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Effects of Early and Delayed Remote Organ Ischemic Preconditioning on the Extent of Renal Ischemia-Reperfusion Injury in a Rat Model

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The PhD defense will be organized at the In Vitro Diagnostic Center, University of Debrecen on September 3, 2021, at 13:00.

1. INTRODUCTION

Renal ischemia-reperfusion (I/R) injury is a common cause of acute renal failure. Many cardiovascular surgeries require interventions of the aorta above the renal arteries, resulting in their partial or complete short-term exclusion, which can alter the blood supply, and thus the supply of oxygen to the kidneys. Decreased renal perfusion may also occur during other surgeries, which increases the risk of damage in chronic renal failure. Depending on the degree of damage, kidney transplantation may be required. Hypoxaemic conditions during organ transplantation followed by sudden recurrent circulation also cause I/R injury, which could impair kidney function and lead to delayed graft failure and rejection.

Ischemic conditions can lead to cell damage and cell death due to reduced or completely eliminated oxygen and nutrient supply, as well as insufficient removal of by-products of cell metabolism. Restoration of circulation, i.e., the early onset of reperfusion, is important for cell survival, but paradoxically, it also causes tissue damage. Due to altered cellular metabolism, cells are more likely to be exposed to oxidative stress, which also occurs with the appearance of fresh, oxygen-rich blood. These two processes together are termed ischemia-reperfusion injury, and a number of parameters affects the degree of injury.

Following ischemia-reperfusion injury, a number of changes are observed both in the damaged organ and in the whole organization. Short- and long-term mediators are released and various neural and biochemical pathways are activated. The circulatory system, including blood and blood flow, is significantly affected. The field of science dealing with the problem of blood flow and its influencing factors is haemorheology. A better understanding of haemorheological changes during I/R injuries can significantly help to assess the extent of the damage and to monitor it more accurately. Influencing haemorheological parameters/changes may allow to reduce the damage.

Prevention of ischemia-reperfusion injury would be the best solution; however, this is rarely possible. Several pharmacological and surgical methods are known to reduce the extent of damages. The essence of condition-based procedures is to help? the cells “to adapt” to I/R injury by alternating short, ischemic and reperfusion stages. Conditioning at or near the target organ and intervention site prior to, during or after I/R injury are also known. Remote organ ischemic preconditioning (RIPC) in experimental surgery has been shown beneficial in reducing I/R injury in many organs, while its efficacy is less clear in clinical routine.

The mechanism of action, as well as the ideal protocol for remote organ ischemic preconditioning are yet unknown. Two different time frames exist for the intervention, and they are based on different mechanisms. There is limited available data in the literature on renal ischemia-reperfusion in haemorheological and microcirculatory events associated with ischemic-reperfusion injury.

2. AIMS OF THE STUDY

1. To establish a rat model to investigate the effects of 45 minutes of unilateral renal ischemia and subsequent 120 minutes of reperfusion.
2. To assess the extent of ischemia-reperfusion injury, based on haemorheological, haematological, microcirculatory, vital and acid-base homeostasis parameters and histological examination.
3. To reduce ischemia-reperfusion injury by using early 1-hour (RIPC-1) and delayed 24-hour (RIPC-24) remote organ preconditioning techniques.
4. To compare the efficacy of early and delayed remote organ preconditioning protocols using the above parameters.

3. MATERIALS AND METHODS

3.1. Experimental animals, groups, surgical techniques

Our research was carried out in conduction with national and European legislation, approved by the University of Debrecen Committee of Animal Welfare (UDCAW), and an official permission was issued by the Hajdú-Bihar County Animal Health and Food Control Station (registration number: 25/2016/UDCAW). The experimental animals were kept at room temperature in conventional cages, dry food and water were provided ad libitum.

Twenty-seven male 12-14 weeks old, Crl: WI, outbred rats (Toxicoop Ltd., Body weight: 301.6 ± 38.6 g) were included in the study. Experimental animals were randomly divided into 4 groups according to treatment protocols. All animals underwent general anaesthesia (40 mg/bwkg Thiopental ip.) 24 hours before surgery, and general anaesthesia (60 mg/bwkg Thiopental ip.) was repeated 1.5 hours before surgery. All interventions were performed on a heated bench, and the animals were restrained in a supinated position at their limbs.

In the control group (n=7), no intervention was performed during the day before surgery. Under anaesthesia, prior to surgery, the left inguinofemoral and abdominal regions were regularly shaved and disinfected with Braunol solution, then isolated with sterile gauze. In the left inguinofemoral region, a 1 cm long incision was performed and the left femoral artery was cannulated (BD NeoflonTM, 26 G) using an operating microscope (Leica Wild M650). Surgical procedures were performed using sterile, standard microsurgical equipments. Following cannulation, a median laparotomy was performed, startint the incision from the processus xiphoideus to the pecten ossis pubis. The left kidney was isolateded and its blood vessels were atraumatically dissected. The intestines were isolated with a warm, physiological saline-soaked gauze for the time of preparation, then placed back into the abdominal cavity and covered similarly. Measurements were taken during the observation period, and blood samples were

taken from the cannulated artery. No further surgical or ischemia-reperfusion procedures were performed in the control group.

The protocol of the ischemia-reperfusion group (I/R, n=7) was the same as that of the control group until left kidney dissection. After atraumatic dissection of the vessels of the left kidney, a microsurgical clip was placed on left renal artery, thus creating unilateral renal ischemia, which was also confirmed by macroscopic colour change. After 45 min of ischemia, the clip was removed and after a 120-minute reperfusion period, the animals were exsanguinated. In distant organ preconditioned groups, under general anaesthesia, the right hind limb was suppressed at the height of the inguinal ligament using an atraumatic tourniquet for 3x10 minutes with 10 minutes of reperfusion periods. In the early remote organ preconditioned group (RIPC-1, n=7), preconditioning was performed 1 hour prior to surgery, while in the delayed remote organ preconditioned group (RIPC-24, n=6), the tourniquet was inserted 24 hours prior to surgery. After preconditioning, the protocols of the preconditioned groups were the same as that of the I/R group.

3.2. Protocol of measurements

At the beginning of surgery (base) and at 30, 60 and 120 minutes of reperfusion (R30, R60, R120), blood samples were drawn (0.3-0.5 mL) from the cannulated left renal artery into K3-EDTA (1.5 mg/mL) anticoagulant-containing blood collection tubes, then laboratory tests were performed. Following blood harvest, the circulating volume was replaced with an equal volume of physiological saline at body temperature. Measurements were taken at the time of blood collection as well as at the end of the ischemic period (I45).

At the end of the 120-minute reperfusion period, biopsy samples were taken from the liver or from the kidneys for histological examination, followed by the animals' exsanguination.

3.3. Vital parameters

Heart rate and mean arterial pressure were measured invasively using the HaemoSys system (Experimetria Ltd., Hungary) connected to the cannulated artery. Respiratory rate was determined by direct counting for 60 seconds. Body temperature was measured in the rectum using a digital thermometer (Rudolf Riester GmbH, Germany), while organ surface temperature was determined at designated anatomical points in the liver and in the kidneys respectively.

3.4. Microcirculation

The microcirculation of the examined organs was examined with a laser Doppler effect device (LD-01 Laser Doppler tissue flowmeter, Experimenta Ltd., Hungary) using a standard pencil head (MNP100XP, Oxford, Optironics Ltd., United Kingdom) with the support of the operating doctor.

Laser-Doppler measurement provides real-time, quantifiable information about the perfusion of a given tissue, a microrheological parameter that significantly affects viability. The method is based on the Doppler-shift phenomenon, which works with coherent laser beam reflected from the moving red blood cells and undergoes a change in wavelength. The rate of change is directly proportional to the number and rate of erythrocytes in motion, but does not correlate with the direction of motion. The reflected laser light is detected by the device and the frequency offset is S.P.E.L. With the help of Advanced Kymograph software (Experimetria Ltd., Hungary) it is possible to represent graphically the parameter characteristic of the microcirculation, which is given by the system in relative flow units (blood flux units, BFU). Depending on the frequency used, the laser beam penetrates to a depth of 1-1.5 mm on an area of 1 mm^2, so that the circulation of about 1-1.5 mm^3 of the examined tissue can be examined.

The measurement may be affected by the temperature of the organ, tissue oxygenation, respiratory and muscle movements, pulsation of any nearby large vessels, elevated serum lipid

levels, haemolysis, external sources of interfering light, or tremor in the person's hand while performing the measurement.

After standardizing the parameters that can be influenced, an approx. 40-seconds period was recorded in the case of the liver and in the middle third of the anterior surface of the kidneys, respectively. From the characteristic patterned curves of the organ, 10-second intervals were later analyzed "off-line" after parameterization of the curve sections.

3.5. Laboratory parameters

3.5.1. Blood gas analysis

Prior to ischemia and at the end of the 120-minute reperfusion period, arterial blood tests were performed using an EPOC Blood Analyzing System (Siemens Healthcare GmbH, Germany). The device determines the acid-base parameters, electrolyte and metabolite values (pH, pO_2 [mmHg], pCO_2 [mmHg], HCO_3^- [mmHg], base deficiency (BE (ecf)) [mmol/L], O_2 saturation [%], sodium (Na^+) [mmol/L], potassium (K^+) [mmol/L], calcium (Ca^{2+}) [mmol/L], chloride (Cl^-) [mmol/L], lactate [mmol/L], creatinine [mg/dL], glucose [mmol/L]).

3.5.2. Haematological parameters

Quantitative and qualitative haematological parameters: erythrocyte count (RBC) [T / L], haemoglobin concentration (Hgb) [g/dL], haematocrit (Htc) [%], mean erythrocyte volume (MCH) [pg], mean erythrocyte volume (MCV) [fl], MCHC [g/dL], white blood cell count (WBC) [G/L], Platelet count (Thr) [G/L] was determined by using an automatic Sysmex K-4500 system (TOA Medical electronics Corp., Ltd., Japan).

3.5.3. Red blood cell deformability

Using a LoRRca MaxSis Osmoscan ectacytometer (Mechatronics BV, The Netherlands), we measured the deformability of red blood cells, i.e. its elongation under shear stress (SS [Pa]), by laser diffraction. For the measurements, 10 µL of blood sample was suspended in 2 mL of isotonic polyvinylpyrrolidone (PVP) -phosphate buffer (PBS) solution

(Sigma-Aldrich Co. USA; PVP-PBS viscosity: 33.6 mPas, osmolality: ~ 300 mOsmol/kg, pH=7.3), then the suspension was pipetted into the measuring chamber. The Couette measuring chamber consists of a straight-walled, rotatable cylindrical cylinder shell (cup) and a matching static cylinder (bob). Between the "bob" and the "cup" there is an annular gap about 0.3 mm wide for the sample. At the start of the measurement, the "cup" begins to rotate around the cylinder, thereby exerting shear on the cells, causing the red blood cells to elongate. Measurements were made from the lower shear stress to the higher shear stress (0.5–30 Pa) under tempered conditions (37 ˚C). During rotation, a laser is projected onto the elongated red blood cells (wavelength: 670 nm). Depending on the degree of elongation, the angle of diffraction changes, which is recorded by a CCD camera and then analysed by an associated software. From the length (A) and width (B) of the diffraction sample, the dimensionless elongation index (EI) can be calculated from the formula (A-B) / (A + B). Plotting the EI values in a coordinate system with the corresponding SS values gives a curve describing the deformability of the red blood cells. The higher the EI value for a given SS, the better the elongation of the cells, i.e., their better deformability. The obtained curve can be calculated by Lineweaver-Burke analysis ($1/EI=SS_{1/2}/EI_{max}\times 1/SS+1/EI_{max}$) the maximum EI ($EI_{max}$) or the shear stress ($SS_{1/2}$) [Pa] corresponding to half of this value, which are the indicators traditionally used to characterize deformability. A linear transformation was used.

3.5.4. Red blood cell osmotic gradient deformability

The osmotic gradient deformability (osmoscan) of red blood cells was determined using a LoRRca MaxSis Osmoscan ectacytometer (Mechatronics BV, The Netherlands). 250 µL of blood samples were suspended in 5 mL of PVP-PBS solution per measurement. The principle of osmoscan measurement is the same as the traditional red blood cell deformability measurement, but in this case the applied shear stress is constant (eg SS=30 Pa), while the osmolality (O) of the suspending medium (O) [mOsm/kg] is 0 to 500 mOsm/kg. to increase.

Plotting the EI-O value pairs in a coordinate system, we get a characteristic curve with well-defined and precisely definable parameters of the osmotic gradient deformability of red blood cells: the smallest elongation index measured in a low osmolality medium (EI min), the maximum elongation index (EI max), half of the EI max in hyperosmolality medium (EI hyper) and the associated osmolality values (O min, O (EI max), O hyper, [mOsm/kg]) and the calculated area under the curve (AUC). From the above parameters, the difference of EI min and EI max (ΔEI) can be further calculated,) the difference of their osmolality values (ΔO) and their quotient (ΔEI / ΔO).

3.5.5. Red blood cells aggregation

Red blood cell aggregation was determined using a Myrenne MA-1 light transmission aggregometer (Myrenne GmbH, Germany). A 20 µl anticoagulated blood sample was applied to a 2˚ convex lens, and then the glass plate on the lid of the sample space dispersed the drop of blood on the lens were folded by the process. The diode above the slide emits infrared light onto the sample while the instrument disaggregates the sample with a 600 s^{-1} velocity gradient rotation. The rotation stops (M mode) or decreases to a speed gradient of 3 $^{s-1}$ (M1 mode), and then in the 5th (5s) or 10th second (10s) of the aggregation process, the detector under the lens detects the intensity of the transmitted infrared light, and the device determines the value of the aggregation index. The higher the light that passes through the sample, the higher the aggregation index, i.e., the higher the aggregation, as the space between the cells and aggregates widens with the formation of aggregation, thus increasing light transmission. A total of four aggregation indices can be determined from one sample (M 5s, M 10s, M1 5s, M1 10s). To determine the index numbers, we performed 4-4 parallel measurements and used their averages in the statistical evaluation.

3.6. Histology

For histological examination, samples were taken from the liver, and ischemic and intact kidneys were also removed, which were examined by periodic acid-Schiff (PAS) staining after paraffin fixation. Histological evaluation of renal sections examined tubular epithelial (TH) nucleus staining, the amount of hyaline globules in TH, hydropic degeneration in TH, tubular necrosis, glomerular stagnation, congestion of peritubular capillaries, iron stasis and the condition of the brush edges, evaluating each parameter with a score of 0-3, with a higher score associated with a less favourable histological picture. Sections of liver samples were evaluated based on stagnation, cell necrosis, and vacuolation observed in the liver sinuses, with values ranging from 0 to 3 points per parameter, with 0 being the most favourable and 3 the most damaged.

3.7. Statistics

Our results are presented as mean ± standard deviation (SD). GraphPad Prism software (GraphPad Software Inc., California, USA) was used for statistical analysis. Depending on the distribution of the data, Student's t-test or Mann-Whitney test was used to compare data from two groups, while data between several groups and within groups were compared by one-way or repeated measures analysis of variance (ANOVA), Dunn, Bonferroni, or Student–Newman–Keuls. using control methods. The significance level was set at $p<0.05$.

4. RESULTS

4.1. Vital parameters

4.1.1. Heart rate

The lowest heart rate was measured in the I/R group, which increased significantly from base by the end of ischemia ($p=0.0112$) and further increased during reperfusion ($p<0.0001$ vs.

R30, R60, R120). A slight increase from the 60th minute of reperfusion was also observed in the Control group (p=0.096 vs. R60, p=0.0085 vs R120). The values of the preconditioned groups did not change during the experiment.

4.1.2. Arterial mean pressure

Mean arterial pressure was stable during surgery in all three groups except the RIPC-1 group. Values in the early preconditioned group increased during the experiment, and were significantly highest by the end of reperfusion. (I45: p=0.0154 vs. I/R; R30: p=0.0074 vs. Control, p=0.0013 vs. I/R, p=0.0228 vs. RIPC-24, p=0.0262 vs. base; R60: p=0.036 vs. RIPC-24; R120: p=0.0115 vs. I/R, p=0.0153 vs. RIPC-24, p=0.0139 vs. base)

4.1.3. Respiratory rate

The lowest respiratory rate was measured in the RIPC-24 group throughout the experiments (base: p<0.0001 vs. Control, RIPC-1, p=0.0003 vs. I/R; I45: p=0.0012 vs. Control, p=0.0359 vs. I/R, p=0.0048 vs. RIPC-1, R30: p=0.0006 vs. Control, p=0.0487 vs. RIPC-1, R60: p=0.0006 vs. Control, p=0.0279 vs. I/R; R120: p=0.0046 vs. Control, p=0.0149 vs. I/R). A small increase was observed in the Control and RIPC-24 groups, respectively. The values of the other two groups were stable.

4.1.4. Temperature

Rectal temperature was stable during the experiment in the Control and I/R groups, while the RIPC-24 group values were significantly lower in the other groups until the 30th minute of reperfusion (base: p=0.0073 vs. Control, p=0, 0057 vs. I/R, p=0.0053 vs. RIPC-1, I45: p=0.0388 vs. I/R, R30: p=0.016 vs. Control, p=0.0033 vs. I/R , p=0.0141 vs. RIPC-1). By the end of the reperfusion period, the RIPC-1 group had the lowest values (p=0.0071 vs. base, p=0.0002 vs. I45, p<0.0001 vs. R30, Control, p=0.0179 vs. R60, p=0.0008 vs. I/R, p=0.0139 vs. RIPC-24).

No significant differences in organ surface temperatures were found within the groups or among the groups for any of the measured organs. A small but continuous increase was observed in the RIPC-24 group, while the values in the I/R and RIPC-1 groups were stable throughout.

4.2. Microcirculation

There was a difference in the relative changes in BFU values describing hepatic microcirculation between the Control and I/R groups, which was significant at R30 ($p=0.0205$), while the values of the preconditioned groups did not change.

In the case of the right kidney, which was not exposed to ischemia-reperfusion injury, no significant change was measured in either group. In the left ischemic kidney, however, a moderate increase was observed during reperfusion in the I/R and RIPC-24 groups,.

In the RIPC-24 group, the increase in BFU lasted until the 60th minute of reperfusion ($p=0.0085$ vs. I45) and then decreased close to base, while in the I/R group, it increased further until the end of reperfusion ($p=0.0011$ vs. I45, $p=0.0322$ vs. RIPC-1).

4.3. Laboratory parameters

4.3.1. Blood gas analysis

No significant difference was found between the pH values of the Control group measured after base and reperfusion. A moderate decrease from base at time R120 was observed in the I/R group, and more significant n the RIPC-1 group ($p=0.014$), while there was a significant increase in the RIPC-24 group ($p=0.024$). There was no significant difference in pO_2 values, only a small decrease in the I/R and RIPC-1 groups and a slight increase in the RIPC-24 group. Similarly, the delayed preconditioned group had the highest respiratory compensation, with the respiratory rate increasing to 120–140% from base in the animals. Among the pCO_2 values, the base of the RIPC-24 group was increased compared to the other

groups (p=0.028 vs. Control, p=0.014 vs. IR), while by the end of the experiment, all groups except the RIPC-1 group showed a decrease from base (Control: p=0.004; I/R: p<0.001; RIPC-24: p<0.001). A decrease in bicarbonate levels from base was observed in all groups (Control: p=0.003; I/R: p<0.001; RIPC-1: p=0.011; RIPC-24: p<0.001), with the slightest decrease in the RIPC-24 group. (p=0.041 vs. Control, p=0.014 vs. I/R, p=0.019 vs. RIPC-1). The greatest base deficiency was in the I/R and RIPC-1 groups.

Relative changes from base in electrolytes measured at the 120th minute of reperfusion were examined. There was no significant change in Na^+ values, that remained in the physiological range. In contrast, K^+ levels increased in all groups, most significantly in the I/R group (p<0.001 vs. base), and the lowest increase was observed in the RIPC-24 group (p=0.008 vs. base, p=0.03 vs. I/R). The measured Ca^{2+} values decreased slightly in all groups, the lowest values were measured in the I/R group (p<0.001 vs. base), while in the RIPC-24 group the calcium ion levels remained significantly higher (p=0.015 vs. I/R). Cl^- level was significantly increased from base in the I/R (p<0.001) and RIPC-1 (p<0.001) groups, which was not seen in the RIPC-24 protocol group (p=0.005 vs. I/R, p=0.007 vs. RIPC-1).

The relative changes from base in metabolite levels obtained when measuring R120. Lactate concentrations were significantly increased in I/R (p<0.001 vs. base, p=0.011 vs. control), RIPC-1 (p=0.013 vs. base, p=0.03 vs. Control) and lowest, but still significantly in the RIPC-24 (p=0.013 vs. base, p=0.03 vs. Control) group. Creatinine concentration was significantly increased in all three groups exposed to ischemia-reperfusion injury (I/R: p=0.008; RIPC-1: p=0.021; RIPC-24: p=0.014). An increase in glucose concentration was observed only in the RIPC-24 group (p=0.016 vs. base).

4.3.2. Haematological parameters

The highest red blood cell count was measured in the RIPC-24 group (base, R30, R60, R120: p<0.0001 vs. Control, I/R; R30, R60: p<0.0001 vs. RIPC-1), while the Control group

had the lowest values (base, R30, R60, R120: $p<0.0001$ vs. RIPC-24; R30, R60: $p<0.0001$ vs. RIPC-1; R30: $p=0.0003$ vs. I/R; R60: $p=0.0002$ vs. I/R).

Haematocrit values were similarly variable, with the highest values in the RIPC-24 group (base: $p=0.0053$ vs. I/R; R30: $p<0.0001$ vs. I/R, $p=0.0011$ vs. RIPC-1, R60: $p<0.0001$ vs. I/R; R120: $p=0.0010$ vs. I/R, $p=0.0339$ vs. RIPC-1).

The most stable white blood cell counts were measured in the preconditioned groups, while a jump was observed in the Control (R60: $p=0.0307$ vs. base, $p=0.039$ vs. RIPC-1) and I/R groups (R30: $p=0.0102$ vs. base, $p=0.0006$ vs. R120, $p=0.0004$ vs. Control, $p<0.0001$ vs. RIPC-1, $p=0.0005$ vs. RIPC-24).

The lowest platelet values were found in the RIPC-24 group (base: $p<0.0001$ vs. I/R, $p=0.0416$ vs. RIPC-1; R30: $p=0.0043$ vs. I/R, $p=0.0279$ vs. RIPC-1; R60: $p=0.0161$ vs. I/R). A decreasing trend was also observed in the I/R group, which became significant by the end of reperfusion ($p=0.0465$ vs. base).

4.3.3. Red blood cell deformability

The elongation index measured at a shear stress of 3 Pa, and the quotient of $SS_{1/2}$ and EI_{max} (deformability index) which characterize the deformability. In the case of EI values measured at 3 Pa, there was a significant difference between the groups already in the basic measurement. Values in the RIPC-1 group were significantly lower than in the Control group ($p=0.002$), and by the end of the observed period, a significant increase from base was observed (R120: $p=0.013$). In contrast, the RIPC-24 group showed a decrease compared to the other groups, which was significant from R60 compared to the Control group ($p=0.043$). This difference was further exacerbated at time R120 ($p=0.001$ vs. Control; $p=0.015$ vs. RIPC-1).
When analysing the $SS_{1/2}/EI_{max}$ ratios, the changes were more significant. Among the initial values, the values of the preconditioned groups were higher than the values of the Control group (RIPC-1: $p<0.001$; RIPC-24: $p<0.00$), and in the case of the early preconditioned group also

from the I/R group (p=0.014). The values of the untreated ischemic group were increased compared to the values of the control group during reperfusion, which was significant from the 60th minute of reperfusion (p=0.041). Values in the preconditioned group were also increased during reperfusion. For R120 measurements, the highest values were obtained in the RIPC-24 group (p<0.001 vs. base; p=0.001 vs. I/R; p=0.002 vs. RIPC-1.

4.3.4. Red blood cell osmotic gradient deformability

There was a significant difference in the osmotic magnitude ectacytometry (osmoscan) values in a few parameters. Significant differences in base O (EI max), O hyper, and AUC values were found between the Control and RIPC-24 groups. These differences were also observed at the end of the reperfusion period and became significant for AUC compared to the other groups. (p=0.004 vs. Control; p=0.026 vs. I/R; p=0.017 vs. RIPC-1).

The ΔO values at the end of reperfusion were significantly lower in the RIPC-24 group compared to the I/R group values (p=0.019), which was not significant for ΔEI.

4.3.5. Red blood cell aggregation

The changes in the aggregation index when using different settings (M 5s, M 10s, M1 5s, M1 10s) are shown in Figure 4. For M 5s mode, the lowest default value was obtained in the RIPC-24 group (p=0.008 vs. Control), in contrast, this group had the highest values during reperfusion. (R30: p<0.001 vs. base, p=0.004 vs. I/R, p=0.003 vs. RIPC-1; R60: p<0.001 vs. base, p=0.03 vs. control; R120: p<0.001 vs. base, p=0.0027 vs. Control, p<0.001 vs. RIPC-1).

At the M 10s setting, the base values of the RIPC-24 group were also the lowest (p<0.001 vs. Control, I/R, RIPC-1), but they were significantly increased during reperfusion (R60: p=0.004 vs. base; R120: p< 0.001 vs. base; p=0.013 vs. R30).

Among the M1 5s measurements, the values of the RIPC-24 group were also the highest during reperfusion (R30: p=0.0018 vs. base, p<0.001 vs. Control, I/R; R60: p=0.0018 vs. base, p=0.0022 vs. Control, p=0.0007 vs. I/R, R60: p=0.008 vs. base, p=0.0022 vs. Control, p=0.0007 vs. I/R; R120: p=0.0008 vs. base, p=0.015 vs. control, p=0.046 vs. I/R).

M1 10s values were highest in the RIPC-24 group in the second half of reperfusion (R60: p=0.0024 vs. Control; R120 p=0.0003 vs. control, p=0.0073 vs. Control). There was also a significant increase in the I/R group by the end of 120 min reperfusion (p=0.027 vs. Control, p=0.042 vs. R30).

4.4. Histological examinations

In the case of the left, ischemic kidney, we obtained significantly higher scores compared to the contralateral, intact kidney in the I/R ($p<0.0001$) and RIPC-1 ($p<0.05$) groups. The lowest score from the left ischemic kidney data was found in the Control group ($p<0.0001$ vs. I/R and RIPC1, p=0.0025 vs. RIPC-24), while the scores were highest in the I/R group. (p=0.0017 vs. RIPC-24). Of the groups exposed to I/R damage, the RIPC-24 group had the lowest scores (p=0.0025 vs. RIPC-1).

The greatest degree of hyaline degradation in tubular epithelial cells, along with the highest number of hyaline globules and damaged glomeruli were in the I/R group. Congestion in the vasa recta was more pronounced in the RIPC-1 group, whereas pathological brush border was observed in all three groups exposed to I/R damage. Nuclear staining of tubular epithelial cells decreased, while tubular necrosis increased with I/R, while these changes were less detectable in the RIPC groups. In kidneys exposed to ischemia, hyaline degradation was increased and foamy lesions appeared in the cytoplasm of tubular epithelial cells, which appeared to a lesser extent in the RIPC groups. In the Control group, there were no significant glomerular, peritubular capillary, or vasa rectal congestion in either side of the kidneys, while sections in the I/R group showed mild to moderate congestion in the ischemic kidneys. The intact kidney was without deviation. In contrast, the RIPC-1 group showed significant congestion in both sides of the kidney, while the RIPC-24 group had little detectable congestion. The brush border of the proximal epithelial cells was completely intact in the kidneys on both sides of the Control group, while the I/R and RIPC-1 groups had subtotal and intermittent

absence of the brush border in the ischemic kidney. In the RIPC-24 group, only smaller gaps were found in the continuity of the ischemic kidney brush border.

The highest histological score was found among liver sections in the RIPC-1 group, where the most pronounced difference was congestion observed in hepatic sinusoids. The lowest score was obtained in the RIPC-24 group.

5. MAIN FINDINGS AND CONCLUSIONS

1. The renal ischemia model used during the experiments was suitable for the short-term study of ischemia-reperfusion injury and for the comparison of the different distant organ preconditioning protocols used using the examined parameters.

2. A significant deterioration in erythrocyte deformability, acid-base and metabolite values, and a significant increase in leukocyte count in the I/R group were observed compared to the control group, as a result of the 45-minute renal ischemia. Histological examination revealed the greatest damage in this group. Microcirculation was increased in the ischemic left kidney. No significant differences in red blood cell aggregation or vital parameters were observed.

3. Preconditioning protocols have mitigated all I/R-caused changes. Among the haematological parameters, a decrease in platelet count and an increase in erythrocyte count were observed, and leukocyte count did not change significantly in these groups. Deformability values were not improved by preconditioning protocols, while histological scores were found to be more favourable compared to the I/R group. Increased circulation in the left kidney was moderated by both RIPC protocols.

4. Comparing the two preconditioning protocols, both methods had a positive effect on several parameters. For haemorheological parameters, the early protocol proved to be more favourable, while for metabolite values and histological examinations, the results of the delayed preconditioned group were better.

To determine the optimal remote organ ischemic preconditioning protocol to be used during renal ischemia reperfusion, further studies are needed to allow long-term follow-up.

6. PUBLICATIONS

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Candidate: Gábor Varga
Doctoral School: Doctoral School of Clinical Medicine
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List of publications related to the dissertation

1. **Varga, G.**, Ghanem, S., Szabó, B., Nagy, K., Pál, N., Tánczos, B., Somogyi, V., Baráth, B., Deák, Á., Matolay, O., Bidiga, L., Pető, K., Németh, N.: Which remote ischemic preconditioning protocol is favorable in renal ischemia-reperfusion injury in the rat?
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   IF: 1.741 (2019)
2. **Varga, G.**, Ghanem, S., Szabó, B., Nagy, K., Pál, N., Tánczos, B., Somogyi, V., Baráth, B., Deák, Á., Pető, K., Németh, N.: Renal ischemia-reperfusion-induced metabolic and micro-rheological alterations and their modulation by remote organ ischemic preconditioning protocols in the rat.
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   DOI: http://dx.doi.org/10.3233/CH-189414
   IF: 1.741

List of other publications

3. Németh, N., Pető, K., Magyar, Z., Klárik, Z., **Varga, G.**, Oltean, M., Mantas, A., Czigány, Z., Tolba, R. H.: Hemorheological and Microcirculatory Factors in Liver Ischemia-Reperfusion Injury - An Update on Pathophysiology, Molecular Mechanisms and Protective Strategies.
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   Clin. Hemorheol. Microcirc. "Accepted by Publisher", 2021.
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5. Magyar, Z., Mester, A., Nadubinszky, G., **Varga, G.**, Ghanem, S., Somogyi, V., Tánczos, 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.
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Clin. Hemorheol. Microcirc. 64 (4), 765-775, 2016.
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