

SHORT THESIS FOR THE DEGREE OF DOCTOR OF PHILOSOPHY (PhD)

**Investigation of hemorheological and microcirculatory alterations
caused by intestinal ischemia-reperfusion
in an experimental model**

by Anita Mester, MD

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UNIVERSITY OF DEBRECEN
DOCTORAL SCHOOL OF CLINICAL MEDICINE

DEBRECEN, 2018

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The Examination takes place at the Department of Ophthalmology, Faculty of Medicine, University of Debrecen

11:00 am, 10 July, 2018

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The PhD Defense takes place at the Lecture Hall of Building “A”, Department of Internal Medicine, Faculty of Medicine, University of Debrecen

1:00 pm, 10 July, 2018

1. INTRODUCTION

Intestinal ischemia-reperfusion (I/R) is a life-threatening clinical disorder associated with several conditions, such as acute mesenteric ischemia, trauma, cardiopulmonary disease, shock, intestinal transplant rejection, volvulus and necrotizing enterocolitis in newborns. Although it is responsible for only 1-2% of gastrointestinal diseases, its significance is that mortality is still extremely high today, reaching 60-80%. This is in part attributable to the lack of early diagnostic markers, so the disease continues to be a challenge for clinicians, which often delays the diagnosis. On the other hand, minimal preventive and therapeutic opportunities and unprompted pathophysiology also contribute to the high mortality rate.

Nowadays, a lot of information is available about the events of the ischemia-reperfusion. During ischemia blood flow of an organ is interrupted and as a result the oxygen supply and adenosine-triphosphate (ATP) production decrease. The cells start to produce energy through an anaerobic metabolism and the accumulated lactic acid will lower the intracellular pH. The restoration of blood flow, also called reperfusion, may cause further local and remote tissue injury due to the formation of reactive oxygen species including hydroxyl radical, superoxide and peroxynitrite ions. During this process, red blood cells are also damaged, as a result of which their deformability is worsened and their aggregation is increased, leading to elevated blood viscosity and flow resistance. Furthermore, red blood cell aggregation may also affect the function of other cells, for example, promotes the activation of endothelial cells and the adhesion of thrombocytes and white blood cells to the vessels. The adverse effect of reperfusion mainly affects the area of the microvasculature, causing microcirculation disorders in the given tissue or organ. The optimal flow properties of the blood and the proper condition of the blood vessels are essential to maintain good microcirculation, therefore the

rheological features of red blood cells play an outstanding role in pathologic conditions.

Aging is a major risk factor in ischemic disorders, like stroke, ischemic heart disease, mesenterial ischemia and many surgical interventions, and I/R injuries also occur more frequently in the elderly population. It is not a negligible fact that mesenteric ischemia is expected to increase with the aging of the population and the increase in the proportion of elderly patients. Unfortunately, as mortality in elderly patients is significantly higher, we can not expect a decrease in mortality in the future.

In addition, it is important to note that cardiovascular risk in men is higher than in women. This is due to the protective effect of estrogens, which is a consequence of complex mechanisms: estrogens participate in gene transcription, intracellular signalling, vasoregulation, and also have anti-inflammatory and anti-oxidant effects. On the other hand, females have more favorable haemorheological properties: they have lower blood viscosity, haematocrit, red blood cell aggregation and better deformability than males.

Despite the vast amount of knowledge and the ongoing research, many issues remain unclear. The significance of *in vivo* changes in haemorheological parameters during pathological processes and when these alterations reach the level that can lead to thrombotic complications, remains in question. Investigation of preventive and therapeutic options is still necessary as hemorheologic therapy is not common practice, although ignorance of rehological factors reflects an outdated approach in medical practice.

2. AIMS AND OBJECTIVES

1. Investigation of systemic haematological and micro-rheological parameters and local microcirculatory effects and their simultaneous changes during a 30-minute intestinal ischemia and subsequent 120-minute reperfusion in rats.
2. Analysis of the effect of 30-minute intestinal ischemia and 120 minutes of reperfusion on distant organs by microcirculatory and histological examinations in the rat.
3. Detection of hematological and micro-rheological differences in 30-minute intestinal ischemia and 120-minute reperfusion in male and female, young and older rats.
4. Investigation of the age- and gender-related differences of histological alterations caused by small intestinal ischemia-reperfusion in rats.

3. MATERIALS AND METHODS

The experiments were approved by the University of Debrecen Committee of Animal Welfare

(permission Nr.: 20/2011 UDCAW) in accordance with national and EU regulations (the Hungarian Animal Protection Act (Law XVIII/1998) and the Edict 63/2010).

3.1. Early systemic micro-rheological and microcirculatory alterations during intestinal ischemia-reperfusion

3.1.1. *Experimental animals and anesthesia*

Female CD outbred rats (body weight: 265.5 ± 26.7 g) were randomly divided into Control (n=7) and Ischemia-reperfusion (n=7) groups. Animals were housed in standard size cages under conventional conditions, received standard rat food and water ad libitum. All the experiments were performed under general anesthesia (Thiopental, 60 mg/bwkg, i.p.). As premedication animals were given atropine-sulphate (0.06 mg/bwkg, s.c.). The animals were placed on a heating pad to support maintaining body temperature.

3.1.2. *Operative techniques and sampling protocol*

After shaving and disinfecting (Betadine) the middle part of the abdomen and the left inguinal region, the left femoral artery was cannulated (BD NeoflonTM, 26G) under operating microscope (LeicaWild M650). Midline laparotomy was performed and the superior mesenteric artery was gently exposed. In the Control group there were no other interventions. In the Ischemia-reperfusion (I/R) group the superior mesenteric artery was clipped atraumatically for 30 minutes and 120 minutes of reperfusion was observed afterwards.

Before the ischemia (Base), just after clip removal (I-30), at the 30th (R-30), 60th (R-60) and 120th (R-120) minute of the reperfusion microcirculatory and temperature measurements were carried out and blood samples (~0.3 ml each time, anticoagulant: 1.5 mg/mlK3-EDTA) were taken from the cannulated artery. After samplings similar volume of physiological saline solution was given. In the Control group the same time points were used for samplings and tests. At the end of the experiments samples were taken from the small intestine, liver, kidney, pancreas and lungs for histological examinations and the animals were euthanized.

3.1.3. Laboratory measurements

3.1.3.1. Hematological parameters

Hematological parameters were measured by Sysmex F-800 microcell counter (TOA Medical Electronics Corp., Ltd., Japan). The test requires about 70 μl of blood. In this study hematocrit (Hct [%]), red blood cell count (RBC [$\times 10^6/\mu\text{l}$]), white blood cell count (WBC [$\times 10^3/\mu\text{l}$]) and platelet count (Plt [$\times 10^3/\mu\text{l}$]) were analyzed.

3.1.3.2. Red blood cell aggregation

Red blood cell aggregation was determined by Myrenne MA-1 erythrocyte aggregometer (Myrenne GmbH, Germany) using light-transmittance method. 20 μl of blood sample is briefly disaggregated with high shear rate (600 s^{-1}) and then the shear rate drops to 0 (M mode) or 3 s^{-1} (M1 mode). Aggregation index values are determined 5 (M 5 s, M1 5 s) or 10 seconds (M 10 s, M1 10 s) after disaggregation. Higher aggregation index values reflect enhanced aggregation.

3.1.3.3. *Red blood cell deformability*

For testing red blood cell deformability LoRRca MaxSis Osmoscan rotational ektacytometer (Mechatronics BV, The Netherlands) was used. 10 µl of blood was gently mixed in 2ml of polyvinylpyrrolidone (PVP) – phosphate buffered saline (PBS) solution (viscosity: 27 mPas, osmolarity: 300 mOsm/kg, pH: ~7.3). The suspension was injected into the device which generates shear stress (SS), while the laser diffraction pattern was analyzed calculating elongation index values (EI) from the longitudinal (A) and horizontal diameter (B) of the ellipsoid laser diffraction pattern: $EI = (A-B) / (A + B)$. The measurements were carried out at 37°C.

Red blood cells are subjected to continuously increasing shear stress (between 0.3 and 30 Pa) resulting in an S-shaped deformability (EI-SS) curve. Since the conventional EI-SS curves may be morphologically different, a global parameter is required, which characterizes the whole curve. Thus, for data comparison the maximal elongation index values (EI_{max}) and the shear stress belonging to the half of it ($SS_{1/2}$) were calculated using Lineweaver-Burk analysis and the ratio of EI_{max} and $SS_{1/2}$ ($EI_{max}/SS_{1/2}$) was also compared.

3.1.4. *Microcirculatory investigations*

Microcirculation was monitored by laser Doppler technique (LD-01 Laser Doppler Flowmeter, Experimetria Ltd., Hungary) using standard pencil probe (Oxford Optronix Ltd., UK) on the antimesenteric surface of the jejunum, on the front surface of the liver and on the middle front surface of the right kidney.

This is a non-invasive method to detect tissue perfusion. The procedure is based on the Doppler phenomenon. The laser beam emitted by the device is reflected by the moving and restrained cells in the tissue, but the laser light reflected from the moving particles undergoes a frequency shift. The device determines blood flux units (BFU [au]) depending on the number and velocity of the moving red blood cells in the examined tissue volume.

The signal was recorded for 20 seconds and the data were analyzed offline using the average values of 10-second long, noise-free, stable periods. By same measurement scheduling, a digital infrared thermometer was applied on a jejunum loop to test surface temperature, and using a rectal probe body temperature was also monitored (Experimetria Ltd., Hungary).

3.1.5. *Histological examinations*

After the last blood sampling, tissue samples were taken from the small intestine, the liver, the pancreas, the right kidney and the lungs for histological examination. The samples were fixed in 5% formaldehyde-solution, dehydrated in a graded series of alcohol, embedded in paraffin, microtomed into 3–5 μm sections and stained with hematoxylin and eosin (H&E).

3.1.6. *Statistical analysis*

Data were expressed as means $\pm$ standard deviation (S.D.). For inter-group comparison Student *t*-test or Mann-Whitney rank sum tests, for intra-group comparison one-way and repeated measures ANOVA tests (Dunn's or Bonferroni's method) were applied, depending on the normality of data distribution. $P < 0.05$ was considered statistically significant.

3.2. Age- and gender-related hemorheological alterations in intestinal ischemia-reperfusion

3.2.1. *Experimental animals and anesthesia*

Young (4 month old) male and female and older (18 month old) male and female Crl:WI rats (ToxiCoop Ltd., Hungary) were involved in the study. The animals were kept in conventional animal facility and received rat chow (Babolna rodent-specific CRLT/N) and water ad libitum. The experiments were performed under continuous general anesthesia (thiopental, 60 mg/bwkg, intraperitoneally).

3.2.2. Operative techniques and sampling protocol

Six experimental groups were formed:

- I. Control young males (n=7; 435.9 ± 75.2 g)
- II. Control young females (n=7; 281.7 ± 27.8 g)
- III. Ischemia-reperfusion (I/R) young males (n=7; 333.3 ± 149 g)
- IV. Ischemia-reperfusion (I/R) young females (n=7; 249.3 ± 25.6 g)
- V. Ischemia-reperfusion (I/R) older males (n=6; 622.2 ± 189.6 g)
- VI. Ischemia-reperfusion (I/R) older females (n=6; 548.7 ± 217 g)

In addition, laboratory data was used from the database of the department as old controls (females n=8, 407.87 ± 37.2 g; males n=7, 743.57 ± 148.6 g).

In the control groups, the left inguinal region and the middle part of the abdomen were shaved and disinfected with Betadine. After isolation, an incision (1 cm) was made on the skin above the left femoral artery. The artery was prepared and cannulated (BD Neoflon, 26 G) under operating microscope (Leica Wild M650). Midline laparotomy was performed, and the superior mesenteric artery was gently exposed by atraumatic preparation. In the I/R groups, the same preparations were carried out, and the superior mesenteric artery was clamped with microvascular clip for 30 min, then a 120-min reperfusion period was observed.

Blood samples (0.3 mL each time, anticoagulant: 1.5- mg/mL K3-EDTA) were taken from the cannulated artery after the surgical preparations (base), at the 30th min of the ischemia, just before the removal of the clip (I-30), at the 30th (R-30), 60th (R-60), and 120th (R-120) min of the reperfusion. In the control groups, the same sampling time points were used. At the end of the experiment, after the last blood sampling, a sample of the small intestine was taken for histological examination and then the animals were euthanized.

3.2.3. Laboratory measurements

Laboratory parameters were measured as described in section 3.1.3.

3.2.4. *Histological examinations*

Tissue samples were taken from the small intestine for histological examination. The samples were fixed in 5% formaldehyde-solution, dehydrated in a graded series of alcohol, embedded in paraffin, microtomed into 3–5 μm sections and stained with Periodic acid-Schiff (PAS) method.

The evaluation of histological samples from the small intestine was based on the Chiu classification commonly used in the literature:

Grade 0: mucosa with normal villi

Grade 1: developing of the sub-epithelial (Grunhagen's) space, usually at the villus apex

Grade 2: extension of the sub-epithelial space with moderate lifting of epithelial layer from lamina propria

Grade 3: massive epithelial lifting down the sides of the villi

Grade 4: denuded villi with lamina propria and dilated capillaries exposed

Grade 5: digestion and disintegration of lamina propria, hemorrhage and ulceration

3.2.5. *Statistical analysis*

The performed statistical analysis is presented in section 3.1.6.

4. RESULTS

4.1. Early systemic micro-rheological and microcirculatory alterations during intestinal ischemia-reperfusion

4.1.1. *Hematological parameters*

Hematocrit values of the Control group did not show important changes, while in the I/R group an increase was observed, being significantly higher during the reperfusion period. Red blood cell count showed similar changes: in the I/R group it was elevated during the reperfusion. White blood cell count in the Control group increased and remained elevated during the ischemia and reperfusion period, as well as in the I/R group, however it was higher in the I/R group at the 60th and 120th minutes of the reperfusion period. Platelet count increased during the ischemia and in the first hour of the reperfusion in the Control and I/R group as well, but there was no significant difference between the two groups.

4.1.2. *Red blood cell aggregation*

Red blood cell aggregation values were significantly higher in the I/R group during the ischemia and remained elevated during the reperfusion period. The aggregation index values were the highest at the end of the reperfusion. Increase of the aggregation could be also detected in the Control group, but it was smaller than in the I/R group.

4.1.3. *Red blood cell deformability*

Elongation index values at a shear stress of 3 Pa were lower in the I/R group by the end of the ischemia and the first hour of the reperfusion, being markedly decreased at the 60th minute of the reperfusion (I/R vs. Control $p=0.018$). The calculated EI_{max} and $SS_{1/2}$ values did not show significant changes in the I/R group.

4.1.4. *Temperature and microcirculation*

In body temperature there were no significant differences between the two groups, however a slight increase could be observed by the 120th minute of the reperfusion in the I/R group.

The small intestine surface temperature did not change in the Control group during the experiment, but in the I/R group a significant decrease could be seen at the end of the 30-minute ischemia (vs. Control $p=0.002$; vs. Base $p=0.002$).

Intestinal microcirculatory blood flux units (BFU) decreased during the ischemia (vs. Base: $p=0.048$) but did not drop to zero, probably due to the collateral circulation of the mesentery. The values were higher after the clamp removal but did not normalize by the end of the reperfusion. On the liver BFU values were lower in the I/R group compared to the Control group, being the lowest at the 30th minute of the reperfusion period. The kidney microcirculatory BFU values decreased by the end of the reperfusion.

4.1.5. *Macroscopic alterations and histology*

Ten minutes after the clamping of superior mesenteric artery, the spasm of jejunum ducts was detected. In addition, at the end of ischemia, the colour of damaged areas changed, dark purple parts could be observed on the intestinal tract.

In the histological samples of the small intestine we could observe lamina propria hemorrhage, superficial epithelial necrosis and ulceration with minimal inflammation compared to the Control group where no damage could be detected. In the liver around the central vein small drops of fat were seen and in the kidney congestion was observed in glomeruli and vasa recta. In the pancreas and the lungs there were no important changes.

4.2. Age- and gender-related hemorheological alterations in intestinal ischemia-reperfusion

4.2.1. Hematological parameters

Hematocrit increased in the younger male groups, but significant difference was not found between the control and I/R groups, whereas in the younger female I/R group, a marked increase could be observed during the reperfusion period. In the older I/R females and males, the hematocrit values were higher compared with the younger I/R groups.

Red blood cell count was higher in male animals. In the younger I/R groups, a significant rise could be seen during the reperfusion in males and females as well. Higher RBC counts were measured in the older I/R groups compared with the younger same gender I/R groups, except for the R-60 values in males.

In case of the white blood cell count, male animals had higher values compared with females. In the young I/R groups, WBC count significantly elevated by the end of the reperfusion period in both genders. In the older female and male I/R groups, the changes were of larger magnitude. During the ischemia and the reperfusion, the increased WBC count of the older I/R females was significant compared with the base values and the younger I/R females. In the older male I/R group, the rise of the WBC count was only marked during the first hour of the reperfusion period. In the younger I/R groups, the WBC count remained elevated; however, in the older I/R groups, it showed a slight decrease by the end of the reperfusion.

In platelet count there was an increase during the first hour of the reperfusion in the control groups and the younger female I/R group, followed by a decrease in the 120th min of the reperfusion, while in the younger male I/R group, the platelet count remained significantly higher. In the older I/R groups, females showed elevated values being significant versus its base ($p=0.046$) and compared with younger I/R females at R-120 ($p<0.001$). In the older male I/R group, these changes could not be seen, the values were similar to the base data.

4.2.2. Red blood cell deformability

The calculated $EI_{\max}$ values were the lowest in the younger male I/R group. In female groups, the younger I/R animals had the highest $EI_{\max}$ value from the beginning, and it remained high during the experiment.

Male groups presented higher shear stress values at half $EI_{\max}$ ($SS_{1/2}$) compared with female groups. The highest values were detected in the older male I/R group. However the younger I/R males also showed elevated values compared with the control group and the female I/R group.

Regarding the $EI_{\max}/SS_{1/2}$ ratio, the same tendency was observed. Older I/R males presented the lowest values and a decrease in the younger I/R male group could be also observed.

4.2.3. Red blood cell aggregation

Aggregation index values increased during the ischemia in the control and I/R groups as well and remained elevated during the reperfusion period being markedly higher in the I/R groups. In the older I/R male group, the values were lower than in the young I/R male group during the ischemia and reperfusion. In control groups, the males had elevated aggregation index values, and significant differences could be detected between the female and male animals.

4.2.4. Histology

During the histological examination of the small intestine Chiu's classification was used. In control groups, intact tissue structure was visible (Chiu-score: 0). In comparison, I/R groups showed important histological alterations. In the histological sections, subepithelial loosening, denuded intestinal villi, necrotic areas, dilated capillaries and inflammatory cells were depicted (Chiu-score: 4), and lamina propria bleeding in one case of the older female I/R group (Chiu-score: 5).

In the I/R groups, significantly higher scores were found versus the young control groups in both sexes. There was no significant difference between males and females or younger and older groups, but older groups had higher values. In all cases, scores of 4 or above were obtained, while younger groups had lower scores (2 and 3) as well.

5. DISCUSSION

5.1. Early systemic micro-rheological and microcirculatory alterations during intestinal ischemia-reperfusion

Intestinal ischemia-reperfusion may lead to life threatening complications through local and remote tissue injury. In our experiment the alterations of hematological, micro-rheological and microcirculatory parameters were investigated during 30-minute intestinal ischemia followed by 120 minutes of reperfusion. This model enables the study of early and acute changes caused by intestinal ischemia-reperfusion.

In our study, hematocrit, red blood cell count, leukocyte count significantly elevated during the reperfusion. The platelet count increased in both groups during the ischemia and first hour of the reperfusion, then decrease was observed. These changes may be associated with the ischemia-reperfusion induced inflammation and acute phase reaction. During the acute phase reaction the platelet count could increase or decrease as well. In the early phase it usually remains unchanged or decreased due to the formation of micro-thrombi.

Our results showed that micro-rheological factors deteriorated during the ischemia and the following reperfusion. It is known that ischemia and reperfusion influence micro-rheological parameters of the blood. Damage occurring during the ischemia may be further exacerbated by the restoration of blood flow. Upon reperfusion oxygen is reintroduced into the tissues where it reacts with the xanthine oxidase to produce reactive oxygen free radicals. Other sources of free radicals are the nitric oxide synthases and the polymorphonuclear cells. Mechanisms leading to enhanced aggregation and increased cell rigidity include free radicals (causing lipid peroxidation, hemoglobin and protein alterations), mechanical stress, changes in acid-base parameters, in lactate concentration, in osmolarity and oxygenation. Interestingly, controlled reperfusion may reduce hemorheological alterations. The increased red blood cell aggregation may be the

consequence of free radical release and elevated fibrinogen levels due to acute injury. Decreased erythrocyte deformability may be caused by local metabolic changes and oxygen free radicals by lipid peroxidation and modified protein structure and function.

It is well documented that hemorheological parameters play an important role in determining microcirculation. The worsening of micro-rheological factors was accompanied by the deterioration of microcirculation of intra-abdominal organs. It was partly due to the decreased deformability and enhanced aggregation and partly the “no reflow” phenomenon that is characteristic for tissue ischemia-reperfusion. Several mechanisms may attribute to the lack of reestablishment of blood flow including intravascular thrombosis, leukocyte and thrombocyte plugging, hemoconcentration, endothelial cell swelling, vasomotor dysfunction and interstitial edema.

Intestinal blood flux units decreased during the ischemia, however the interruption of blood flow was not total. Megison et al. showed that the reduction in flow after superior mesenteric artery occlusion ranged from 44 to 97% and the individual variation was high due to the variability of collateral flow. In our experiment, we did not observe high standard deviations. Most animal models use 30 minutes of ischemia by clamping the superior mesenteric artery, but there are several models of intestinal ischemia, e.g. the superior mesenteric artery occlusion with collateral ligation, embolization, low-flow ischemia and segmental mesenteric vascular occlusion, which all has its appropriate purpose, advantages and disadvantages.

Examination of microcirculation may be useful in the diagnosis of various diseases, in assessing the severity of circulatory disturbance, in the selection of adequately perfused sections during intestinal anastomoses, in the evaluation of therapy and its efficacy. Many invasive and non-invasive methods are suitable for measuring microcirculation, one of the most commonly used is the laser Doppler flowmetry. This is a simple, easy-to-use method for continuous, rapid and real-

time tracking of perfusion changes. However, there are several limitations of the laser Doppler flowmetry as well, including drying or movement of the tissue, temperature, instability of the device, and too much pressure on the tissue, which were tried to be minimized during the measurements.

During the experiment we investigated the surface temperature of the small intestine, which decreased significantly in the I/R group at the end of the ischemia and then increased during the reperfusion, approaching the baseline values. During intestinal I/R, determination of the viability of the intestine is extremely difficult. This is predicted on the one hand by clinical considerations (colour, peristalsis, pulsation, bleeding) and on the other hand there are a number of other objective methods which, however, are not widely used in practice. Malafaia et al. studied the temperature of the small intestine with infrared camera in rats during and after ischemia to determine viability. In their study, intestinal temperature decreased during ischemia and reactive hyperaemia was observed during reperfusion in those cases where the intestine was viable clinically and histologically no or only slight changes were present. Reactive hyperaemia usually occurs during the first minutes of the reperfusion, in our experiment we could not observe it, although we did not monitor the temperature continuously. According to the histopathological analysis the changes were reversible in our study.

The above-described pathophysiological processes have an effect on local and remote cells as well, therefore intestinal I/R injury is also frequently associated with liver, kidney and lungs failure. In our study, the histology and laser Doppler measurements revealed the damage of the liver and kidney of the remote organs.

5.2. Age- and gender-related hemorheological alterations in intestinal ischemia-reperfusion

Intestinal injury due to I/R plays a significant role in many clinical conditions, and it is also an important cause of mortality in surgical patients. Among the abdominal organs, the intestine is particularly sensitive to I/R injury.

The microrheological parameters of blood, i.e., red blood cell aggregation and deformability have a major role in tissue perfusion; therefore, their investigation may be useful in experimental surgery and microsurgery. Several pathophysiological processes may deteriorate these parameters, including I/R injuries. During the reperfusion, oxygen is reintroduced to the tissues, and the release of free radicals may lead to the damage of the membrane of red blood cells causing the impairment of erythrocyte deformability and aggregation.

More and more studies suggest that gender and age have a great influence on the response to I/R injury and on hemorheological parameters as well. To test the hypothesis that aging and male gender are associated with greater susceptibility to I/R injury, we investigated the effects of intestinal I/R on systemic hematological and microrheological parameters in a rat model. Our main findings were the following: (1) Hct increased significantly in the younger female I/R group. In the older I/R groups, the values were higher compared with the younger animals. (2) RBC count was higher in male animals versus females and older I/R animals versus younger I/R groups. (3) In case of WBC count, male animals had higher values compared with females. In the older female and male I/R groups, the changes were of larger magnitude. (4) Plt count elevated in the younger I/R animals in case of the male groups, whereas in the female groups, older animals showed higher values. (5) The impairment of red blood cell deformability was observed mainly in the male and older I/R groups. (6) Enhanced erythrocyte aggregation was seen in all groups, being more expressed in the female I/R groups.

The elevated hematocrit, red blood cell, white blood cell, and platelet levels are the signs of acute phase reaction caused by the I/R. In the ischemic bowel, the activated white blood cells, endothelial cells, and platelets produce cytokines,

resulting in the upregulation of cell adhesion molecules, which play a significant role in platelet aggregation and the formation of microthrombi in the vessels leading to the deterioration of microcirculation.

Several mechanisms, including oxidative stress, may change the rheological properties of red blood cells by causing lipid peroxidation, hemoglobin, and protein alterations. Reactive oxygen species are generated during the aging process (free radical theory of aging) and the reperfusion period, as well.

Aging is a major risk factor for ischemic disorders, including stroke, ischemic heart disease, ischemic bowel disease, and many surgical procedures. I/R injuries affect mainly the elderly population, and some studies also presented evidence that the mechanism of I/R injury differs in older and younger animals. Therefore, it should be desirable to choose older animals in I/R models; however, usually younger animals are used in these experiments because of several reasons, such as greater availability and lower cost.

The average life expectancy of laboratory rats is 2.5-3 years. If we would like to correlate rat ages with human ages, it is important to take into consideration that relative ages are different depending on the stage of life. For example, a 4-month-old rat is considered a 10-year-old human, whereas 18 months of rat age equals to 45 human years.

According to the free radical theory of aging, the process of aging is (partly) due to the oxidative stress causing damage to biologically important targets. Age-related changes in different organs after I/R have been demonstrated in several studies.

Aging is also associated with a deterioration of the hemorheological parameters: increased blood and plasma viscosity, enhanced RBC aggregation, and impaired RBC deformability.

Hemorheological differences regarding gender were observed in many studies in humans, too. Compared with male blood, female blood has lower viscosity, hematocrit and red blood cell aggregation, and higher erythrocyte

deformability. Furthermore, it has been revealed that the age distribution of erythrocytes is significantly different in females and males: the female blood has more young RBCs than male blood. It has been demonstrated that “old” RBCs compared with “young” RBCs have increased mechanical fragility, rigidity, and elevated aggregability. These properties of the blood may contribute to the higher risk for the development of cardiovascular diseases in males.

It has been also shown that estrogen has a protective effect against I/R injury in heart, brain, and limb. Estradiol therapy during small intestinal I/R reduced local and distal (lung) lesions in male rats. The mechanisms underlying this effect are quite complex: estrogens are involved in gene transcription and intracellular signaling, have anti-inflammatory and anti-oxidant activities, and take part in vasoregulation as well. However, some studies found that estrogen replacement therapy increased the cardiovascular incidences in women. This discrepancy underlines the importance of further research in this field.

Any surgical procedure or disorder that involves the interruption of blood flow to an organ or tissue along with the restoration of blood supply can result in I/R injury, and it may lead to local and remote tissue damage. The investigation of the microrheological parameters may provide important information about the pathomechanism of intestinal I/R injury and potential therapeutic options. Furthermore, our study draws the attention of physicians that elderly patient may suffer more severe injuries.

6. SUMMARY OF MAJOR RESULTS AND CONCLUSIONS

1. We demonstrated that in rats hematocrit, red blood cell count and white blood cell count significantly increased during 30 minutes of small intestinal ischemia and the subsequent 120-minute reperfusion. Micro-rheological parameters deteriorated during the observation period. The microcirculation values of the small intestine decreased during ischemia and did not fully normalize at the end of the reperfusion.
2. It has been shown that during intestinal ischemia-reperfusion the microcirculatory values of the liver and kidney decreased of the remote organs and histological examinations also showed alteration in case of these organs.
3. We demonstrated that hematological and micro-rheological parameters show gender- and age-related differences in rats during intestinal ischemia-reperfusion. The highest hematocrit and red blood cell counts were found in the older male I/R group at the end of the reperfusion, and this group had the worst red blood cell deformability values during the study.
4. We have shown that mesenteric ischemia-reperfusion caused significant tissue damage. According to the histological analysis, I/R induced changes were the most severe in the older groups.

The worsening of micro-rheological parameters during ischemia-reperfusion may contribute to microcirculatory disturbances of local and remote organs. A better understanding of the pathophysiology of the small intestine I/R may serve to develop further haemorheological therapies. The observed gender and age-related differences may provide useful information for planning further experiments and evaluating results.

7. PUBLICATIONS



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Registry number: DEENK/100/2018.PL
Subject: PhD Publikációs Lista

Candidate: Anita Mester
Neptun ID: HFWA3B
Doctoral School: Doctoral School of Clinical Medicine

List of publications related to the dissertation

1. Mester, A., Magyar, Z., Molnár, Á., Somogyi, V., Tanczos, B., Pető, K., Németh, N.: Age- and gender-related hemorheological alterations in intestinal ischemia-reperfusion in the rat. J. Surg. Res. 225, 68-75, 2018.
DOI: <http://dx.doi.org/10.1016/j.jss.2017.12.043>
IF: 2.187 (2016)
2. Mester, A., Magyar, Z., Somogyi, V., Tanczos, B., Stark, Y., Cherniavsky, K., Bidiga, L., Pető, K., Németh, N.: Intestinal ischemia-reperfusion leads to early systemic micro-rheological and multiorgan microcirculatory alterations in the rat. Clin. Hemorheol. Microcirc. 68 (1), 35-44, 2018.
DOI: <http://dx.doi.org/10.3233/CH-170278>
IF: 1.679 (2016)

List of other publications

3. Magyar, Z., Mester, A., Nadubinszky, G., Varga, G., Souleiman, G., Somogyi, V., Tanczos, B., Deák, Á., Bidiga, L., Mihai, O., Pető, K., Németh, N.: Beneficial effects of remote organ ischemic preconditioning on micro-rheological parameters during liver ischemia-reperfusion in the rat. Clin. Hemorheol. Microcirc. "Accepted by Publisher", 2018.
IF: 1.679 (2016)
4. Pető, K., Németh, N., Mester, A., Magyar, Z., Souleiman, G., Somogyi, V., Tanczos, B., Deák, Á., Bidiga, L., Frecska, E., Nemes, B.: Hemorheological and metabolic consequences of renal ischemia-reperfusion and their modulation by N,N-dimethyltryptamine on a rat model. Clin. Hemorheol. Microcirc. [Epub ahead of print], 2018.
DOI: <http://dx.doi.org/10.3233/CH-170361>
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5. Magyar, Z., Molnár, Á., Nachmias, B. D., Mann, D., Somogyi, V., Mester, A., Pető, K., Németh, N.:
Impact of groin flap ischemia-reperfusion on red blood cell micro-rheological parameters in a
follow-up study on rats.
Clin. Hemorheol. Microcirc. [Epub ahead of print], 2017.
DOI: <http://dx.doi.org/10.3233/CH-170277>
IF: 1.679 (2016)
6. Józsa, T., Klárik, Z., Kiss, F., Tóth, E., Mester, A., Hargitai, Z., Changchien, Y. C., Fossum, M.,
Németh, N.: Morphological and microcirculatory evaluation of the rat testis after detorsion
with or without a capsular release with a tunica vaginalis flap.
Asian J. Androl. 18 (3), 462-466, 2016.
DOI: <http://dx.doi.org/10.4103/1008-682X.157546>
IF: 2.996
7. 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.
Clin. Hemorheol. Microcirc. 57 (4), 339-353, 2014.
DOI: <http://dx.doi.org/10.3233/CH-131724>
IF: 2.242

Total IF of journals (all publications): 14,141

Total IF of journals (publications related to the dissertation): 3,866

The Candidate's publication data submitted to the iDEa Tudóstér have been validated by DEENK on
the basis of Web of Science, Scopus and Journal Citation Report (Impact Factor) databases.

23 April, 2018



8. ACKNOWLEDGMENT

I would like to thank my supervisor, Dr. Norbert Németh, Head of Department, who helped my work since I was medical student and to carry out my PhD research work under his supervision. Thank you for your advice, encouragement and support.

I thank Prof. Dr. István Furka and Prof. Dr. Irén Mikó for learning from them.

I am grateful to the PhD students of the Department, Dr. Zsuzsanna Magyar, Viktória Somogyi and Bence Tánczos, for their friendships and that during my research work they were great help in the experiments and laboratory measurements.

Thanks to Dr. Katalin Pető and Dr. Ádám Deák, who I could turn to for advice at any time.

I thank all the employees of the Department, especially Rozália Gödényné, from whom I have received a lot of help in my work.

Thank you, Dr. László Bidiga, for your time and help in histology.

Last but not least, I would like to thank my Family for their support and providing a calm background for my studies. Finally, I am grateful for the patience and support of my husband during every day.