Thesis for the degree of doctor of philosophy (Ph.D.)

MORPHOLOGICAL AND HEMORHEOLOGICAL EXAMINATIONS
OF EXPERIMENTAL ARTIFICIAL ARTERIO-VENOUS SHUNTS

Tímea Hevér, M.D.

Supervisor: Norbert Németh, M.D., Ph.D.

UNIVERSITY OF DEBRECEN
Doctoral School of Clinical Medicine
Debrecen, 2011
MORPHOLOGICAL AND HEMORHEOLOGICAL EXAMINATIONS OF EXPERIMENTAL ARTIFICIAL ARTERIO-VENOUS SHUNTS

By Timea Hevér, M.D.

Supervisor: Norbert Németh, M.D., Ph.D.

Doctoral School of Clinical Medicine, University of Debrecen

Head of the Examination Committee: Prof. András Berta, M.D., Ph.D., D.Sc.
Members of the Examination Committee: Prof. György Wéber, M.D., Ph.D., C.Sc.
Prof. József Balla, M.D., Ph.D., D.Sc.

The Examination takes place at Library of the Department of Ophthalmology, Medical and Health Science Center, University of Debrecen
12 a.m. 31 March 2011

Head of the Defense Committee: Prof. András Berta, M.D., Ph.D., D.Sc.
Reviewers: Miklós Szokoly, M.D., Ph.D., Ph.D.
János Mátýus, M.D., Ph.D.
Members of the Defense Committee: Prof. György Wéber, M.D., Ph.D., C.Sc.
Prof. József Balla, M.D., Ph.D., D.Sc.

The Ph.D. Defense takes place at the Lecture Hall of the 1st Department of Medicine, Institute for Internal Medicine, Medical and Health Science Center, University of Debrecen
2 p.m. 31 March 2011
1. INTRODUCTION

Arterio-venous (AV) anastomosis may develop in developmental malformation, tumors, or traumatisation. The artificially created AV shunts, fistulas may be required during hemodialysis treatment, in which they must meet several criteria: easy manageability, repetitive access of circulation, ensuring proper blood flow, long-term patency, and low complication ratio.

Nowadays, arterio-venous shunts named by Cimino, Brescia and Appel are used for prolonged hemodialysis. These shunts provide effective dialysis access sites. The most frequently used vascular anastomosis type is the end-to-side technique.

The artificial AV fistula provides a low-resistance flow connection between the high-pressured arterial and low-pressured venous systems. At the venous side of the shunt, the presence of hypertension indicates an increased shear stress on the vessel wall, which initiates the transformation of the vessel wall by impacting the effect on the endothelium as part of the maturation process of the shunt, but the increased shear stress, change blood flow (semi-turbulent, turbulent blood flow) can lead to the development of stenosis, occlusion and vessel wall deformability around the anastomosis.

The morphologic, functional state and the maturation of artificial AV shunts are affected by several factors. The age of the patient, chronic diseases and disorders affecting the vascular system are preoperative-, the technique of making the anastomosis (angle of anastomosis, size and geometry of the shunt) and its location are intaoperative-, low venous blood flow velocity and narrowed vessel diameter are postoperative hemodynamical influencing factors.

The safe technique of the shunt execution, its geometry, increasing surgical safety, and the factors affecting the maturation process of the shunt are recognized with the help of many experimental models. During the planning of these models and evaluation of their results, it is important to select the small and the large experimental animals appropriately taking into account the
consideration of operative technique, the possible postoperative follow-up investigations and the differences of the given anatomy and physiologic characteristics of animal species.

It is also possible to create end-to-end, end-to-side, and side-to-side vascular anastomoses on small laboratory animal experimental models with the application of microsurgical technique placed in various anatomical locations similarly with the clinical appliance. At the selection of the localization, it is an important aspect, that to what degree the created AV shunt lades the circulation.

Most of the AV shunts made with microsurgical method on experimental models represent the development of a fistula with significant size, which may lead to venous hypertension or cardiomegaly. The creation of a technically well feasible shunt model may be important because in term of hemodynamics and physiology they represent a smaller load, but the models are suitable for the study of AV maturation (thus, the questions arousing during the clinical appliance).

Hemorheologic parameters (blood and plasma viscosity, hematocrit, fibrinogen concentration, red blood cell aggregation, red blood cell deformability) are significant determinants of the blood flow, which determine in a complex way not only the velocity of blood flow, but also the shear stress acting on the endothel along the different levels of circulation and mainly in the microcirculation.

In the aspect of artificial AV shunt, little hemorheologic informations are available and many questions are unanswered. How much does the presence of the shunt affect the micro-rheologic parameters in the aspect of local and systemic circulations? Is it demonstrable in the hemorheologic parameters, mainly in micro-rheologic factors the AV difference, and to what extent does it change with the creation of the shunt and during the maturation process? What effect does this have on the microcirculation of the tissue?

During our experiment, we researched the answers in the aspect of the AV shunt model created with microsurgical methods.
2. AIMS

1. To perform end-to-side and side-to-side femoro-femoral and sapheno-saphenous anastomoses as *preliminary operation technique study* to develop a safe technique with the review of the literature dealing with microsurgical technique performed experimental animal artificial arterio-venous (AV) shunt models.

2. *To perform a sapheno-saphenous artificial end-to-side AV shunt model* on rats, that would not disturb significantly the systemic circulation and easy to perform using microsurgical skills, to study the maturation of the shunts.

3. To examine the *arterio-venous (aorto-caval) hemorheological* –especially red blood cell deformability and red blood cell aggregation– *basic differences on rats* that change after creating the AV shunt and substantially less known about it.

4. To do *microcirculational, hemodynamical and hemorheological* examination of the matured sapheno-sapheous end-to-side AV shunts according to the functional state and local and systemic circulation, microrheological changes of the shunt on the performed rat model complex.

5. *To examine morphologically* the above mentioned matured shunts, *to analyse the histological changes of the vessel wall* in comparison with saphenous vessels of the non-operated side and the intact structural saphenous vessels of the Control animals.
3. MATERIALS AND METHODS

The experiments were conducted under the law XXVIII. 1998. “Animal protection and sparing” and were approved by the University of Debrecen Committee of Animal Research (permission Nr.: 37/2007 and 6/2008).

3.1. Previous operation technique studies, performance of the artificial end-to-side sapheno-saphenous arterio-venous shunt model

The anesthetized (sodium-thiopenthal, 60 mg/kg, i.p.; atropinum sulphuricum, 0.05 mg/kg, sc.) CD outbred rats’ (n=7; body weight: 407.86±92.4 g) medial thigh skin was incised. Then saphenous vessels were separated from the surrounding nerve and soft tissues.

In the artery approximately 1.5-fold length of the vein diameter incision was made with scalpel blade. Incision was performed in the vein with sharp-sharp microsurgical scissor then the first everting stitch (10/0-s polypropylene) was placed close to the corner. Stitches were symmetrically guided on both sides to the half part of the arterial incision without turning the anastomosis. After the lateral stitches the continuous suture line was finished on the medial side, which is easier to approach. Finally both of the thread was knotted ensuring the equable stretching of the suture.

Using this method the anastomosis of AV shunt model for experiments can be securely applied.

3.2. Examination of arterio-venous hemorheological base differences

Basic arterio-venous differences of the hemorheological parameters are less known, despite of blood samples are taken from different localization in numerous experimental models. We examined on rats the base aorto-caval hemorheological differences to partially ascertain the question and understand the presumed changes after performing the AV shunt model.
3.2.1. **Experimental animals, anesthesia and sampling**

Twelve female CD rats (bodyweight: 328.91±53.68 g) were anesthetized using sodium-thiopenthal (60 mg/kg, i.p.). A midline laparotomy was performed and the infrarenal part of the abdominal aorta and the caudal caval vein were gently explored. The abdominal aorta and the caudal caval vein were punctured separately for blood sampling (1.5 ml per vessels) using a 26 G needle and a connecting syringe that contained sodium-EDTA as anticoagulant (1.5 mg/ml).

3.2.2. **Laboratory tests**

3.2.2.1. Blood pH and gas analysis

In a closed system, the blood samples were immediately filled into the ABL555 Radiometer Copenhagen blood gas analyzing automata (WIP Kft., Budapest), which determines blood pH, pCO2 and pO2 values [mmHg].

3.2.2.2. Hematological parameters

The haematological parameters were determined by a microcell counter (Sysmex F-800 microcell counter, TOA Medical Electronics Corp., Ltd., Japan).

3.2.2.3. Hemorheological parameters

*Red blood cell deformability*

The red blood cell deformability measurements were performed using a RheoScan-D200 slit-flow ektacytometer (RheoMeditech, Seoul, Korea). The sample preparation was made by taking 6 µl of arterial or venous blood sample into 0.6 ml of a viscous isotonic solution (in normal PBS) of 360 kDa polyvinylpyrrolidone (viscosity = 30.51 mPa.s, osmolarity = 327 mOsm/kg ; pH = 7.37).

The parameter obtained during the measurements was the elongation index, which depends on shear stress (SS [Pa]). Increased EI-SS curve means better red blood cell deformability. EI values were used by different shear stress
values for the easier comparison of EI-SS curves and for parameterization of individual EI-SS curves Lineweaver-Burke analyses were performed to calculate the maximal elongation index (EI_{max}) and the shear stress at half EI_{max} (SS_{1/2} [Pa]): 1/EI = SS_{1/2} / EI_{max} x 1/SS + 1/EI_{max}.

Red blood cell aggregation

Red blood cell aggregation was determined using a Myrenne MA-1 erythrocyte aggregometer (Myrenne GmbH, Germany). The method is based upon the measurement of light transmittance through the blood sample: aggregating red blood cells increase the light transmittance through the blood sample, the aggregation process, and rate became traceable.

3.2.3. Statistical analyses

For comparison between arterial and venous blood samples Student t-test or Mann-Whitney rank sum test were made according to the normality of data distribution. A p value of <0.05 was considered as statistically significant.

3.3. Complex morphological, hemodynamical and hemorheological examinations of sapheno-saphenous AV shunts

Shunt examinations were performed by using the sapheno-saphenous AV anastomosis described in 3.1.

3.3.1. Experimental animals, anesthesia

Twenty-two healthy outbred CD rats (Crl:CD1 BR, bodyweight: 336.8 6 71.75 g) were subjected to the study. For the operations and the investigations the animals were anesthetized using sodium-thiopental (60 mg/kg, i.p., Thiopental®, Biocheme GmbH, Austria) and atropinum sulphuricum (0.05 mg/kg, s.c.).
3.3.2. Operative techniques and sampling

Control group (n=6)

In these animals no shunt operation was made, and they served as normal control. In the 8th postoperative week microcirculation, hemodynamical examinations and taking blood samples were performed in anesthesia.

AV shunt group (n=16)

Shunt side: On the left side, the medial thigh region was shaved and prepped with Betadine®. At lower medial thigh region (above the gracilis muscle) a 1.5-cm skin incision was used to approach the medial saphenous vessel. After ligation and transsection of vessel branches, clips were applied to stop blood flow in both vessels. Distal venous part was ligated and then transsected.

After performing arteriotomy with surgical blade 11, the arterial lumen was washed out with sodium-heparin solution (10 U/ml). The venous end was cut in a 45° angle then the end-to-side arterio-venous anastomosis was sutured using continuous suture with 10-0 monofilament polyamide suture material.

During operations careful microsurgical techniques and operating microscope were used (Leica® Wild M650, LEICA Ltd., Germany). Video recordings were made about the removal of the clips to check visually the function of anastomosis and “empty-and-refill” tests were carried out. During the test, blood wasatraumatically pressed from the venous limb of the shunt with microsurgical clips, and then the clip closer to the shunt was removed. In case of well functioning shunts the previously empty venous limb was refilled. The shunts were observed for 5 min for possible bleeding, then the skin was closed using 5-0 polyglycolic-acid suture material.

Non-operated side: On the right side no operation was made. This limb remained intact serving as a control side for the investigations. Postoperative care included daily wound care and observation (behaviour, limb motion, and
color of the paw). Limb motion and color of the paw examination was done weekly from the second postoperative week. To evaluate the state of the shunt after a maturation period, between the 8th and 12th postoperative week the operated animals were anesthetized again and subjected to a complex investigative protocol, including macroscopical, microcirculatory, hemodynamic, laboratory, and histological examinations.

3.3.3. Investigation of limb microcirculation

For non-invasive imaging of skin blood perfusion of the limbs (thighs and paws) a laser Doppler scanner (Perimed® Periscan PIM II Laser Doppler Perfusion Imager, Perimed AB, Sweden; University of Debrecen, Medical and Health Science Center, 3rd Department of Internal Medicine) was used.

This device uses a laser beam (wavelength: 670 nm, beam diameter: 1 mm) and measures the total local microcirculatory blood perfusion including the perfusion in capillaries (nutritive flow), arterioles, venules, and shunting vessels, based upon the laser Doppler technique, expressing blood perfusion unit (BFU), as a dimensionless parameter.

The laser source was placed 20 cm above the rat limbs. The investigated areas (region of interest, ROI) were the medial thigh region (from the line of inguinal ligament to the knee) and the paws both on non-operated and shunt sides of the AV group, and left side in the Control group. On the scan images (three tests per investigated area) the ROIs were selected by the Perimed software and their mean BFU were calculated, as quantification of the signal intensity.

3.3.4. Macroscopical investigation

In all operated animals 2 cm incision on the medial thigh was performed on both sides in anaesthesia and under operating microscope, the control and the shunted vessels were explored by careful tissue preparation. The patency of anastomoses was confirmed by observation of vessel pulsation.
A camera was connected to the operating microscope and photos were made for an off-line measurement of the vessel diameters (orientation distance: a surgical needle with known size) and shunt geometry (image analysis: software Image-Pro Express 5.0, Media Cybernetics Inc., USA).

3.3.5. Investigation of hemodynamics

The right carotid artery was cannulated (Intramedic Clay Adams1 Brand, Polyethylene, ID: 0.76 mm, OD: 1.22 mm) and heparinised (10 U/ml sodium-heparin). Mean arterial pressure (MAP [mmHg]) and heart rate (HR [min⁻¹]) were measured using Statham P23-DB transducers attached to an electro-manometer, recorded, and evaluated by Hemosys Software (Experimetria Ltd., Hungary).

Both on the shunt and non-operated sides the blood flow of saphenous artery and vein parts were investigated three times by a Transonic T206® device (Transonic Systems Inc., Ithaca, USA; flow probe: 1RB2759, University of Debrecen, Medical and Health Science Center, Institute of Pharmacology and Pharmacotherapy), presenting the blood flow rate (BFR) in ml/min.

3.3.6. Laboratory examination of arterio-venous blood samples

Blood samples (0.2–0.3 ml each, anticoagulant in syringe: Na3-EDTA, 1.5 mg/ml; needle: 26G) were obtained from the arterial and venous part of the shunt, as well as from the contralateral, non-operated saphenous artery and vein.

In Control group only the left side was used for blood sampling.

Hematological parameters, red blood cell deformability and red blood cell aggregation were determined according to 3.2.2.2. and 3.2.2.3. methods.

3.3.7. Histological investigations

In all AV shunt group animals by the end of the measurements the entire shunt and the contralateral saphenous artery and vein were excised for
histological examination. In Control group only left side was used for tissue sampling. Then the anesthetized animals were exterminated by exsanguination.

The biopsy material was fixed in 4% buffered formaldehyde solution, dehydrated in a graded series of alcohol, embedded in paraffin, microtomed into 3-5 µm step sections. Hematoxylin-eosin (H&E) and van Gieson staining were used. Morphometrical analyses determined the thickness of intima and media.

3.3.8. Statistical analysis

The statistical analyses were carried out using Student’s t-test or Mann-Whitney rank sum test depending on data distribution. The statistically significance level were considered to be when p < 0.05.

4. RESULTS AND DISCUSSION

4.1. Examination of arterio-venous hemorheological base differences

4.1.1. Blood pH and gas analysis

As it was expected in a physiological point of view blood $pO_2$ values were significantly (p<0.001) higher in aorta, and $pCO_2$ in caudal caval vein blood samples: the $pO_2$ values in aorta were 110.24±4.01 mmHg, and 54.37±4.07 mmHg in caudal caval vein; the $pCO_2$ values in aorta were 42.69±2.78 mmHg, and 59.9±1.8 mmHg in caudal caval vein.

The pH values did not show important arterio-venous differences (abdominal aorta: 7.22±0.01, caudal caval vein: 7.24±0.02; p=0.561).

4.1.2. Hematological parameters

White blood cell count, red blood cell count and hematocrit values were significantly higher in venous blood sample than in abdominal aorta blood samples. White blood cell showed the biggest arterio-venous difference with relatively big variation. Platelet count was slightly increased in venous blood.
Hemoglobin concentration, mean corpuscular volume, mean corpuscular hemoglobin content and its concentration as well as mean platelet volume did not show important arterio-venous differences.

4.1.3. Hemorheological parameters

4.1.3.1. Red blood cell deformability

Concerning comparative data of EI-SS curves, there were slight alteration in EI at 3, 5, 10 and 20 Pa: in venous samples EI values were slightly higher than in abdominal aorta blood samples. At 10 and 20 Pa the difference was significant. The calculated \( E_{\text{I max}} \) was minimally higher and SS1/2 values were smaller in venous samples.

4.1.3.2. Red blood cell aggregation

Both at 5 and 10 seconds the M1 values were higher in arterial samples, reaching significant arterio-venous difference at 5 seconds (2.03±0.14 vs 1.46±0.11, \( p=0.003 \)). At 10 seconds the arterio-venous difference was close to the significant level (\( p=0.068 \)).

Several methodical study reports that the oxygenation state of the blood samples can influence the laboratory results; therefore it is important to standardize the sampling and their handling conditions. Although the difference of partial pressure values of blood gas are well known, the difference between AV hemorheologic parameters have not yet been elucidated.

During animal experiments, it is often required to sample blood from vessels with different localization for the comparison of the local and systemic changes. In this issue, it is important particularly at ischemia-reperfusion and as well as at the studying of the disturbances of different organs’ circulation, where the hemorheologic parameters may be informative.
It is known, that red blood cell deformability and red blood cell aggregation are influenced by microenvironmental factors, osmolarity, pH, and oxygenation state of hemoglobin molecules.

In the blood samples from the abdominal aorta and vena cava or the rat, the red blood cell deformability measured with slit-flow ektacytometer and red blood cell aggregation measured with aggregometer operating on the principle of light transmission are not the same. These data also support the recommendation, that the methodical standardization of the experiments and the appropriate planning of the control measuring are required for the evaluation of the obtained results.

To understand more accurately the in vivo arterio-venous hemorheologic differences, further investigations are required with newer investigating methods and the appliance of instruments, which can reliably detect even small changes in microrheologic parameters.

### 4.2. Complex morphological, hemodynamical and hemorheological examinations of sapheno-saphenous AV shunts

#### 4.2.1. Investigation of limb microcirculation

In AV shunt group animals, the region of their thighs showed slight but not significant increase of BFU values compared with the Control group; however, the BFU values of shunt and non-operated side were almost identical in AV shunt group.

On the shunt side the BFU values of the paw were smaller versus the non-operated side. Both in Control and Experimental groups the paw BFU values were significantly smaller compared with the thigh.

#### 4.2.2. Macroscopical morphological investigation

After shunt maturation period in anesthesia anastomoses was found in the same angle than at operation time ($44.5\pm2.81^\circ$). In operated animals the
diameter of saphenous artery and vein of the shunts was near the same: 687.81±162.61 µm in artery and 676.69±185.05 µm in vein. The values on non-operated side were 548.27±82.52 µm in artery and 510.7±73.43 µm in vein. The connective tissue cicatrix was observed around the site of the operation. In two cases, excessive scarring was found with the presence of specifically enhanced collateral vasculature, however next to a functioning shunt. With one shunt, the loosening of the stitch and its “migration” was found. In this case, the suture material (polyamid) was the same just like in the other anastomosis. At the site of one the functioning shunt, fibrin deposition like lesion was discovered, which was most probably due to the micro-damage caused by the applied clip for compression, or it was a clot.

4.2.3. Investigation of hemodynamics

In Control group mean arterial pressure (MAP) was 129.2±15.5 mmHg, and heart rate (HR) was 403.1±97.8 min⁻¹. In AV shunt group: MAP=139.1±17.8 mmHg; HR=399.7±102.2 min⁻¹. There was no significant inter-group difference.

In AV shunt group animals blood flow rate values of saphenous artery and vein at both sides were significantly lower compared with Control group’s values ($p<0.001$). However, at shunt side the values were significantly greater both in venous and arterial limbs of the fistula compared with the non-operated side saphenous vessels ($p<0.001$ and $p=0.006$, respectively).

The arterial to venous ratio of blood flow rate values was much lower at shunt side (1.2±0.13; $p=0.002$ vs. Control group) versus non-operated (1.59±0.29) or compared with Control group values (1.49±0.05).

4.2.4. Hematological parameters

In general, hematological parameters did not express important difference. White blood cell count showed larger variability at shunt side (standard deviation increased) and the arterio-venous difference was smaller compared to
non-operated side. Platelet count on shunt side, both in arterial and venous samples was slightly lower compared with non-operated side.

4.2.5. Hemorheological parameters

In venous limb of the AV fistula, aggregation index (21.3±0.67) was significantly larger versus shunt artery (18.91±0.59, \( p=0.017 \)) and non-operated side vein (13.89±1.64, \( p=0.016 \)), as well as compared with Control group’s venous values (15.03±0.74; \( p<0.001 \)).

In the artery of the fistula aggregation index values were significantly higher compared to non-operated side artery (8.73±1.88, \( p=0.023 \)) and versus Control group’s arterial values (14.21±0.79; \( p<0.001 \)).

In the non-operated side only the arterial values differed from the Control group (\( p=0.015 \)), and AV difference was found to be significant (\( p=0.047 \)).

On AV shunt side the elongation index (EI) values were lower and the arterio-venous difference was smaller compared with non-operated side values. In general, the operated animals showed worse red blood cell deformability than the Control group: EI values at shear stress of 3 Pa were significantly lower in shunt side artery and vein and in non-operated side artery (\( p<0.001 \)).

Calculated EI\(_{\text{max}}\) was markedly lower in shunt side. Calculated SS\(_{1/2}\) values were higher in operated animals both in arteries and veins of shunted and non-operated side, however, significant difference were found only in the non-operated side artery (\( p=0.014 \) vs. Control group). At shunt side SS\(_{1/2}\) differences from Control group values were well observable in arterial as well as venous blood samples, however did not reach significant level (artery: \( p=0.085 \); vein: \( p=0.07 \)).

4.2.6. Histological investigations

Compared with the non-operated side, the venous limb of the AV fistula was found to arterialized. The vein was found to be enlarged and its wall was
strengthened with increased number of smooth muscle elements and significant amount of connective tissue especially collagen elements, which became visible using van Gieson staining. In four cases fixed thrombi were found at the venous limb of anastomoses.

The intima and media layers in the venous limb of the fistula were thickened in AV shunt group compared with the Control group and non-operated side.

The intima layer was significantly thickened in the venous limb of the fistula compared to non-operated vein values (3.6±2.2 µm; \(p=0.006\)) and Control group values (2.69±0.78 µm; \(p<0.001\)). The intima layer was significantly thinner in the venous limb (5.04±2.99 µm) compared to arterial limb of the shunt (14.55±20.26 µm; \(p=0.003\)). Intima thickness values of the arterial limb of the fistula (14.55±20.26 µm) were significantly bigger compared to the non-operated side artery (4.13±1.92 µm; \(p<0.001\)) and Control group (2.63±0.56 µm; \(p<0.001\)). On the non-operated side only the arterial values differed compared to the Control group values (\(p=0.009\)) and showed arterio-venous difference (\(p=0.005\)).

The media layer was significantly thickened in the venous limb of the fistula (14.81±8 µm) compared to the non-operated side venous values (10.65±3.5 µm; \(p=0.002\)). The media thickness values in the arterial limb of the fistula (61.44±15.28 µm) and non-operated side artery values (65.22±16.26 µm; \(p<0.001\)) were significantly bigger compared to Control group values (49.3±8.45 µm; \(p=0.001\)). As it was expected, a significant arterio-venous difference was found in media layer thickness (\(p<0.001\)).

In the clinical practice, matured AV shunt is required for the safe blood sampling, which enables repeated needle puncture during hemodialysis.

The physiology, maturation (4-6 weeks), arterialization and neointimal formation of the AV fistula are widely studied. During the arterialization the
number of smooth muscle cells is increased, neointima formation takes place in wall of the shunt’s venous limb. Ultrastructural investigations prove the increase in amount of extracellular matrix elements (collagen, elastin, and proteoglycans) around the smooth muscle tissue.

We followed the animals in our investigation of the matured shunt experiment model until the 8-12th postoperative weeks, which time period assured the conduct of the complete maturation process of the shunts. We wanted to provide enough time for the maturation of the shunts and for the development of their compensated circulatory relations in our model.

We observed in our experiment, that the diameter of the vessels increased on the side of the shunt by the examined postoperative period, the diameter of the arterial and the venous limb became nearly the same. During the histological investigations the arterialization of the dilated venous wall was seen. Congruously with the medical literature, the intima and as well as the media layers were thicker due to accumulation of collagen and smooth muscle.

The direct blood flow measurements, conducted on the saphenous vessels (saphenous artery and medial saphenous vein), measured increased flow velocity on the shunt side and showed only a slight arterio-venous difference compared to the non-operated side and control values, where the difference in arterio-venous flow velocity was well measurable.

Presumably, the less known arterio-venous hemorheologic differences were modified with the formation of AV shunt. The results showed that (I) the values of red blood cell aggregation index were increased in the blood samples from the medial saphenous vein compared with the values of the saphenous artery; (II) the values of the aggregation index were elevated on the side of the shunt, significantly increased in the venous samples and showed decreasing arterio-venous difference; (III) the significant degree of arterio-venous difference of red blood cell deformability disappeared and showed lower values of elongation index.
In conclusion it can be said that the presented artificial saphenous-saphenous end-to-side AV shunt model can be safely prepared with microsurgical methods, in the view of its anatomical localization it provides easy accessibility, and the system circulation is not burdened. In the aspect of artificial saphenous-saphenous end-to-side AV shunts, next to the factors of the blood flow and morphological factors, the micro-rheologic parameters changed as well, which are important factors not only at the site of the shunt, but in the determination of microcirculation.

Our experiments disclose the basis of further research in the investigation of factors influencing the maturation of the shunt and the avoidance of possible complications, which may provide useful information for the clinical practice. It is important in the more detailed understanding of the local and systemic hemorheologic relationship in their endothelial function and in the further investigation of resulting changes in the microcirculation.
7. SUMMARY OF THE IMPORTANT RESULTS AND CONSEQUENCES

1. For the examination of the morphological changes, the microcirculation and less studied hemorheological changes of shunts, we executed a new end-to-side artificial sapheno-saphenous arterio-venous shunt model on rats that was securely performable using microsurgical technique; anatomical localization provided easy access, and did not burden substantially the systemic circulation.

2. We established as new result during the examination of less known hemorheological basic arterio-venous (aorto-caval) difference with its expected changes that elongation index values of rats were slightly decreased, red blood cell aggregation index values increased at arterial side (abdominal aorta) compared to venous side (caudal caval vein).

3. Our developed end-to-side artificial sapheno-saphenous AV shunt caused small change in tissue microcirculation values of the thigh and paw skin at shunt side compared to the non-operated and control animals’ microcirculation values. The hemodynamic parameters only locally showed difference with increased blood flow values and decreased arterio-venous difference at AV shunt side.

4. It is established as new result that elongation index parameters decreased in arterial and venous blood samples of AV shunt compared to the non-operated side and values of control animal, showed decreasing arterio-venous difference. Red blood cell aggregation index values increased in saphenous artery and medial saphenous vein at the shunt side compared to non-operated and control values, but especially at venous side.

5. The dilatation of both limb of the AV anastomosis during the maturation of shunt was detected with morphological and histological investigations. Similarly the medical literature, the intima and media layers’ thickening was observed in arterial and venous limb of the shunt with increased number of smooth muscle cells and collagen elements. The intima layer
was thickened to a great extent in on the arterial side of the limb, while the venous side showed thickening in its media layer.

6. Our results supplied experimental data for examination of arterio-venous shunt’s morphological and blood flow parameters. The executed end-to-side artificial sapheno-saphenous AV shunt model can be the prototype for further researches to answer the questions that are important in clinical aspect, to examine the factors that influence shunt’s maturation, complications, the local and detailed investigation of systemic hemorheological relations.
Ph.D. thesis based in extenso publications


Other in extenso publications


Articles based on the performed work of the Department:


*Articles based on international cooperation:*


**Cumulative impact factor: 8.943**
Jelölt: Dr. Hevér Tímea  
Neptun kód: JGGTFG  
Doktori Iskola: Klinikai Orvostudományok Doktori Iskola

A PhD értekezés alapjául szolgáló közlemények

IF: 1.244 (2009)  
Full Text:  
http://hdl.handle.net/2437/111640  
http://dx.doi.org/10.1002/micr.20784

IF: 0.965 (2009)  
Full Text:  
http://hdl.handle.net/2437/111639

További közlemények

IF: 1.78 (2009)  
Full Text:  
http://hdl.handle.net/2437/97975  
http://dx.doi.org/10.3233/CH-2010-1308

IF: 1.244 (2009)  
Full Text:  
http://hdl.handle.net/2437/95920  
http://dx.doi.org/10.1002/micr.20707

4032 Debrecen, Egyetem tér 1. e-mail: publikaciok@lib.unideb.hu
IF: 0.965 (2009)
Full Text:
http://hdl.handle.net/2437/93666

IF: 1.78
Full Text:
http://hdl.handle.net/2437/93354
http://dx.doi.org/10.3233/CH-2009-1178

IF: 0.965
Full Text:
http://hdl.handle.net/2437/93286

Full Text:
http://hdl.handle.net/2437/88302

A DEENK Kenézy Élettudományi Könyvtár a Jelölt által a Publikációs Adatbázisba feltöltött adatok bibliográfiai és tudománymetriai ellenőrzését a tudományos adatbázisok és a Journal Citation Reports Impact Factor lista alapján elvégezte.

Debrecen, 2011.01.17