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Choosing an Optimal Suture Material for Prehepatic Portal Hypertension Modeling

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Abstract: If the catheter size, the thickness of a thread, the place for ligation were determined for the partial portal vein ligation (PPVL), the question of the suture material remains open. The aim of the study was to determine the optimal suture material (silk or prolene) for PPVL. The experiment was performed on 25 male adult outbred rats. The PPVL was carried out using the catheter 20G with thread 4-0. Portal pressure was measured before and 15 days after ligation. The measurements were performed by the differential manometer Testo 510 (Germany), which was connected to the catheter 24G, located in the distal part of the superior mesenteric vein. The animals were divided into two groups: the first group (n=12) – portal vein (PV) was ligated with silk, and the second group (n=13) – PV was ligated with prolene. The average values of portal pressure before PPVL in experimental animals were 7,50 ± 0,20 mmHg. All animals survived in the first group. 7 rats in the second group died on the 1-2 day after surgery because of PV thrombosis. There were no significant differences in the average values of portal pressure in the comparison groups after 15 days: the first group (n = 12) - 11,44 ± 0,06 mmHg, the second group (n = 6) - 11,24 ± 0,04 mm Hg. Despite the same type of portal hypertension model and its equal severity, the use of prolene thread caused a greater propensity to PV thrombosis.

Keywords: Portal Hypertension, Partial Portal Vein Ligation, Prolene, Silk

1. Introduction

Various experimental models are used to study complex pathophysiological disorders that are observed in portal hypertension (PH). These models allow us to evaluate the pathological processes and to develop different methods for their adequate correction, which is not always possible to do in clinical conditions. Prehepatic portal hypertension is usually reproduced in rats by partial portal vein ligation (PPVL) [1]. According to the literature, the catheter size, the thickness of a thread, the place for ligation and the suture material are important for the creation of the model [2]. The most common are the catheter 20G (0.9 mm), the thickness of a thread 3/0 – 4/0 and ligation above the confluence of the splenic vein into the portal vein. The question of the suture material remains open. In particular, some studies indicate that the use of prolene leads to portal vein thrombosis, which is not observed when using silk [3]. However, the undeniable advantages of prolene are biological inertness and minimal tissue reaction to the thread [4]. We assumed that these properties of the thread will reduce its impact on the development of the studied pathological processes and increase the effectiveness of PPVL. The aim of the present study was to determine the optimal suture material (silk or prolene) for PPVL.

2. Materials and Methods

2.1. Ethics

All animal-related procedures were performed in accordance with the guidelines of the Independent Ethics Committee of South Ural State Medical University (SUSMU), the city of Chelyabinsk, Russia, and with the United States National Academy of Sciences Principles for

Research Involving Animals.

2.2. Animals

We used 25 male outbred rats, weighing 250 ± 20 g each, obtained from the SUSMU vivarium. Animals were kept in plastic bin cages, measuring 50 cm × 35 cm × 18 cm and lined with wood chips, under a 12-h dark/light cycle (lights on from 7 a.m. to 7 p.m.), at a controlled temperature of 22 ± 2˚C. The rats were fed commercially available chow and had access to water *ad libitum*.

2.3. Experimental Design

2.3.1. Groups

The animals were divided into two groups: the first group (n=12) – PV was ligated with silk, and the second group (n=13) – PV was ligated with prolene.

2.3.2. Induction of Portal Hypertension

The animals were initially anesthetized with Zoletil® 100 (Virbac, France). Anesthesia occurs within 6-7 minutes after a single injection (30 mg/kg i.p.). This dissociative anesthetic agent was chosen because it has a little influence on systemic hemodynamics, according to the conducted research [5]. After reaching the surgical stage of anesthesia, the upper midline laparotomy was performed and the bowels were gently retracted with a gauze pad soaked in saline. The portal vein was isolated from surrounding tissue above the confluence of the splenic vein and the superior mesenteric vein, and a 20G catheter was placed in front of it to establish PPVL. Both the portal vein and the catheter were tied using 4/0 silk or 4/0 prolene and the catheter was withdrawn creating a calibrated portal vein stenosis [6] (Figure 1).

2.3.3. Portal pressure Measurement

Portal pressure was measured before ligation of the portal vein and after 15 days. Measurements were performed by the differential manometer Testo 510 (Germany), which was connected to the 24G catheter, located in the distal part of the superior mesenteric vein. Pressure measurement lasted for 5 min, and the average value was regarded as the portal pressure. Hemostasis after catheter removal was performed using “Sulfacrilate” surgical glue (MedIn, Russia).

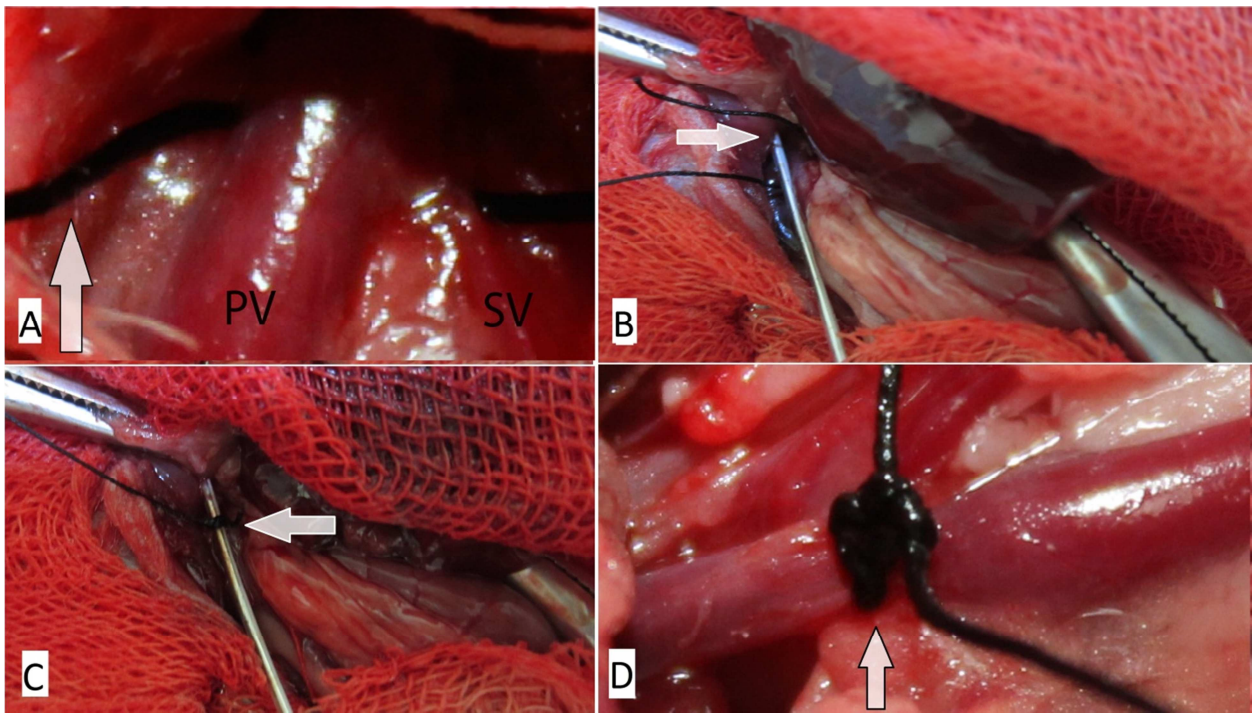


Figure 1. *A – 4/0 silk thread is located under the portal vein (PV – portal vein, SV – splenic vein, arrow - 4/0 silk thread); B – the 20G catheter is placed in front of the portal vein (arrow); C - the portal vein and the catheter are tied (arrow); D - the catheter is removed to create a calibrated portal vein stenosis (arrow).*

2.3.4. Euthanasia

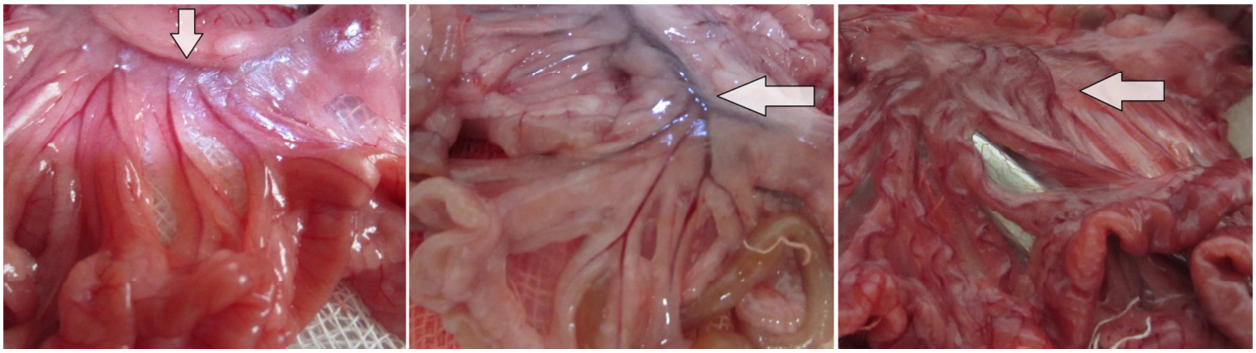
On day 15 after surgery, animals were anesthetized with Zoletil® 100 as described above. After a midline laparotomy, small intestine and spleen were carefully removed, signs of portal hypertension were evaluated macroscopically and the portal pressure was measured. The animals were then killed by exsanguination under deep anesthesia. In the case of the premature death of an animal, its cause was established at autopsy. The portal vein was dissected to determine the presence or absence of thrombosis.

2.3.5. Statistical Analysis

All data are presented as mean ± SE. Statistical significance was calculated using STATISTICA 10.0 for Windows. Student's t-test was used to compare damage between groups. The critical level for rejection of the null hypothesis was considered to be a P value of < 0.05 (i.e., a significance level of 5%).

3. Results

The average values of portal pressure before PPVL in experimental animals were 7.50 ± 0.20 mmHg. All animals survived in the first group. 7 rats in the second group died in the 1-2 day after surgery because of PV thrombosis. After 15 days, deformation and mesenteric venous engorgement were observed in all surviving animals as macroscopic signs of portal hypertension (Figure 2).



(a) (b) (c)

Figure 2. *Macroscopic signs of portal hypertension: (a) – intact mesentery of small intestine (arrow – superior mesenteric vein); (b) - congestion of mesenteric vein occurring immediately after ligation (arrow); (c) - deformation and mesenteric venous engorgement after 15 days from the creation of the model.*

Portal pressure was significantly elevated in both groups on the 15th day after the first operation ($P < 0.001$). There were no significant differences in the average values of portal pressure in the comparison groups: the first group ($n = 12$) – 11.44 ± 0.06 mmHg, the second group ($n = 6$) – 11.24 ± 0.04 mmHg ($P > 0.05$).

4. Discussion

4.1. Pathogenetic Substantination of PPVL in Prehepatic Portal Hypertension Modeling

The choice of PH modeling method depends on the purpose of the study. PPVL allows studying pathophysiological mechanisms and a variety of complications of portal hypertension more selectively because it causes minimal morphological changes in the liver.

4.1.1. Gastropathy

Gastropathy in PH is characterized by pathological damage of vessels located in the submucosa of the stomach, including the so-called "gastric petechiae" and the absence of an inflammatory reaction [7]. Molecular mechanisms involved in the pathogenesis of this complication are studied insufficiently. However, it is known that the basis of its origin is an imbalance between the decreasing antioxidants and the overproduction of reactive oxygen species. That leads to oxidative stress. Nitric oxide, which level rises in PH, promotes hyperdynamic circulation and overproduction of peroxynitrite, interacting with the reactive oxygen species, thereby damages the mucous membrane [8].

It has been shown that PPVL in rats leads to increased activity of NOS [9], the mRNA expression of endothelin type A receptors, significant vasodilatation, and increased blood flow in the gastric mucosa [10].

4.1.2. Enteropathy

Increased levels of TNFα and IL-1β and decreased levels of IL-10 in the small intestine of PPVL rats indicate the existence of an inflammatory process associated with goblet cell hyperplasia. It may be responsible for the remodeling of the intestinal epithelium [11]. It is also noted the connection between the presence of hyperplasia and the dilation of the superior mesenteric vein distal branches (3rd and 4th order) [12].

Other manifestations of malabsorption are bacteria overgrowth and bacterial translocation, which testifies to the existence of the peculiar to portal hypertension intestinal microbiome. It is involved in the processes of splanchnic and systemic changes [13]. In rats with PPVL was found the increase in bacterial translocation to mesenteric lymph nodes [14].

4.1.3. Adaptation of Intrahepatic Microcirculation and Functions of the Liver to Prehepatic Portal Hypertension

After PPVL, the proper hepatic artery becomes the main source of blood supply to the liver, and compensatory changes in the hepatic arterial system play an important role in maintaining the intrahepatic microcirculation [15]. The liver adapts to the reduction in portal blood flow through the remodeling of the hepatic arterial bed [16]. Although it undergoes minimal morphological changes, there are hepatic steatosis and changes in lipid and carbohydrate metabolism at the biochemical level. In this situation, they are similar to those that occur in the chronic inflammatory process described in the human's metabolic syndrome and sepsis [17].

4.1.4. Hepatic Encephalopathy

Hepatic encephalopathy is a complication of liver cirrhosis and portal vein thrombosis in humans, therefore the study of the mechanisms of its occurrence is important. In rats with

PPVL, the increase in blood-brain barrier permeability was confirmed on the 14th day after the operation. Restoration of its normal permeability on the 40th day correlated with the independent gradual decrease in portal pressure to normal levels. It may be the mechanism of reversibility of hepatic encephalopathy. Ammonia and hemodynamic changes could be triggers of brain barrier damage and its recovery [18].

4.1.5. Extrahepatic Angiogenesis

Until recently it was considered that the development of collateral circulation in PH is due to the opening of pre-existing portosystemic shunts in response to the increase in portal pressure. However, recent studies have shown the key role of vascular remodeling and angiogenesis as a mechanism of adaptation in this process [19]. It was shown in animal model of prehepatic portal hypertension, that the blockade of VEGFR-2 with anti-VEGFR-2 monoclonal antibody for 5-7 days and inhibition of VEGF/VEGFR-2 signalization using autophosphorylation inhibitor VEGFR-2 for 5 days after the operation resulted in 50% reduction of portosystemic collateral vessel formation [20, 21]. Blockade of NAD(P)H also contributed to this owing to the reduced splanchnic expression of VEGF, VEGFR-2 and CD31 [22].

4.1.6. Hemodynamic Changes in PPVL

On the 1st day after PPVL, an increase in the portal pressure is caused by a sharp increase in the resistance to portal blood flow through the portal vein due to its stenosis. After 4 days, the formation of portosystemic collaterals leads to a decrease in portal venous resistance and increased portal venous flow is beginning to play a major role in maintaining portal hypertension because of the development of hyperdynamic splanchnic circulation ("forward flow" theory), which finally established on the 8th day. The portal pressure at this time is about 15 mmHg, that is 50% higher than the normal value, and remains elevated the entire period of observation (up to 6 months) [23]. About 99% of the blood flows through portosystemic shunts, portal venous flow increases by 50% while the splanchnic vascular resistance reduces [24]. These hemodynamic changes may lead to the development of varicose veins of the esophagus in a variety of experimental animals [25].

4.2. Analysis of Known methods of Partial Portal Vein Ligation in Prehepatic Portal Hypertension Modeling

The classical experiment is performed under general anesthesia and under aseptic conditions, and the microsurgical technique of PPVL in rats involves the use of an operating microscope [26]. The abdominal cavity is opened through a midline incision and a 20G blunt-end needle is placed alongside the length of the portal vein. One ligature of 3/0 silk placed proximal to the bifurcation of the vein is tied around both the needle and the portal vein. The needle is then removed, and the portal vein is allowed to reexpand [27].

Published data show the importance of selecting the constriction rate of the portal vein. Indeed, the complete ligation of the portal vein as well as using a 26G (diameter = 0.45 mm), a 23G (diameter = 0.6 mm) or a 22G (0.7 mm) leads to high mortality, which is obviously not acceptable for these experiments. Using a 21G needle (diameter = 0.8 mm), the mortality drops to 20%-30%. Using a 19-g needle (diameter = 1.0 mm), the survival rate increases, but the portal vein pressure does not reach an ideal level to meet the experimental demand. A 20G catheter (0.9 mm) is optimal. Its application allows us to induce portal hypertension with minimal mortality, regardless the initial diameter of the vein and the vessel constriction rate [28].

In addition to the constriction rate of the portal vein, the thickness of a thread is an important factor influencing the resistance to portal blood flow. The portal venous pressure is unstable and usually low for the very thin thread, because the resistance of a tube is positively related to the obstructive length. Conversely, for the very thick thread, it is difficult to make a tight knot. The use of silk 4-0 or 3-0 is the most common. At the same time, the results were not satisfied with the death of all the animals, when the polypropylene thread 5-0 was tried.

The present study showed that portal vein thrombosis occur twice as frequently when using prolene than using silk of the same thickness. This result can be explained by the infliction of possible injury to the endothelium by the thread and thrombosis with the loss of the animal. The difficulty in making the knot with prolene must also be considered, once the knot adjustment to the catheter requires more tensile force to be applied by the surgeon. In this experiment, the force was standardized as much as possible. In addition, the breaking strain of prolene is almost three times greater than that of silk [29]. Therefore, the thread is stretched more when tying the knot and is shortened with the catheter extraction, reducing the diameter of the vein more than necessary. As a result, there is a strong tendency to thrombosis of the PV.

5. Conclusion

Despite the same type of portal hypertension model and its equal severity, the use of prolene thread caused a greater propensity to PV thrombosis.

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