RESEARCH ARTICLE

Changes in the microvasculature of small bowel mesentery in rats with prehepatic portal Hypertension: the preliminary study *in vivo*

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Neoangiogenesis in the small intestine mesentery is the trigger of portosystemic collateral circulation in portal hypertension. It leads to the severe complications, in particular, bleeding from esophageal varices. However, the types of blood vessels involved in this process have not been established. The aim of this study was to determine which type of the mesenteric microvessels participates in angiogenesis in portal hypertension. The present study included 12 adult outbred female rats divided into two groups: sham-operated (n = 5) and experimental (n = 7). Intravital microscopy allowed assessing the mesenteric microcirculation of rats in both groups during the first laparotomy and relaparotomy on the 15th day of the experiment. In contrast to the sham-operated animals, we induced prehepatic portal hypertension in rats of the experimental group by partial portal vein ligation during the first operation. The portal pressure in rats of the experimental group at the time of the second operation was significantly higher than in sham-operated rats: 12,53 ± 1,26 mm Hg and 9,34 ± 0,14 mm Hg, respectively (p<0,01). There was a significant increase in the number of capillaries (p <0,05) and total vascular density (p <0,05) in rats of both groups on the 15th day of the experiment. At the same time, a greater increase in the capillary network was in rats of the experimental group (p <0,05), whereas there was no significant difference in the vascular density values of the other types of blood vessels. Therefore, changes in the mesenteric microvasculature in portal hypertension lay in the increase in the vascular density values, which occur mostly at the expense of capillaries. This data allows to understand the pathogenesis of portal hypertension deeper, which is essential for the development of the treatment aimed at prevention of portosystemic shunting leading to the severe complications, such as bleeding from esophageal varices.

Keywords: angiogenesis; portal hypertension; intravital microscopy; microcirculation

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Introduction

Portal hypertension (PH) is characterized by multiple organ vascular disorders including microvascular disturbances in splanchnic territory [1]. Recently, it was found that they initially occur in the small intestine and its mesentery, and include the increase in vascular permeability, vasodilation, and the development of new blood vessels [2, 3]. According to the literature, it is the trigger of portosystemic collateral circulation, which leads to the severe complications, in particular, bleeding from esophageal varices [4]. However, the types of vessels involved in this process have not been established currently. The aim of this study was to determine which type of mesenteric microvessels participates in angiogenesis in portal hypertension.

Materials and methods

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 "Guide for the Care and Use of Laboratory Animals" (eighth edition, NIH Publication, 2011).

Animals

Studies were performed in 12 adult outbred female rats weighing 250 ± 50 g, which were divided into two groups: sham-operated (n = 5) and ligated (n = 7). Animals were kept under a 12-h dark/light cycle (lights on from 7 a.m. to 7 p.m.) and at a controlled temperature of 22 ± 2 ˚C in plastic bin cages measuring 50 cm × 35 cm × 18 cm and lined with wood chips. They obtained commercially available chow and had access to water *ad libitum*.

Intravital microscopy

The mesenteric microcirculation of rats in both groups was examined during the first laparotomy and relaparotomy on the 15th day of the experiment using intravital microscopy described by A.M. Geerts *et al*. [5]. Surgical stage of anesthesia was reached with intraperitoneal injection of Zoletil (Virbac, France) at a dose of 30 mg/kg. All procedures were made under strict sterile conditions. After performing an upper midline laparotomy, the cecum was gently exteriorized with a gauze pad soaked in saline, and the closest site of the mesentery was placed on a glass slide. The 10x objective of a Nikon Eclipse 50i microscope (Nikon, Japan) was positioned at random above the mesenteric "window." The microscopical stage was driven through a meander consisting of five steps of 1mm in the X direction and five steps of 1mm in the Y direction. A video (Nikon D3200, Japan) was cropped with Adobe Premiere Pro CC software, and the shots of each step were stuck with Adobe Photoshop CC software to obtain a 25 mm^2 snapshot. Vascular density of each type of blood vessels (arterioles, metarterioles, precapillary arterioles, capillaries, postcapillary venules, and venules) and total vascular density were calculated as the length of vessels per area of the obtained image (L/A, cm/cm^2). The outlines of each vessel were encircled manually using a "Pen" tool of Aperio Imagescope software (Leica Biosystems Inc., the USA) following the direction of the vascular wall. All the data were converted to Excel spreadsheets and used later for statistical analysis.

Induction of portal hypertension

In contrast to the sham-operated animals, prehepatic portal hypertension was induced in rats of the experimental group by PPVL during the first operation after intravital microscopy. 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 and the catheter was withdrawn creating a calibrated portal vein stenosis [6].

Portal pressure measurement

Portal pressure was measured in rats of both groups during relaparotomy on the 15th day of the experiment right after intravital microscopy. Measurements were performed by the differential manometer Testo 510 (Germany), which was connected to the 24G catheter inserted into the distal part of the superior mesenteric vein. Pressure measurement lasted for 5 min, and the average value was regarded as the portal pressure. Heparin was administered intravenously before the measurement at a dosage of 30 U/100 g of body weight to prevent thrombus formation.

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%).

Table 1. The value of portal pressure in rats of both groups on the 15th day of the experiment

№	Portal preassure (mm Hg)	
	Sham-operated	Partial portal vein ligated
1	9,47	12,5
2	9,36	11,785
3	9,18	14,055
4	9,39	14,25
5	9,12	11,295
6		12,75
7		11,085
Mean value ± SE	9,30 ± 0,17	12,53 ± 1,26*

*p<0,01

Table 2. The initial values of vascular density of small bowel mesentery in experimental animals of both groups

A/L, cm/cm^2	Sham-operated	Partial portal vein ligated
Arterioles	0,09 ± 0,08	0,36 ± 0,52
Metarterioles	1,54 ± 0,37	3,71 ± 2,64
Precapillary arterioles	1,57 ± 1,02	2,70 ± 1,53
Capillaries	28,43 ± 10,96	38,35 ± 7,01
Postcapillary venules	5,08 ± 0,47	4,16 ± 2,22
Venules	2,40 ± 1,19	3,66 ± 1,89
Total	39,11 ± 11,14	52,95 ± 9,30

p>0,05.

Table 3. The values of vascular density of small bowel mesentery in sham-operated rats on the 15th day of the experiment

A/L, cm/cm2	Sham-operated	
	The initial values	15th day
Arterioles	0,09 ± 0,08	0,38 ± 0,12
Metarterioles	1,54 ± 0,37	1,47 ± 0,48
Precapillary arterioles	1,57 ± 1,02	3,45 ± 0,33
Capillaries	28,43 ± 10,96	75,13 ± 2,27*
Postcapillary venules	5,08 ± 0,47	5,28 ± 2,97
Venules	2,40 ± 1,19	1,59 ± 0,65
Total	39,11 ± 11,14	87,29 ± 5,88*

*p<0,05.

Table 4. The values of vascular density of small bowel mesentery in partial portal vein ligated rats on the 15th day of the experiment

A/L, cm/cm2	Partial portal vein ligated	
	Initial values	15th day
Arterioles	0,36 ± 0,52	1,08 ± 1,13
Metarterioles	3,71 ± 2,64	4,24 ± 3,36
Precapillary arterioles	2,70 ± 1,53	2,90 ± 1,76
Capillaries	38,35 ± 7,01	103,61 ± 19,29*
Postcapillary venules	4,16 ± 2,22	8,87 ± 6,05
Venules	3,66 ± 1,89	8,45 ± 8,65
Total	52,95 ± 9,30	129,15 ± 26,80*

*p<0,001.

Table 5. The values of vascular density of small bowel mesentery in experimental animals of both groups on the 15th day of experiment

A/L, cm/cm2	Sham-operated	Partial portal vein ligated
Arterioles	0,38 ± 0,12	1,08 ± 1,13
Metarterioles	1,47 ± 0,48	4,24 ± 3,36
Precapillary arterioles	3,45 ± 0,33	2,90 ± 1,76
Capillaries	75,13 ± 2,27	103,61 ± 19,29*
Postcapillary venules	5,28 ± 2,97	8,87 ± 6,05
Venules	1,59 ± 0,65	8,45 ± 8,65
Total	87,29 ± 5,88	129,15 ± 26,80*

*p<0,05.

Results

The portal pressure in PPVL rats was significantly higher than in sham-operated animals: 12,53 ± 1,26 mm Hg and 9,30 ± 0,17 mm Hg, respectively (p<0,01), which confirms the development of PH after PPVL (table 1).

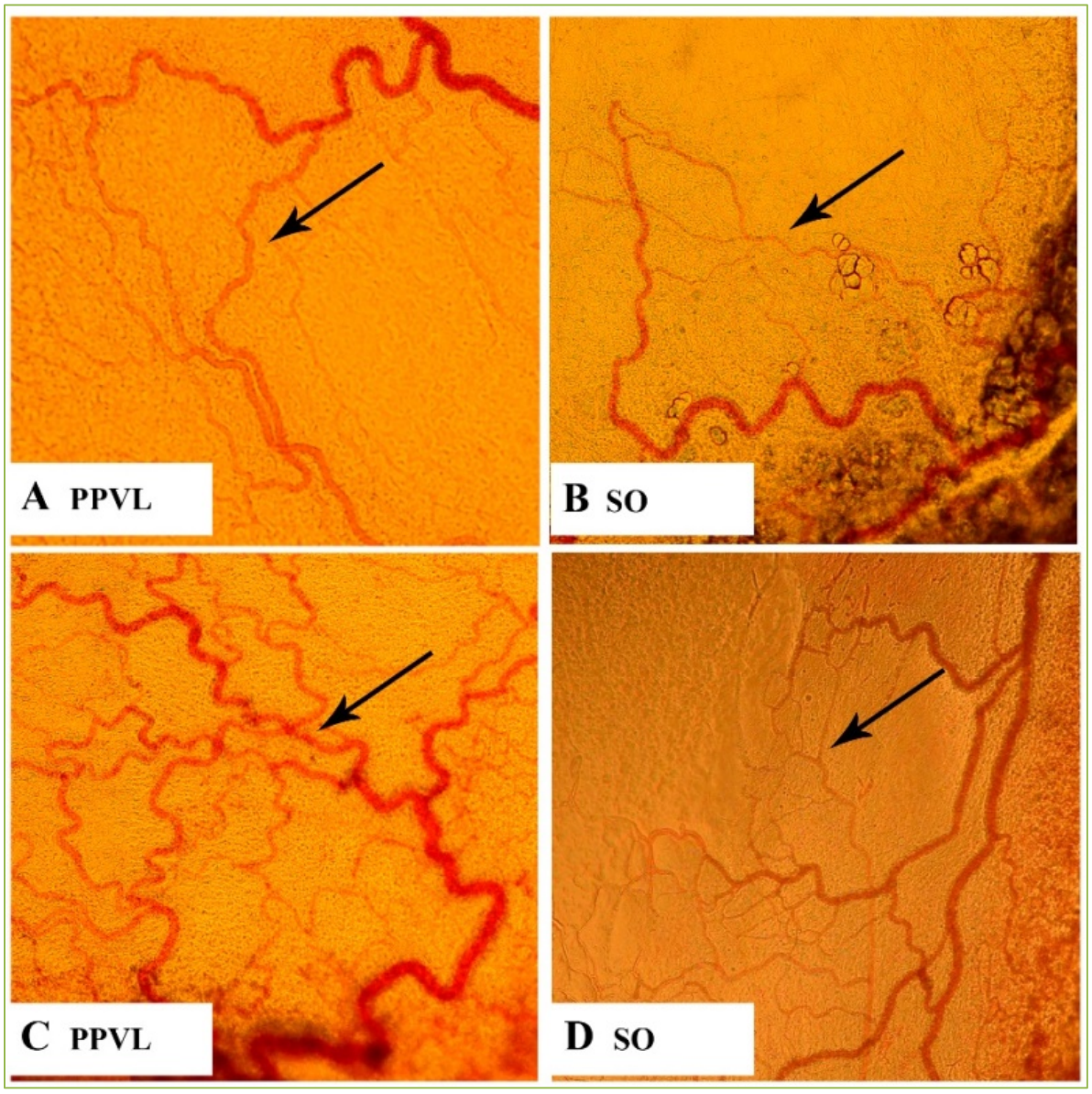

Figure 1. Intravital microscopy (10x magnification): the mesenteric microvasculature (arrow) in both PPVL and SO rats on different days. Normal blood vessels in PPVL (A) and SO (B) rats that was identified during the first operation. On the 15th day there was irregularly organized, tortuous, and engorged blood vessels in PPVL rats (C) and not altered vessels in SO rats (D).

On the 15th day of the experiment, gross structural changes were observed in the mesenteric microvasculature of PPVL rats at the background of increased portal pressure. They consisted in an irregular arrangement, engorgement, and tortuosity of blood vessels (figure 1).

In the same period, the extended dark areas were found along the venules, which is explained by the layering of their walls twisted in the form of a loop (figure 2). The capillaries also formed a shape resembling loops and spirals (figure 3).

According to the results of intravital microscopy conducted during the first operation, no statistically significant differences were found in vascular density between comparison groups (table 2).

There was a significant increase in the number of capillaries ($p < 0,05$) and total vascular density ($p < 0,05$) in rats of both groups on the 15th day of the experiment (table 3,4). At the same time, a greater increase in the capillary network was observed in PPVL rats ($p < 0,05$), whereas no significant differences were revealed in vascular density values of the other types of blood vessels (table 5).

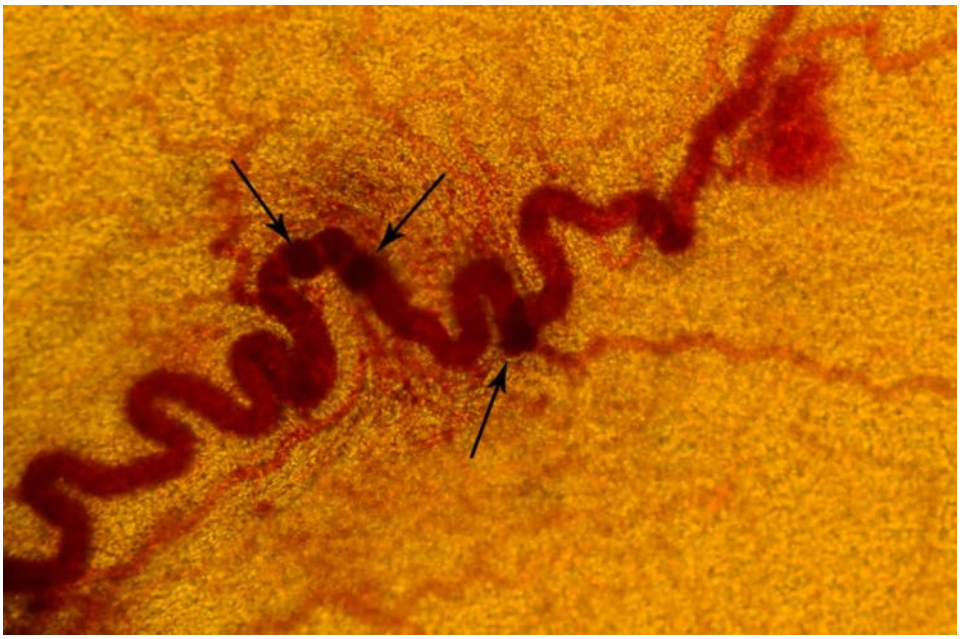

Figure 2. Intravital microscopy (10x magnification): a venule of small bowel mesentery (arrows) in PPVL rats on the 15th day of the study.

Discussion

Increased vascular resistance to portal blood flow in PH causes hemodynamic congestion in the splanchnic vascular bed, which may be an inductor of the cascade of events leading to the development of hyperdynamic circulatory status and portosystemic collateral circulation. In recent years, it was established that angiogenesis plays an important role in this process, initially occurring in the mesentery of the small intestine. Various experimental techniques are used for its studying: (1) implantation of placed into the polyester mesh and type I collagen fullfilled Teflon ring into the peritoneal cavity of rats followed by its extraction on the 16th day, immunohistochemical staining and video morfometry of obtained sections [7]; (2) fixation of the mesentery on the slide followed by FITC-labeled CD31 immunofluorescent staining [8]; (3) quantification of the leukocyte adhesion, hemorrhages and vascular obstruction using intravital video microscopy [9]; (4) placement of the mesentery on a plexiglass plate, intravital microscopy to calculate vascular density, subsequent fixation, and immunohistochemical staining with antibodies to CD31, VEGF, and eNOS [5].

However, the type of small bowel mesenteric blood vessels, which are directly involved in angiogenesis, has not been established.

In the present study, we evaluated changes in the mesenteric microvasculature in rats with prehepatic PH

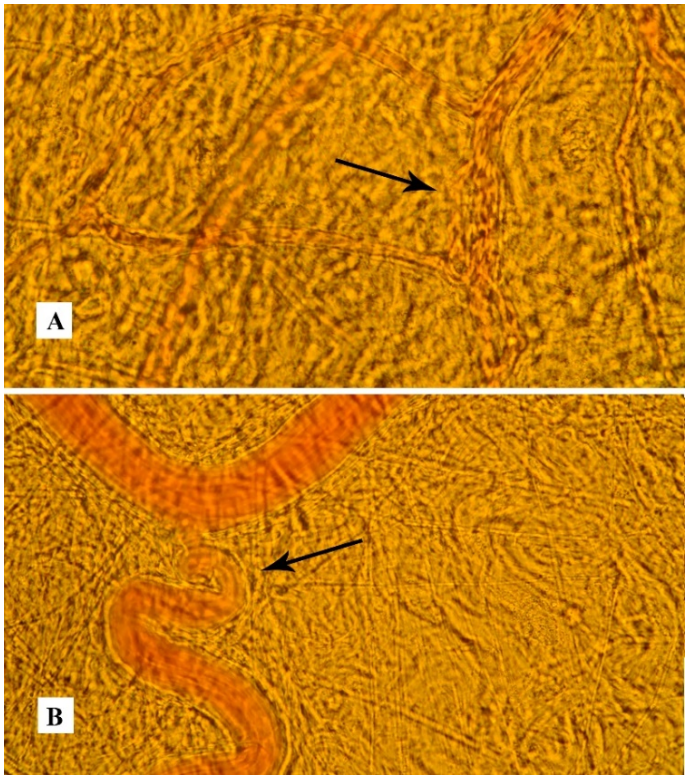

Figure 3. Intravital microscopy (100x magnification): capillaries (arrow) of the small intestine in PPVL rats on the 15th day of the study. The capillary in the form of loop (A), the capillary in the form of spirals (B).

model created by partial portal vein ligation in comparison with the control group. A feature of the rat mesenteric microvasculature is the «bridge type» structure where a

metarteriole is a short way from arterioles to venules. Arterioles (⌀ = 30-60 μm) may dichotomically divide into metarterioles (⌀ = 15-25 μm). The end of a metarteriole (⌀ = 10-15 μm) may be connected to a venule. Frequently, the end of a metarteriole divides into capillaries merging to form venules. Metarterioles may branch out into precapillary arterioles, which also may directly merge with venules, forming an arteriolovenular anastomosis. Precapillary arterioles (⌀ = 10-15 μm) are dichotomically divided into capillaries (⌀ = 5-7 μm) forming a network and then postcapillary venules (⌀ = 15-25 μm), that merge to become larger venules (⌀ = 30-75 μm) [10]. These data were used to differentiate the types of blood vessels.

On the 15th day of the experiment, gross structural changes were observed in the mesenteric microvasculature of PPVL rats at the background of increased portal pressure. All types of blood microvessels were engorged and dilated. Postcapillary venules and especially larger venules were tortuous and formed short loops, whereas capillaries produced an irregularly arranged and dense network. In sham-operated rats, their density was less pronounced and the other structural changes were absent.

Quantitative evaluation showed that the greatest changes occurred in the capillary bed. Its density was significantly increased in PPVL rats compared to SO ($p < 0,05$), which cannot be said about the other types of blood vessels. In addition, a greater number of capillaries anastomosed immediately with large venules. It may indirectly indicate an increase in portal inflow. Although the diameter of venules and arterioles has risen as the result of increased portal pressure, this has not led to a significant increase in their vascular density. In this connection, we can assume that they are not involved in neoangiogenesis in PH.

Thus, density of the mesenteric microvasculature in PPVL rats was reliably higher than in SO rats. However, these changes were statistically significant only for capillaries. Consequently, they are the first vessels responsible for the beginning of portosystemic shunting formation. This data allows us to understand the PH pathogenesis deeper, which is essential for the development of the treatment aimed at prevention of PH severe complications, such us bleeding from esophageal varices.

Conclusion

Changes in the mesenteric microvasculature in portal hypertension lay in the vascular density increase, which occurred mostly at the expense of capillaries. Further immunohistochemical studies with videomorphometry may allow confirming these results.

Conflicting interests

The authors have declared that no conflict of interests exist.

Author contributions

All authors of this research paper have directly participated in the planning, execution & analysis of this study. All authors of this paper have read and approved the final version submitted.

Abbreviations

PH: portal hypertension; SO: sham-operated; PPVL: partial portal vein ligation; VEGF: vascular endothelial growth factor; Enos: endothelial nitric oxide synthase; SE: standard error; μm: micrometers.

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