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MICROCIRCULATORY AND MICRO-RHEOLOGICAL INVESTIGATIONS OF A NOVEL PORTO-CAVAL SHUNT MODEL CREATED BY MICROSURGICAL METHODS

by Zoltán Klárik, MD

Supervisor:

Norbert Németh, MD, PhD

UNIVERSITY OF DEBRECEN

DOCTORAL SCHOOL OF CLINICAL MEDICINE

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By Zoltán Klárik, MD

Supervisor: Norbert Nemeth, MD, PhD

Doctoral School of Clinical Medicine, University of Debrecen

Head of the **Examination Committee**:	Prof. András Berta, MD, PhD, DSc
Members of the Examination Committee:	Zsuzsanna Bereczky, MD, PhD
	Andrea Ferencz, MD, PhD

The Examination takes place at the library of the Department of Ophtalmology, Faculty of Medicine, University of Debrecen, 8 June 2015, at 11 a.m.

Head of the **Defense Committee**:	Prof. András Berta, MD, PhD, DSc
Reviewers:	Prof. Lajos Bogár, MD, PhD, DSc
	Miklós Szokoly, MD, PhD
Members of the Defense Committee:	Zsuzsanna Bereczky, MD, PhD
	Andrea Ferencz, MD, PhD

The PhD Defense takes place at the Lecture Hall of Bldg. A, Department of Internal Medicine, Faculty of Medicine, University of Debrecen, 8 June 2015, at 1 p.m.

1. INTRODUCTION

The artificial porto-caval shunts are special vessel anastomosis, which connect the portal and systemic circulation under appropriate indication. These shunts totally or partially divert the blood from the portal system, thus decrease the portal hypertension and the development of its complications. Accordingly, there are selective and non-selective variants of these shunts.

Portal hypertension leads to sever hepatic failure, in which cases liver transplantation is the only therapeutic option. To treat the escalation of complications such as bleeding caused by esophageal varix, endoscopic sclerotherapy and transjugular intrahepatic porto-systemic shunt are frequently used today. In emergency situation, where these therapeutical ways are not effective surgical porto-caval shunts are the only choice.

For analyzing the complex functional and morphological changes, numerous porto-caval shunt models have been developed in laboratory animals. Lee and Fisher created the first microsurgical porto-caval shunt in the rat in 1961. It was an end-to-side anastomosis model, which had been widely applied in variety of research models. Since then several further refined models were developed and still used today. Besides the microsurgical background, the knowledge of the shunt's complication on experimental animals is an important aspect when we choose the anastomosis model and the animal species. The porto-caval anastomosis models are frequently used for the study of hepatic encephalopathy and liver atrophy.

While creating such artificial anastomoses, the surgical safety is an important aspects: patency, geometry and long-term effectiveness of these shunts have proved to be significant. The monitorization of intraoperative microcirculatory changes may provide important information on the tissue and organs perfusion, especially while creating a vessel anastomosis. To ensure adequate tissue perfusion, the micro-rheological (red blood cell deformability and aggregation) play a significant role, therefore the investigation of the

correlation between the changes of these parameters and the microcirculation may be very useful.

The red blood cell deformability – passive shape changing capability – is essential for the passage through the capillaries, and as well as the red blood cell aggregation – the cells reversible interconnection – can influence the hemodynamics. A long lasting ischemia can severely affect the hemodynamics and cause irreversible changes in a tissue. In a stagnant blood of an excluded region, several physical and metabolic changes can occur, which can exert its effect to the whole body during reperfusion. The micro-environmental changes cause increased aggregation and decreased deformability, which lead to microcirculatory deterioration. The decreased blood pH level worsens the aggregation and deformability, at alkali pH levels the red blood cells lose their biconcave shape and their volume deceases. Changes in the blood gas parameters can also influence the blood micro-rheological characteristics. In the last decade, by using modern hemorheological devices, increasing amount of data became available on regional hemorheological properties as well as local versus systemic changes of red blood cell deformability and red blood cell aggregation in various pathophysiological conditions, including circulatory disorders, ischemia-reperfusion and vascular anastomosis.

However, the data in the literature are still unclear and controversial. There is also a lack of data about intraoperative microcirculatory changes in the organs that are affected by hypoperfusion or ischemia during creating artificial anastomosis. Furthermore, several experimental models suggested that besides arterio-venous and local versus systemic alterations, marked aorto-porto-caval hemorheological differences have to be taken under consideration when planning experiments and evaluating results.

Taking account the above described differences, it would be interesting to know the physiological hemorheological parameters (red blood cell deformability and aggregation) of the portal circulation and how these values

differ from the arterial and systemic venous blood samples' hemorheological characteristics. It is not known, how the ischemia-reperfusion arising during a creation of porto-caval shunts modulates the micro-rheological differences and the microcirculation of the affected organs. These issues are little know, although it might have a significant clinical relevance,

2. AIMS

1. The investigation of less known aorto-porto-caval hemorheological differences in particular the red blood cells deformability and red blood cell aggregation.
2. Creating a new selective porto-caval model with microsurgical methods in the laboratory rat, where only the rostral mesenteric veins is utilized and the portal vein with its splenic vein is left intact.
3. The examination of multiple organs' (small intestine, liver, and right kidney) intraoperative microcirculation before and after a meso-caval localized end-to-side porto-caval anastomosis.
4. The pre-assessment of hematological and hemorheological parameters on the 1^{st} and 14^{th} postoperative days as a possible follow-up investigation after the artificial shunts.

3. MATERIALS AND METHODS

The experiments were approved and registered by the University of Debrecen Committee of Animal Research (permission Nr.: 37/2007. and 6/2008. UD CAR), in accordance with the relevant Hungarian Animal Protection Act (Law XVIII/1998) and EU Directives (EEC 63/2010).

3.1. Investigation of aorto-porto-caval micro-rheological differences

3.1.1. Experimental animals and blood sampling protocol

Thirteen healthy male (n=8; 381.5 ± 13.4 g) and female (n=5; 292.2 ± 20.9 g) Sprague-Dawley rats (Janvier Co., France) were subjected to the study. Under general anesthesia (60 mg/kg, i.p., Thiopenthal®, Biocheme GmbH, Austria) midline laparotomy was performed and the infrarenal part of the abdominal aorta and the caudal caval vein as well as the portal vein were gently prepared and isolated using atraumatic, microsurgical methods.

Blood samplings were carried out by 26 G needle connected to a syringe (anticoagulant: Na-EDTA, 1.5 mg/ml). The vessels were punctured in the following order: portal vein, caudal caval vein, abdominal aorta. 0.6-0.8 ml blood was withdrawn per vessel. At the end of the experiment the animals have been sacrificed by exsanguination.

The samples were immediately taken into the laboratory to complete the measurements within the possibly shortest time (total *in vitro* time < 30 min).

3.1.2. Laboratory examinations

3.1.2.1. Lactate level, blood pH and gas analysis

A blood gas analyzer automate (ABL555 Radiometer Copenhagen, Denmark) was used to determine lactate concentration (mmol/l), blood pH as well as pCO2 and pO2 values [mmHg]. The samples, in closed system, were immediately filled into the device without direct contact with air.

3.1.2.2. Hematological parameters

A semi-automated microcell counter (Sysmex F-800, TOA Medical Electronics Co., Japan) was used to determine the general hematological parameters.

3.1.2.3. Red blood cell deformability

A LoRRca MaxSis Osmoscan device (Mechatronics BV, The Netherlands) was used to measure red blood cell elongation index in the function of shear stress, together with red cell osmotic properties.

Red blood cell deformability measurements: trying to avoid direct contact with the laboratory air 5 µl of blood was taken into 1 ml of isotonic polyvinyl-pyrrolidone solution (360 kDa PVP in normal phosphate buffered saline; viscosity = 28.8 mPa.s, osmolality = 305 mOsm/kg ; pH = 7.36) and gently mixed. The suspension was injected into the bob-cup system of the device, without air-bubbles. The LoRRca generated shear stress (SS) range from 0.3 to 30 Pa in computer-controlled grades, while the laser diffraction pattern was continuously analyzed: elongation index (EI) is equal to (L-W)/(L+W), where L is the length and W is the width of the diffractogram (reflecting deformed cells) at a constant shear stress. EI increases with red blood cell deformability. The measurements were carried out at constant temperature of 37 °C.

For comparison of individual EI-SS curves Lineweaver-Burk analyses were performed, calculating the maximal elongation index (EImax) and the shear stress at half EImax (SS1/2 [Pa]) values, according to the following formula: 1/EI = SS1!2/ EImax x 1/SS + 1/EImax

SS1/2 increases with decreasing red blood cell deformability. For comparison we used self-made calculation (shear stress range: shear stress range: 0.95-30 Pa) and the LoRRca software-given data, too (shear stress range: ~1.69-30 Pa).

Osmoscan function: 250 µl blood was taken into 5 ml PVP solution then gently mixed. The device generated a continuous shear stress of 30 Pa on the

sample, while continuously measuring the elongation index. During the measurements the device changes the osmolality of the medium using gradual mixtures of PVP solutions of 0 and 500 mOsmol/kg.

The measured and calculated parameters by the device: maximal elongation index values measured at shear stress of 30 Pa (maximal EI), minimal elongation index values measured at shear stress of 30 Pa and at low osmolar environment (minimal EI), measureable elongation index values at shear stress of 30 Pa and high osmolar environment (EI hyper), osmolality at minimal EI, osmolality at maximal EI (‘optimal‘ osmolality), osmolality at EIhyper and the area under the individual osmolality-elongation index curves.

3.1.2.4. Red blood cell aggregation

A Myrenne MA-1 erythrocyte aggregometer (Myrenne GmbH, Germany) was used to measure red blood cell aggregation. The technique is based on light transmittance method. Since the LoRRca device needs approximately 1 ml of blood for aggregation measurement, we could not use that function in this experiment. The Myrenne aggregometer requires approximately 20 µl of blood, only. After disaggregating blood sample by 600 s^{-1}, the shear rate drops to zero (M index) or to a low, 3 s-1 shear rate (M1 index). According to the changes in light transmittance (disaggregation: low light transmittance, aggregation process: increasing light transmittance), the instrument calculates the aggregation index values at the 5th or 10th second of the aggregation process. The indices (M 5 s, M1 5s, M 10 s, M1 10 s) increase with enhanced red blood cell aggregation.

3.1.3. *Statistical analysis*

For the statistical analysis we used SigmaStat (Systat Software Inc., San Jose, California, USA) software. For comparison of arterial, venous or portal venous blood samples paired t-test/Wilcoxon signed rank test, as well as t-

test/Mann-Whitney rank sum test were carried out according to the data distribution normality. A p value of <0.05 was considered as statistically significant.

3.2. Morphological and intraoperative microcirculatory investigation of end-to-side porto-caval shunt

3.2.1. Experimental animals, anesthesia

Ten Sprague-Dawley rats (bodyweight: 340.9 ± 24.52 g) were subjected to the study. The experimental animals were anesthetized using sodium-thiopental (60 mg/kg i.p., Thiopenthal, Biocheme GmbH, Austria). For microsurgical operations a Leica Wild M650 operative microscope was used, and video recordings were made.

3.2.2. Operative technique

As a preliminary investigation, in three (300-350 g, male) anatomical study was performed, during which we prepared and mobilized the main branches of the and the suprarenal section of caudal caval vein, then the Lee ad Fisher shunt model was performed.

The microsurgical technique provided a design of a selective porto-caval shunt, in which only the rostral mesenteric vein (rMV) was sutured, as end-to-side anastomosis, into the caudal caval vein (CCV), while the other main branch of the portal vein (splenic vein) was left intact.

After median laparotomy and anteposition of the intestines, careful dissection of the portal vein (PV) and its tributaries were prepared. The CCV was mobilized between the two renal veins. At the bifurcation of the portal vein the proximal part of the rMV was ligated with 8/0 braided silk. Clips were applied onto the intestinal part of the rMV and to the CCV proximally and distally. The rMV was washed out with physiological saline solution diluted

with sodium-heparin (10%) and positioned toward the CCV in order to minimize the tension.

On the isolated anterior wall of the CCV, venotomy was performed with the length equal to the diameter of the mesenteric vein. The site of the venotomy was located as lateral as possible. Firstly, the posterior wall of the anastomosis was continuously sutured, while on the anterior wall simple interrupted sutures (6-7 stitches) were used with 10/0 polyamide monofilament suture material. After releasing the clips, the shunt patency was checked.

The intestines then were re-positioned and after completing the intraoperative microcirculatory measurements, the abdominal wall was closed in two layers.

3.2.3. Morphometric analyses

The vessel geometry parameters were determined off-line from the video-recordings: outer diameter of the CCV and the rMV, as well as of the legs of the completed end-to-side anastomoses together with their angle. The offline measurements were carried out with Adobe Photoshop CS5 software. The angle of the anastomosis at the heel was measured with the same software. The axis of the veins was drawn and the angle was calculated where the lines were connected.

3.2.4. Intraoperative microcirculatory investigation

A non-invasive laser Doppler tissue flowmetry was used (LD-01 laser Doppler tissue flowmetry monitoring system, Experimetria Ltd., Hungary) with a standard pencil probe (MNP100XP, Oxford Optronix Ltd., UK) placed on the surface of the liver's middle lobe, on the antimesenteric surface of a jejunum segment, as well as on the anterior surface of the right kidney. Based upon the Doppler shift effect when the laser beam is reflected from moving red blood cells, the device determines blood flux units (BFU), which were registered for

20 seconds after stabilization of the signal. Using single-channel laser Doppler flowmetry, it is important to set the standards for the evaluation,26,28-30 we have chosen the evaluation of the average BFU values of a standard time period of 20 seconds.30

The laser Doppler measurements were carried out before (Base) and shortly after applying the clips, before and just after releasing the clamping (Reperfusion 0') as well as in the 30th minutes of the reperfusion (reperfusion 30').

3.2.5. Laboratory examinations

In the experiment, from the last two animals individually laboratory test were performed on the 1st and 14th postoperative days. These animals after the operation were rehydrated with 5 ml of NaCl (0.9%) solution, and postoperaive analgesia was given (Flunixin, 20 mg/kg, s.c.).

Under general anesthesia, re-laparotomy was done and blood samples were collected. As it was described in *3.1.2.2.* and *3.1.2.3.* sections, from the blood the hematological and hemorheological parameters were determined.

3.2.6. Statistical analysis

For statistical analysis SigmaStat (Systat Software Inc., San Jose, California, USA) software was used. Data are presented as mean ± standard deviation (S.D.), it is indicated if median ± standard error (S.E.) is shown. The comparison of intraoperative laser Doppler flowmetry data obtained from various measurement sites were carried out with Student's t-test or Mann-Whitney rank sum test, while the changes during the time-frame of experiment within measurement sites were analyzed by one-way ANOVA methods (Bonferroni's or Dunnett's test), depending on the data distribution. The significance level were considered when $p<0.05$.

4. RESULTS AND CONCLUSIONS

4.1. Investigation of aorto-porto-cavalis haemorheologic differences

4.1.1. Lactate concentration, blood pH and gas values

Lactate concentration was slightly but significantly higher in arterial blood samples ($p=0.001$ by t-test and $p=0.007$ by paired t-test vs. venous sample; and only $p=0.064$ by t-test and $p=0.073$ by paired t-test vs. portal venous sample). Blood pH was also higher in arterial blood ($p=0.002$ by t-test and $p<0.001$ by paired t-test vs. venous sample; and $p=0.008$ using t-test and $p=0.006$ by paired t-test vs. portal venous sample), while in systemic venous and portal venous samples the values were almost identical.

Blood gas values reflected the physiological expectations, pO_2 was the highest in arterial blood samples ($p<0.001$ both by t-test and paired t-test vs. venous sample; $p<0.001$ both by t-test and paired t-test vs. portal venous sample) and pCO_2 in systemic venous samples ($p=0.028$ using t-test and $p=0.016$ by Wilcoxon test vs. venous sample; and only $p=0.063$ by t-test and $p=0.082$ using paired t-test vs. portal venous sample).

4.1.2. Hematological parameters

Total leukocyte count was significantly lower in arterial blood compared to both systemic venous ($p=0.013$ by Mann-Whitney test and $p<0.001$ by paired t-test) and portal venous blood ($p=0.009$ using t-test and $p=0.002$ by paired t-test). Although neither one reached the significant level, both red blood cell count and hematocrit were moderately elevated in systemic venous and portal venous blood samples. Mean corpuscular volume did not differ markedly, however, in venous samples slightly decreased.

Platelet count moderately elevated in systemic venous and portal venous samples without significant difference. The other tested hematological parameters did not differ essentially.

4.1.3 Hemorheological parameters

4.1.3.1. Red blood cell deformability

Although the standard deviations overlapped, the differences were clearly observable between EI-SS curves: the highest EI values could be measured in arterial, the lowest values in systemic venous blood samples, while the portal venous values lay in between.

The parameterizational data also reflected these differences. EImax values were significantly lower in arterial samples compared to systemic venous blood ($p=0.004$ by Mann-Whitney test and $p=0.003$ using Wilcoxon test; software-calculated values: $p=0.003$ by Mann-Whitney test and $p=0.005$ using Wilcoxon test). SS1/2 values were higher in arterial blood compared to systemic venous blood ($p=0.023$ by paired t-test; software-calculated values: $p=0.043$ by paired t-test). The difference between arterial and portal venous blood did not reach the statistically significant level ($p=0.071$ by paired t-test).

The minimal EI values (at the lowest osmolality where swelled red blood cells still exist) were slightly higher in systemic venous and portal venous blood samples compared to the arterial blood. Maximal EI values did not differ essentially, however, in venous blood samples it was moderately higher. The EI hyper (at the highest osmolality where shrunk erythrocytes are still existing) were moderately higher in both venous sample-types compared to arterial blood.

The *osmolality at the maximal EI ('optimal' osmolality)* was a bit higher in systemic venous blood compared to arterial samples. In portal venous blood these values were the lowest, almost reaching the significance level versus systemic venous vales ($p=0.072$ by paired t-test).

4.1.3.2. Red blood cell aggregation

In arterial blood M values at the 5th second showed the lowest values (1.15 ± 0.46) compared to caudal caval venous (1.26 ± 0.52) and portal venous blood samples (1.21 ± 0.58) but without significant difference.

M1 values at the 5th second were higher in arterial blood samples (2.03 ± 0.88) compared to the venous blood (1.87 ± 0.85). In portal venous blood we measured the highest values (2.95 ± 2.03; p=0.055 vs. venous blood, using t-test).

M index at the 10th second showed marked differences. Caudal caval vein blood samples had the lowest values (2.27 ± 0.83), being significant compared to both arterial (4.38 ± 2.51; p=0.001 by Mann-Whitney test) and portal venous blood samples (4.71 ± 1.98; p<0.001 by using Mann-Whitney test).

M1 index at the 10th second did not show significant differences. However, arterial blood samples had slightly higher values (5.27 ± 3.13) compared to the venous blood (5.03 ± 2.24), while portal venous samples showed the highest values.

4.1.4. Discussion and conclusions

In this current study we aimed to analyze the possible aorto-porto-caval differences of red blood cell deformability (together with osmoscan data), red blood cell aggregation, hematological parameters as well as blood pH, lactate concentration, pCO_2 and pO_2 values.

The osmoscan data did not show important aorto-porto-caval differences. However, it could be observed that samples from the portal veins often showed more expressed fluctuating values and moderately flattened plot over 350-400 mOsm/kg. Since very little data are available with the new osmoscan, we could not find explanation for these results.

Micro-rheological variables, such as red blood cell deformability and red blood cell aggregation may show arterio-venous and porto-caval differences in the rat. The appropriate control examinations thus are important in experimental surgical and microsurgical research models (e.g.: artificial porto-caval shunt models). For better understanding the significance and the background of these differences, further comparative studies are needed.

4.2. Intraoperative microcirculatory and morphological investigations of end-to-side porto-caval shunts

4.2.1. Technical experiences and morphometric data

The created mesocaval end-to-side venous anastomosis was functioning well; there were no bleeding at the site of the anastomosis. Its patency was maintained and no stenosis was observed. The operation time was 48.75 ± 11.26 min. The longest phase was the preparation and positioning of the vessels to be, providing situation without tearing, stretching or rupturing the intima. During the procedure (when clips were being applied) the changing of organs' color was well observable. The small intestine showed venous congestion and the liver became paler.

The presented geometry of the shunt occurred due to the anatomical localization of the given vessels, since there was a persisted risk of the rMV to tear at various angulation positioning. In order to minimalize such intraoperative complication, the anastomosis could be created tension-free and at the angulation of 77.13 ± 5.84°.

The outer diameter of the rMV as well as the CCV dilated just above or below the shunt compared to the normal situation (state before applying the clips) (Table 1). Dilatation of the rMV was not significant, and the CCV diameter was increased significantly above ($p<0.001$ vs. normal; $p=0.05$ vs. anastomosis site) and below ($p<0.001$ vs. normal; $p=0.004$ vs. anastomosis site) the shunt.

4.2.2. Intraoperative microcirculatory investigation

The changes of blood flux unit (BFU) are shown on Figure 3. At base level the values of liver (32.64 ± 13.68), jejunum (35.83 ± 13.67) or kidney (36.14 ± 17.71) did not differ significantly among each other. When applying the clips according to the operative protocol, the intestinal BFU values as well as renal ones decreased, and as we expected, it was more expressed on the jejunum.

After clamping the intestinal values (17.61 ± 9.09) were significantly lower compared to its base ($p<0.001$), as well as versus liver (29.13 ± 14.45, $p<0.001$) and kidney values (24.75 ± 10.86, $p=0.001$). Renal BFU decreased significantly compared to its base ($p<0.001$).

Just before releasing the clamps the lowered values were more expressed, keeping similar relations between the investigated organs (on the jejunum: 12.03 ± 9.19, $p<0.001$ vs. base and liver, $p=0.036$ vs. kidney; on the liver: 23.26 ± 13.37, $p<0.001$ vs. base, $p=0.02$ vs. kidney; on the kidney: 16.07 ± 10.91, $p<0.001$ vs. base).

Just after releasing all the clips, the BFU values started to increase, but not in the same manner in the organs. The renal BFU values recovered almost completely (31.87 ± 12.59), while intestinal values (21.26 ± 17.07, $p<0.001$ vs. base, $p<0.001$ vs. kidney) as well as hepatic BFU values (24.43 ± 12.06, $p<0.001$ vs. base, $p<0.001$ vs. kidney) were dropped behind the renal data. During the investigated reperfusion period, the intestinal values were remained lower compared to the hepatic values, however, the p values were close to the significance level (at reperfusion 0': $p=0.059$; at reperfusion 30': $p=0.051$).

4.2.3. Hematological parameters

The red blood cell count, hemoglobin concentration, as well as hematocrit decreased on the 1st postoperative day and by the end of second week compared to the control data of the Department's Hemorheological Research Laboratory.

4.2.4. Hemorheological parameters

4.2.4.1. Red blood cell aggregation

The aggregation increased on the first day, especially at the 10th second of the aggregation process in case of M and M1 index parameters. At the 5th and 10th second the M parameters were lower compared to the control values on the

14^{th} postoperative day, while the M1 parameters were close to the control values.

4.2.4.2. Red blood cell deformability

The deterioration of red blood cell deformability was well visible on the 1^{st} and 14^{th} postoperative day in the samples from the examined rats. There were a slight alteration in the EI at 3 Pa, EI_{max} and $SS_{1/2}$ values compared to the control in the 1^{st} postoperative day, and by the 14^{th} postoperative day the deformability showed significant deterioration.

4.2.5. Discussions and conclusions

Experimental researches focusing on vascular anastomosis have high clinical importance hence the investigation of surgical safety, the morphological vascular changes and the long term effectives of these procedures are inevitable. Studying these aspects is necessary for a better understanding of the characteristic of such decompressive porto-systemic shunts.

The presented end-to-side mesocaval anastomosis between the caudal caval vein and the rostral mesenteric vein is a renewed microsurgical model for creating a selective porto-caval shunt. Although, the operative technique is not simple, due to the anatomical distances, but it gives the possibility to redirect the retrograde flow existing in case of portal hypertension back to the systemic circulation via bypassing the liver.

During the design of the surgical protocol our aim was to create a situation where we can minimize the tension between the structures. Therefore, additional vessel ligations were needed to further mobilize the mesenteric vein, so the approximation of the anatomical structures was under less tension. Using the applied suturing technique the veins were not stretched and the anastomosis was secure. At the toe of the anastomosis, the first knot of the continuous suture line was a surgical knot.

Several hepatic pathological deteriorations could be studied due to the complication after the creation of such shunts. The diverted blood from the liver directly into the systemic circulation can cause hepatic encephalopathy alongside with hepatic morphological and functional changes. During the postoperative follow-up, we found prominent alterations in the blood samples compared to the control values, even though by the 14th postoperative day in some parameters (RBC count, Hct, MCV, M1 5 sec and 10 sec aggregation index, and EI at 3 Pa) were close to the control values. After two weeks the animal behavior changed, consumed less food, which resulted severe weight loss. All of these could be contributed to the metabolic changes caused by the shunt.

In this model a selective porto-caval shunt was achieved by anastomosing the rostral mesenteric vein into the caudal caval vein with a modified microsurgical technique in laboratory rat, where the shunts were well feasible and stable geometry was formed. The mesocaval shunt is also a competent model to study hepatic encephalopathy without a significant liver atrophy. The intraoperative laser Doppler measurements provided useful information for the elaboration of a more secure surgical technique with the assessment of microcirculatory changes on the areas exposed to hypoperfusion and/or ischemia-reperfusion.

5. SUMMARY OF MOST IMPORANT RESULTS AND CONCLUSIONS

1. We were the first to describe aorto-porto-caval basic hemorheological differences in the rat. The lowest elongation index values were recorded in the arterial samples, the highest were in the systemic venous blood samples. The parameters from the portal vein fall between the two. The aggregation index M parameters at the 10^{th} seconds of the aggregation process were significantly lower in the systemic venous blood compared the arterial and portal venous samples. The highest aggregation index M1 values at the 5^{th} seconds were measured in the samples from the portal vein.

2. By using microsurgical methods, we securely developed a new, meso-caval localized end-to-side porto-caval shunt model for the investigation of morphologic, intraoperative microcirculatory and postoperative hemorheological changes.

3. After the examination of multiple organs' (small intestine, liver and right kidney) intraoperative microcirculation we could conclude that the necessary venous (caudal caval vein and rostral mesenteric vein) temporary ligation caused varying degrees of microcirculatory disturbance: the most significant deterioration was observed in the parameters of small intestine and the liver. In the reperfusion period the values of the involved organs normalized compared to base values but not in the same manner. During the intraoperative investigation of the vessels' calibers, the caudal caval vein significantly dilated above and below the shunt.

4. In the presence of porto-caval shunt the hematological and hemorheological parameters worsened according to the results of the preliminary investigation (between the 1^{st} and 14^{th} postoperative days).

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List of publications related to the dissertation

1. **Klárik, Z.**, Tóth, E., Kiss, F., Mikó, I., Furka, I., Németh, N.: A modified microsurgical model for end-to-side selective portacaval shunt in the rat. Intraoperative microcirculatory investigations.
Acta Cir. Bras. 28 (9), 625-631, 2013.
IF:0.57

2. **Klárik, Z.**, Kiss, F., Mikó, I., Németh, N.: Aorto-porto-caval micro-rheological differences of red blood cells in laboratory rats: Further deformability and ektacytometrial osmoscan data.
Clin. Hemorheol. Microcirc. 53 (3), 217-229, 2013.
DOI: http://dx.doi.org/10.3233/CH-2012-1531
IF:2.215

List of other publications

3. Tóth, C., **Klárik, Z.**, Kiss, F., Tóth, E., Hargitai, Z., Németh, N.: Early postoperative changes in hematological, erythrocyte aggregation and blood coagulation parameters after unilateral implantation of polytetrafluoroethylene vascular graft in the femoral artery of beagle dogs.
Acta Cir. Bras. 29 (5), 320-327, 2014.
DOI: http://dx.doi.org/10.1590/S0102-86502014000500006
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4. Tóth, C., Kiss, F., **Klárik, Z.**, Gergely, E., Tóth, E., Pető, K., Ványolos, E., Mikó, I., Németh, N.: Following-up changes in red blood cell deformability and membrane stability in the presence of PTFE graft implanted into the femoral artery in a canine model.
Korea-Aust. Rheol. J. 26 (2), 209-215, 2014.
DOI: http://dx.doi.org/10.1007/s13367-014-0023-3
IF:0.632 (2013)

5. Németh, N., Kiss, F., **Klárik, Z.**, Tóth, E., Mester, A., Furka, I., Mikó, I.: Simultaneous investigation of hemodynamic, microcirculatory and arterio-venous micro-rheological parameters in infrarenal or suprarenal aortic cross-clamping model in the rat.
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Total IF of journals (all publications): 12,847
Total IF of journals (publications related to the dissertation): 2,785

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