (IBM Corp., Armonk, NY) and EZR (R-statistics) packages. The statistical significance was set at the level $p < 0.05$.

Results and discussion

In the experiment at the stage of research development and the method in the rats of study group 1 with PV diameter of 1,3 ± 0,007 mm 24G and 22G were chosen as optimal for ligation. In four rats which died within 24 hours after surgery PPVL resulted into critical PHP decrease, while two rats, in which 22G catheter was used, survived. Other four animals died for technical issues during the surgical procedure. The correlation of these processes is reflected mathematically in the indicator of the DPVN, which was set to assess the critical PHP decrease (Table 2). According to formula, median DPVN was 70,54% (95%CI 69,13-71,87) in rats which died after PPVL with 24G catheter. DPVN in two rats which survived was 52,07% and 53,45% correspondingly. The autopsy of four rats that died during 24 hours after surgery showed hemorrhagic exudate in the abdominal cavity, significant venous hyperemia of the intestinal vessels, its swelling. Rats liver was pallor, with decreased elasticity. SV expansion and an increase of spleen size compared to preoperative were noted. Functional disorders after critical PPVL that caused death is associated first of all with severe hypoglycemia and acute liver failure.

After obtaining high mortality rate in study group 1, identifying the critical meaning of PHP, the second study group was formed (n = 30) with 6 weeks old rats. The study group 2 was divided into three subgroups: subgroup 1 – PPVL with 22G catheter (n = 15), subgroup 2 – false ligation (n = 10), subgroup 3 – control subgroup, with no surgeries performed in animals (n = 5). PV diameter was 1,4 mm (95%CI 1,36-1,42) in subgroup 1, and 1,39 mm (95%CI 1,38-1,42) in subgroup 2; with no difference found between diameters in subgroups (p = 0,686). Catheter diameter was 0,9 mm. No mortality was observed in both subgroups; according to set mathematical model, median DPVN was 58,67% (95%CI 56,3-59,82) (Table.3).

Thus, during our experimental study, we developed a stable and long-term model of PPH in

Table 2. Characteristics of animals and DPVN in study group 1

Study group 1 (n = 10), 4 weeks old rats, catheters 22G+24G			
	PPVL (n = 6)		Without PPVL or sham surgery (n = 4)
Indicator	22G (n = 2)	24G (n = 4)	
PV diameter	1,3 ± 0,007 mm		–
DPVN	52,07% and 53,45%	70,54% (95% BI 69,13-71,87)	–
Survival	2	0	–
Mortality	0	4	4

Table 3. Characteristics of animals and DPVN in study group 2

Study group 2 (n = 30), 6 weeks old rats, catheter 22G			
Indicator	PPVL (n = 15)	False ligation (n = 10)	Control group (n = 5)
PV diameter	1,4 mm (95%CI 1,36-1,42)	1,39 mm (95%CI 1,38-1,42)	–
DPVN	58,67% (95% CI 56,3-59,82)	–	–
Survival	15	10	5
Mortality	0	0	0

ISSN 2786-6661
eISSN 2786-667X

rats. We also observed a significant hair loss, bluish skin, low physical activity, decreased appetite compared to rats of subgroups 2 and 3. Four weeks after PPVL surgery an ultrasound examination of the abdominal cavity was performed, where we saw that spleen was markedly enlarged in all rats of subgroup 1, collateral blood circulation in porta hepatis and formation of venous cavern, hepatofugal PV blood flow were seen in 11 rats (73,3%) of subgroup 1, the density of liver tissue was increased in comparison with healthy rats and rats which underwent false ligation (Fig.4).

Animal models made it possible to study the hemodynamic changes, both visceral and systemic blood circulation disorders, specific for PH in detail. PPH models can be used for visceral and hyperdynamic blood circulation studying, while cirrhosis models allow to study changes in intrahepatic microcirculation that lead to increased portal perfusion resistance (Abraldes, Pasarín & García-Pagán, 2006). In vivo studies are necessary to study the pathophysiology of hyperdynamic blood flow which can be provided by PPVL PPH model (Vorobioff, Bredfeldt & Groszmann, 1983; Colombato, Albillos & Groszmann, 1992). Moreover, mentioned PPH model can be easily reproduced, and does not require significant financial input; PH develops and becomes demonstrative in a short period of time. In particular, PH with hyperdynamic blood circulation and portosystemic shunts can be registered or visualized in rats one week after PPVL (Abraldes, Pasarín & García-Pagán, 2006).

Increased pressure in the PV basin can be obtained by PPVL together with raised blood flow resistance in the PV (Silva et al., 2013). By means of catheter with diameter of 0.9 mm, it was possible to use a mathematical calculation that determined the DPVN $(1 - r^2\pi/\pi R^2)$ x 100%). Mentioned 0.9 mm catheter allowed to narrow the PV with an diameter of 1.4 mm by an average of 58.67%. Such a narrowing causes a rapid increase of the portal blood pressure PV. Its stabilization is observed after two weeks but remains higher than the basal value even in the period when the dilatation of the tributaries of the PV is already observed. A catheter with a diameter of 1 mm have not resulted into significant and clear changes of PHP, though the mortality of animals decreased (Wen et al., 2009).

PPVL models are important because complete ligation leads to a fatal outcome in all experimental animals provoked by the termination of the PHP (Cheever & Warren, 1963), On the other hand PHP as an indicator is extremely important as it gives the possibility to calculate the vital initial parameters. With a 0.9 mm (22G) catheter, DPVN of 58.67% was obtained in our experiment, and Rodrigues et al. (2014) reached DPVN of 55.8% with a survival rate of 100% in both studies. At the same time, Wen et al, using a 0.7 mm diameter (24G) catheter, achieved a survival rate of 80% reaching DPVN of 88.9% (Wen

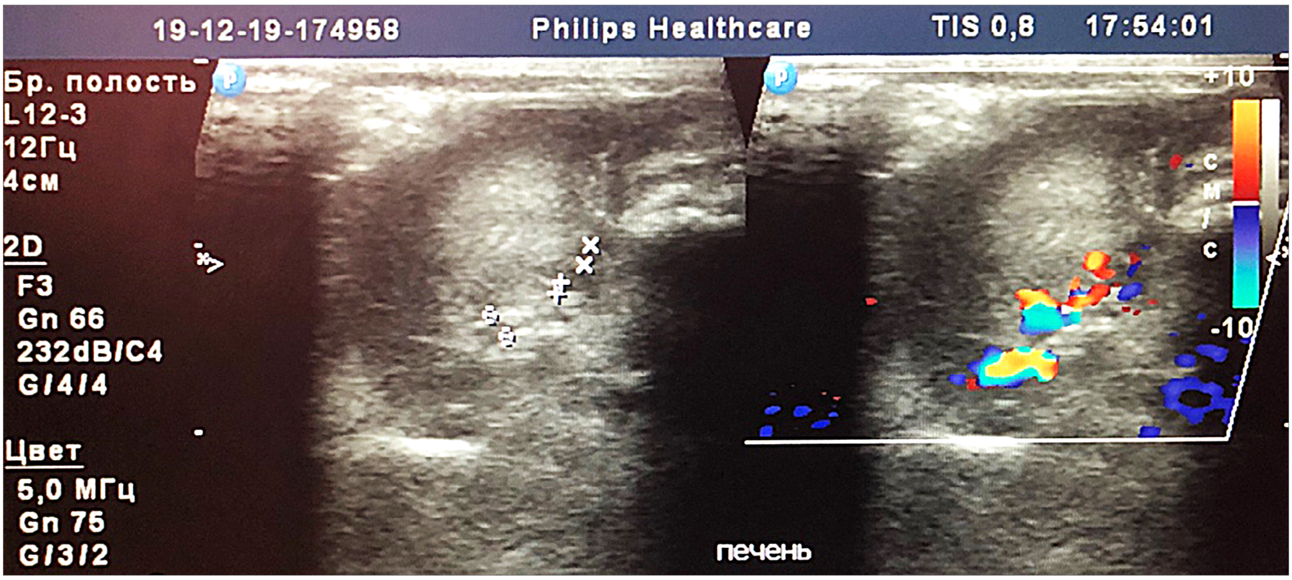

Figure 4. Ultrasound image obtained in a rat 4 weeks after PPVL: PV cavern and hi density parenchyma can be seen

ISSN 2786-6661
eISSN 2786-667X

et al., 2009). In this studies included animals were with larger initial body weight and age. All rats that survived during the study period had adequate portal blood inflow, as indicated by the absence of mortality at the end of the experiment.

The experiment is planned to be continued further and result with the studying the gross anatomy and changes of the liver after PPVL, and performing liver biopsy followed by the histological picture and research by means of western blot for apoptosis and hypoxia proteins expression.

Conclusion

Clear regularities between PPVL and a critical lethal PHP reduction in rats aged 4 weeks were obtained. These results were confirmed in 6 weeks old rats (as evidenced by DPVN of 52.07-53.45% and 58.67% respectively). Thus, we managed to create the stable prolonged PPH model with all its clinical manifestations. DPVN of 56,3-59,82% can be considered optimal in 6 weeks old Wistar rats to obtain stable and time-prolonged PPH model. Rats aged 4 and 6 weeks are equally suitable for PPH modeling by means of PPVL with 22G catheter, but younger age can lead to mortality for technical reasons while the main stage of the surgery.

Financing

The represented results of the work are a part of scientific study №0122U001363 which is funded by the Health Ministry of Ukraine from the state budget.

Conflict of interests

The authors have no conflict of interest to declare.

Consent to publication

The authors have read and approved the final version of the manuscript. Authors agreed to publish this manuscript.

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Модель допечінкової портальної гіпертензії у щурів

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Анотація: *в ході роботи було поставлено та досліджено методику моделі розвитку допечінкової портальної гіпертензії (ДПГ) у щурів лінії Вістар з використанням часткової перев'язки ворітної вени (ЧПВВ) з подальшим аналізом стабільності та пролонгованості цієї моделі в часі з використанням показника ступеня звуження ворітної вени (СЗВВ) та залежності від нього портопечінкової перфузії (ППП). Було виявлено, що у щурів віком 4 тижні з масою тіла 99,6 ± 2,0 г критичним було зниження ППП вже при СЗВВ 69,13%, а при СЗВВ 53,45% летальності не було; у групі щурів віком 6 тижнів з масою тіла 155 ± 3,5 г, де медіана СЗВВ склала 58,67 (95%ВІ 56,3-59,82) після ЧПВВ, летальності не було. Одночасно було відмічено затримку фізичного розвитку щурів в порівнянні з контрольною підгрупою та підгрупою з хибним лігуванням та чіткі зміни при ультразвуковому дослідженні.*

Ключові слова. Гіпертензія, лапаротомія, лігування, щурі.