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Simultaneous investigation of hemodynamic, microcirculatory and arterio-venous micro-rheological parameters in infrarenal or suprarenal aortic cross-clamping model in the rat

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Abstract. We aimed to investigate hemodynamic, microcirculatory and hemorheological consequence of infrarenal or suprarenal aortic cross-clamping (IRAXC, SRAXC) in the rat. We hypothesized that the magnitude of the changes are different. Twenty-one male rats were randomized into Control, IRAXC or SRAXC groups. Under anesthesia the right carotid artery was cannulated for monitoring heart rate and mean arterial pressure, then median laparotomy was performed. In AXC groups the abdominal aorta and the caudal caval vein were atraumatically clamped for 60 minutes below or above the renal vessels. Before and just after the ischemia, in the 30th and 60th minutes of the reperfusion besides hemodynamic test, laser Doppler flowmetry was used on the liver's, small-intestine's and the kidney's surface, then arterial (cannulated carotid artery) and venous (lateral tail vein) blood samples were taken for determining hematological, acid-base, erythrocytes' deformability, osmoscan and aggregation parameters. We found that when hemodynamic changes were prominent, microcirculatory or hemorheological parameters did not show such large differences. However, every parameter changed in various manners, showing more or less differences between IRAXC and SRAXC groups. Although the largest deviations were observable in SRAXC group, the acid-base and hemodynamic alterations were much more expressed than the micro-rheological ones. Further investigations of *in vivo* relations-correlations of changes in hemodynamic, microcirculatory, metabolic and hemorheological factors need further studies providing simultaneous monitoring possibilities.

Keywords: Infrarenal or suprarenal aortic cross-clamping, ischemia-reperfusion, red blood cell aggregation, red blood cell deformability, microcirculation, hemodynamics, rat model

1. Introduction

In vascular surgery cross-clamping of the abdominal aorta at various levels can be necessary, depending on the localization of vascular disease and the surgical intervention itself. The outcome and the surgical safety (e.g., clamping time) of infrarenal versus suprarenal aortic cross-clamping thus is still among the field of interest, having important clinical aspects. In the last decades the percentage of vascular surgical

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interventions requiring suprarenal cross-clamping obviously increased [27]. Among the predictors of the outcome in these cases, the position and the duration of the clampings are important factors [23, 24, 29, 37, 50, 52, 57].

Depending on the level and duration of the clamping, these interventions cause serious impact, resulting in extended ischemic and reperfusionic alterations in the affected organs [e.g., 22, 36]. Its hemorheological component has not been investigated so much yet, only a few data are available in the literature. In a pilot study we started to investigate this question, together with enzymological investigations, focusing on renal and liver functions [33]. Other studies showed hemorheological changes that follow hind limb, bowel or renal ischemia-reperfusion in various experimental models [39], thus, it is supposed that the rheological changes can be different depending on the level of the aortic cross-clamping.

The hemorheological parameters show significant changes in several pathological processes [3]. The micro-rheological changes, such as the characteristics of red blood cell deformability and aggregation become more widely studied with the latest measuring methods [4, 7, 15, 48]. However, the border of reversibility and irreversibility of these changes is still unclear, and as well as the *in vivo* rheological alterations raise further questions to be answered [2, 3, 20, 42]: *inter alia,* during the ischemia-reperfusion processes, when clamping and releasing of vessels are necessarily associated with definitive surgical interventions [8, 23, 39]. Further interesting issues are the related arterio-venous (aorto-caval) micro-rheological alterations [26].

Since hemorheological parameters play important role determining the microcirculatory pattern [2, 11, 12, 19, 20, 28, 31, 46, 47, 49, 54], the combined investigations of hemodynamics and the microcirculation of a given tissue together with testing the micro-rheological parameters of the circulating blood have important meanings.

In this study we aimed to investigate hemodynamic, microcirculatory and hemorheological consequence of infrarenal or suprarenal aortic cross-clamping in the rat. We hypothesized that the magnitude of the changes are different between infra- or suprarenal level, and also supposed, that these alterations are associated with each other. We also expected that the results may provide valuable information on the multi-organ involvement of the ischemia depending on its extent and also on the correlation of the synchronous changes in the micro-rheological, microcirculatory and hemodynamic parameters.

2. Materials and methods

2.1. Experimental animals and study design

The experiments were approved and registered by the University of Debrecen Committee of Animal Research (registration Nr.: 20/2011/UD CAR), in accordance with the Hungarian Animal Protection Act (Law XVIII/1998).

Twenty-one adult (7–8 months old) male Sprague-Dawley rats (Janvier Co., France) (bodyweight: 554.04 ± 27.77 g) were randomly divided into three equal experimental groups: Control (C) group, Infrarenal Aortic Cross-Clamping (IR AXC) group and Suprarenal Aortic Cross-Clamping (SR AXC). All the experiments were carried out under continuous general anesthesia (Thiopenthal® 60 mg/kg, i.p.).

2.2. Operative techniques and sampling protocol

In the *Control group* (C, $n = 7$) the front and the right lateral region of the neck as well as the middle region of the abdominal wall had been shaved and disinfected with Betadine®. After isolation, the skin on

the neck over the right carotid artery was horizontally incised (∼1 cm) and the right common carotid artery had been cannulated (BD Neoflon^{TM}, 26 G) under operating microscope (Leica Wild M650), for providing invasive intraoperative hemodynamic measurements. Via the cannula the animals received ∼100 U/kg sodium-heparin during the experiment. A midline laparotomy was performed, and by atraumatic preparation, the abdominal aorta and the caudal caval vein had been gently exposed.

In the *Infrarenal Aortic Cross-Clamping group* (IR AXC, $n = 7$) the same preparatory procedure was carried out, and both of the abdominal aorta and the caudal caval vein had been atraumatically clamped for 60 minutes just under the renal vessels, using microvascular clips. After 60 minutes, the clips were removed, and 60 minutes of reperfusion period was observed.

In the *Suprarenal Aortic Cross-Clamping group* (SR AXC, $n = 7$) besides the same preparation and procedure, the abdominal aorta and the caudal caval vein had been clamped for 60 minutes above the renal vessel, but just below the celiac trunk.

After surgical preparation (Base), just after the 60-minute clamping period (I-60), as well as at the 30th and 60th minutes of the reperfusion (R-30 and R-60) -using the parallel time periods in Control group- hemodynamical, microcirculatory measurements were carried out and blood samples were taken for laboratory investigations.

For laboratory tests each time both arterial and venous blood samples were collected (0.6 ml per each time) from the cannulated right common carotid artery and via puncturing the caudal caval vein, using a 26 G needle distally from the site of the microvascular clip application (anticoagulant: 1.5 mg/ml K_3-EDTA). After the last blood sampling biopsies were taken from the liver, the kidneys and from a jejunum segment for later histological examinations. In the end of the experiment period, the animals were euthanized.

2.3. Hemodynamic and microcirculatory investigations

Through the cannulated right common carotid artery heart rate (HR [1/min]) and mean arterial pressure (MAP [mmHg]) values were recorded by a circulatory monitoring hardware-software system (Haemosys configuration, Experimetria Ltd., Hungary). For this system, a LD-01 laser-Doppler tissue flowmetry monitoring device was attached (Experimetria Ltd., Hungary), determining microcirculatory blood flux units (BFU), which were registered for 20 sec after the stabilization of the signal. We used a standard pencil probe (MNP100XP, Oxford Optronix Ltd., UK), which was placed on the anterior surface of the liver, on the surface of the right kidney and on the antimesenteric surface of the jejunum just prior to each blood samplings. The HR, MAP and LD data were analyzed offline, using the average values of the 20-sec recorded, stable periods.

Rectal temperature was also recorded by a SEN-06-RTH1 stick temperature probe (Experimetria Ltd., Hungary).

2.4. Laboratory investigations

For testing *hematological parameters*, a Sysmex F-800 microcell counter (TOA Medical Electronics Co., Ltd., Japan) was used. The tests require approximately 70 μl of blood. In this study white blood cell count (WBC [$\times 10^3/\mu$l]), red blood cell count (RBC [$\times 10^6/\mu$l]), hematocrit (Hct [%]) and platelet count (Plt [$\times 10^3/\mu$l]) were analyzed.

An ABL555 blood gas analyzer automate (Radiometer Copenhagen, Denmark) was used to determine *blood pH and lactate concentration* [mmol/l].

Determining *red blood cell deformability* parameters, a LoRRca MaxSis Osmoscan device (Mechatronics BV, The Netherlands) was used to measure red blood cell elongation index in the function of shear stress and osmotic gradient ektacytometry parameters.

For *regular red blood cell deformability tests* 5 μl blood sample was gently mixed in 1 ml of isotonic polyvinyl-pyrrolidone solution (360 kDa PVP in normal phosphate buffered saline; viscosity = 27 mPa.s, osmolarity = 290–300 mOsm/kg; pH ∼ 7.3). The suspension was injected into the bob-cup system of the device without air bubbles. The device generates shear stress (SS) range from 0.3 to 30 Pa, while the laser diffraction pattern is being analyzed, calculating elongation index (EI) values: EI = (L − W)/(L + W), where L is the length and W is the width of the diffractogram. EI increases with red blood cell deformability [4, 15]. The tests were carried out at constant temperature of 37°C. For data reduction and comparison, EI values at 3 Pa as well as calculated maximal elongation index at infinitive shear stress (EI_{max}) and the shear stress values at half of it ($SS_{1/2}$ [Pa]) were used, according to the Lineweaver-Burk analyses: $1/EI = SS_{1!2}/EI_{max} \times 1/SS + 1/EI_{max}$ [5]. Furthermore, ratio of $SS_{1/2}$ and EI_{max} were also compared ($SS_{1/2}/EI_{max}$), as suggested by Baskurt and Meiselman [6].

For the *osmotic gradient ektacytometry (osmoscan) measurements* 250 μl blood was gently mixed in 5 ml iso-osmolar PVP solution. During ektacytometry measurements a constant shear stress of 30 Pa was used, while the osmolarity of the sample continuously changed when the device was aspirating 0 or 500 mOsmol/kg PVP solutions into the measurement chamber, and so the EI values were continuously registered in the function of osmolarity [15]. Also based on initial experiences [26, 40] in this study we analyzed the maximal elongation index values at the peak of the EI-osmolarity curve, the osmolarity at this maximal EI ('optimal' osmolarity).

A Myrenne MA-1 erythrocyte aggregometer (Myrenne GmbH, Germany) was used for measuring *red blood cell aggregation*. The measurements require approximately 20 μl of blood for determining aggregation index values M (shear rate: 0 s^{-1}) and M1 (shear rate: 3 s^{-1}) 5 or 10 seconds after disaggregation. The M 5 s, M1 5 s, M 10 s, and M1 10 s index values increase with enhanced red blood cell aggregation [4, 7, 15].

2.5. Statistical analysis

Data are presented as mean ± standard deviation (S.D.). Student *t*-test or Mann-Whitney RS test were used for inter-group comparison and one-way ANOVA tests (Dunn's or Bonferroni's method) for intra-group comparison, depending on the data distribution. At time point of 'R-60' statistical tests were not performed, because of the decreased case number (lethal events) in the SR AXC group.

A p value less than 0.05 was considered as statistically significant.

3. Results

3.1. Hemodynamic parameters

The heart rate (HR [1/min]) showed moderate decrease over the experimental period in all groups. However, after an initial lowering by the end of the ischemia, markedly in Control group, the SR AXC group expressed gradual decrease (at R-30: p) (Fig. 1A).

In parallel, the mean arterial pressure (MAP [mmHg]) continuously decreased in the experimental period in all group, by the largest manner in the SR AXC group, where the values fell by the end of

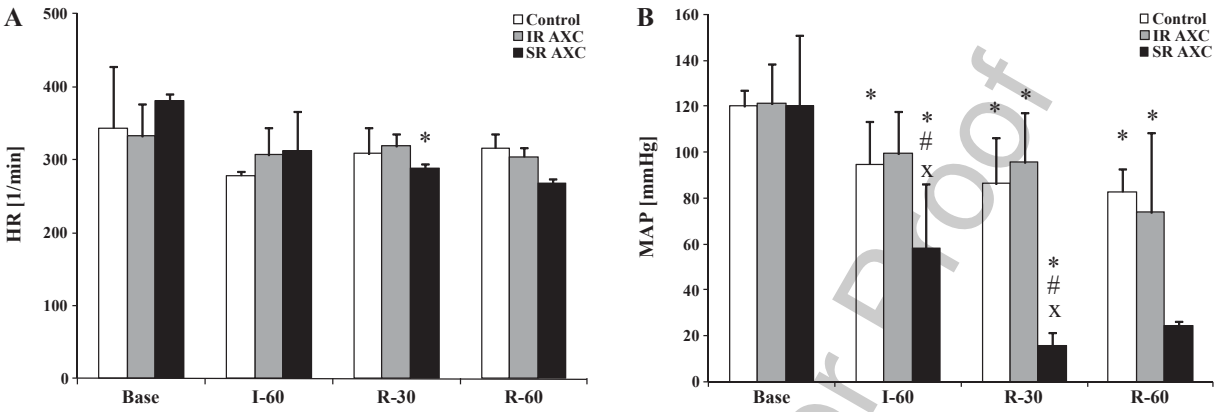

Fig. 1. Changes of heart rate (HR [1/min]) (A) and mean arterial pressure (MAP [mmHg]) (B) in the Control, the Infrarenal Aortic Cross-Clamping (IR AXC) and the Infrarenal Aortic Cross-Clamping (IR AXC) groups. means ± S.D.; Base = before ischemia; I-60 = the end of the 60-minute ischemia; R-30 = the 30th minute of the reperfusion; R-60 = the 60th minute of the reperfusion. *$p < 0.05$ vs. Base; # vs. Control; X vs. IR AXC.

ischemia ($p < 0.001$ vs. Base, $p = 0.001$ vs. Control and $p < 0.001$ vs. IR AXC) and showed further drop in the reperfusion period (at R-30: $p < 0.001$ vs. Control and IR SXC) (Fig. 1B). In this group these changes led to three lethal events before the R-30 measurement point, and further two until the end of the experimental period. In IR AXC group one animal died by the R-60 point.

3.2. Microcirculatory investigations

Interestingly the changes of blood flux units (BFU) did not show such large differences, except for certain territories. On the liver surface BFU mildly decreased by the end of the ischemic period, showing significant difference versus the base values both in IR AXC and SR AXC groups ($p < 0.001$ and $p = 0.001$, respectively). During the reperfusion the values were close to the base, except for the R-60 data, where BFU were lower compared to the Control, mostly in the survivor animals of the SR AXC group (Fig. 2A).

On the bowel surface BFU values decreased during the ischemic period in both aortic cross-clamping groups (in IR AXC group $p < 0.001$ vs. its base values), which was followed by the relative increase over the reperfusion period. At the 30th minutes of the reperfusion BFU values were higher compared to the Control values, too (in IR AXC group: $p < 0.001$; in SR AXC group: $p = 0.006$), and at the 60th minutes microcirculatory blood flux units resulted in the highest values in the SR AXC group ($p = 0.013$ vs. its base, $p = 0.001$ vs. Control) (Fig. 2B).

As expected, the kidney microcirculatory BFU values obviously differed between infra- and suprarenal cross-clamping groups. In SR AXC group definitely low values were detected by the end of the ischemia ($p < 0.001$ vs. base values, as well as compared to the Control and IR AXC groups). During reperfusion, the values dropped behind the IR AXC group. as well as during the observed reperfusion period in SR AXC group (Fig. 2C).

In parallel with the microcirculatory measurements the body temperature were also monitored, which moderately decreased over the experimental period in all groups. However, in SR AXC group the decrease in body temperature were in a bigger magnitude (Fig. 2D).

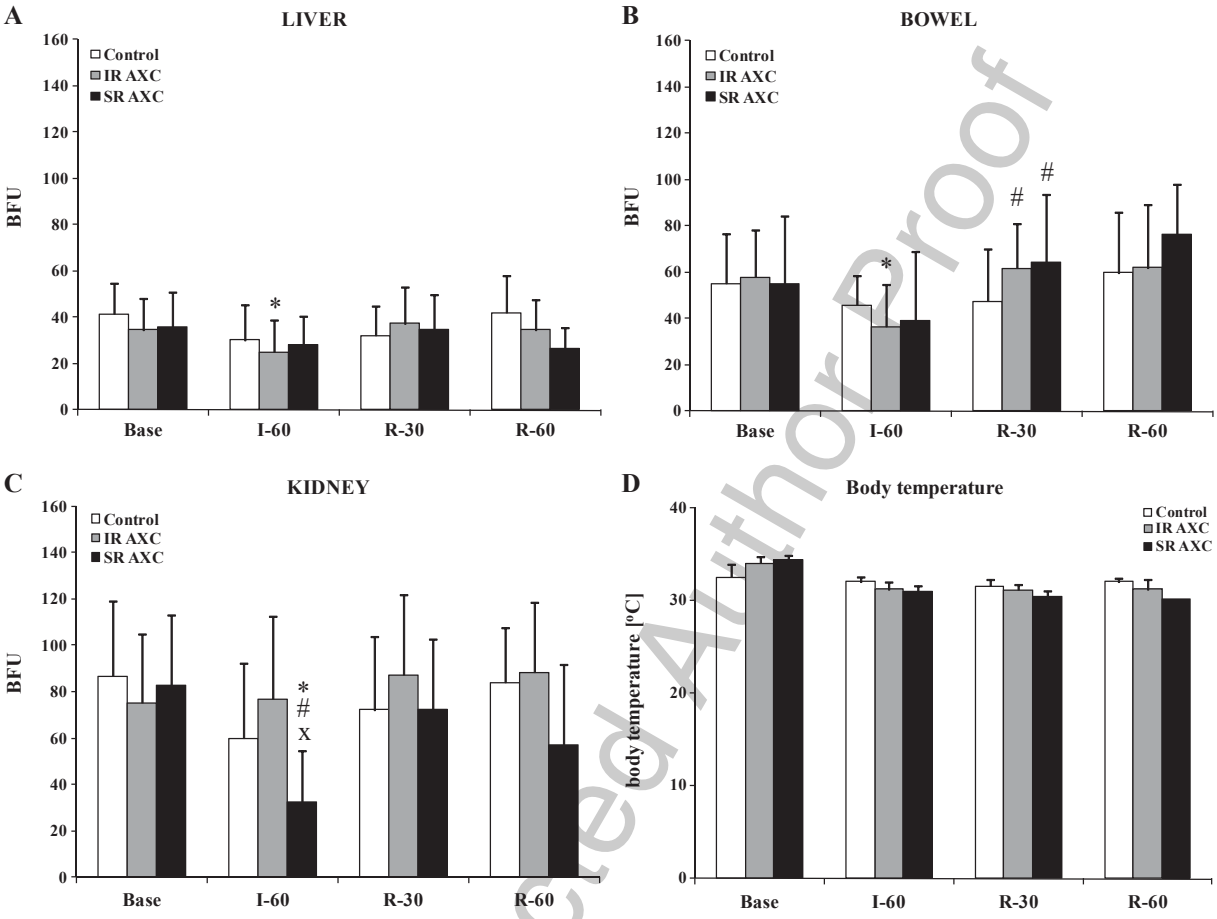

Fig. 2. Changes of blood flux units (BFU) measured on the surface of the liver (A), small bowel (B) and the right kidney (C) and alterations in body temperature (°C) (D) in the Control, the Infrarenal Aortic Cross-Clamping (IR AXC) and the Infrarenal Aortic Cross-Clamping (IR AXC) groups. means ± S.D.; Base = before ischemia; I-60 = the end of the 60-minute ischemia; R-30 = the 30th minute of the reperfusion; R-60 = the 60th minute of the reperfusion. *$p < 0.05$ vs. Base; # vs. Control; X vs. IR AXC.

3.3. Hematological parameters

White blood cell count showed only moderate and minimal increase during the reperfusion period in the IR AXC group, and decreased in SR AXC group both in arterial and venous blood samples, without significant differences. However, the survivor animals had low leukocyte count values at R-60 point (SR AXC base values: artery: $8.28 \pm 2.27 \times 10^3/\mu l$; vein: $8.66 \pm 1.16 \times 10^3/\mu l$; values at R-60: artery: $3.2 \pm 0.14 \times 10^3/\mu l$; vein: $3.8 \pm 0.01 \times 10^3/\mu l$).

After an initial increase in red blood cell count and hematocrit, a slight decrease was observed in IR AXC group, while in the SR AXC group the lowest hematocrit values were measured over the experimental period. Important difference was found only at the end of the ischemic period, where venous hematocrit values of SR AXC group ($34.67 \pm 7.32\%$) significantly differed from the base values ($48.05 \pm 3.89\%$, $p = 0.009$) as well as from the I-60 values of the IR AXC group ($49.3 \pm 4.58\%$, $p < 0.001$).

Platelet count of Control group did not show important changes. In IR AXC group it was continuously higher over the reperfusion period, while SR AXC group expressed a decreasing tendency. Significant difference was not found, however, similarly to the leukocyte and red blood cell count, survivor animals of the SR AXC group showed relatively lower platelet count (artery: $679 \pm 19.8 \times 10^3/\mu l$; vein: $501.5 \pm 21.9 \times 10^3/\mu l$) compared to their base values (artery: $1088.4 \pm 364.5 \times 10^3/\mu l$; vein: $1117 \pm 290.9 \times 10^3/\mu l$), versus the Control group (R-60 artery: $853.7 \pm 76.8 \times 10^3/\mu l$; vein: $768.5 \pm 113.4 \times 10^3/\mu l$) or the IR AXC group (R-60 artery: $970.2 \pm 52.3 \times 10^3/\mu l$; vein: $1054.1 \pm 274.9 \times 10^3/\mu l$).

3.4. Blood pH and lactate concentration

The pH values decreased in the reperfusion period in both aortic cross-clamping groups, being the mostly expressed in SR AXC group. At the end of the ischemia the differences were found to be significant compared to the base values (artery: $p = 0.003$; vein: $p < 0.001$), to the Control group (vein: $p < 0.001$), as well as versus the IR AXC group (artery: $p = 0.008$; vein: $p < 0.001$). Arterio-venous difference were also found at I-60 in SR AXC group ($p = 0.008$). At the 30th minute of the reperfusion these alterations were more intense, showing further significant differences versus base (artery: $p < 0.001$; vein: $p < 0.001$), Control (artery: $p = 0.01$; vein: $p < 0.001$) and IR AXC groups (artery: $p = 0.02$; vein: $p < 0.001$). The direction of the changes were similar both in arterial and venous blood samples, however, the values were the lowest in the venous blood (Fig. 3A, B).

In parallel, blood lactate concentration [mmol/l] markedly increased during the reperfusion after releasing the clamps, showing the highest values in the survivor animals of the SR AXC group. At the end of the ischemia lactate concentration of SR AXC group significantly rode versus base values (both in artery and vein: $p < 0.001$), Control (both in artery and vein: $p < 0.001$) and IR AXC group (both in artery and vein: $p < 0.001$). Arterio-venous difference was also found to be significant, the rise in lactate concentration was the highest in venous samples ($p = 0.003$). At the 30th minute of the reperfusion a stepwise increase was observed, which was significant versus base (both in artery and vein: $p < 0.001$), Control (both in artery and vein: $p < 0.001$) and IR AXC group (both in artery and vein: $p < 0.001$), as well as compared to the I-60 values within the group (artery: $p = 0.004$; in vein almost significant: $p = 0.06$) (Fig. 3C, D).

3.5. Red blood cell deformability (regular and osmotic gradient ektacytometry)

Elongation index values at a shear stress of 3 Pa decreased by the end of the 60-minute ischemia in the SR ACX group, both in arterial and venous blood samples (Fig. 4). The differences were significant versus base (in artery: $p = 0.024$) and Control values (in artery: $p = 0.048$, in vein almost significant: $p = 0.059$) reach the significant level. By the 30th minute of the reperfusion, EI values slightly increased (in artery: Control vs. IR AXC $p = 0.005$; Control vs. SR AXC $p = 0.007$), but the calculated EI_{max} lowered both in infrarenal and suprarenal cross-clamping groups. The $SS_{1/2}$ values of IR AXC and SR AXC groups were moderately increased by the end of ischemia, but during the reperfusion these values rather decreased compared to the Control group.

Using the $SS_{1/2}$ / EI_{max} ratio, the same tendency was observed, expressing more obvious differences at the I-60 measurement point, mostly in venous blood samples of SR AXC group (Fig. 5). The $SS_{1/2}$ / EI_{max} values increased in SR AXC group ($p = 0.018$ versus base both in arterial and venous blood samples), than showed a marked decrease by the 30th minutes of the reperfusion (in arterial blood: $p = 0.019$ vs. base and $p = 0.02$ vs. Control; in venous blood: $p = 0.012$ vs. I-60 values, $p = 0.024$ vs. Control).

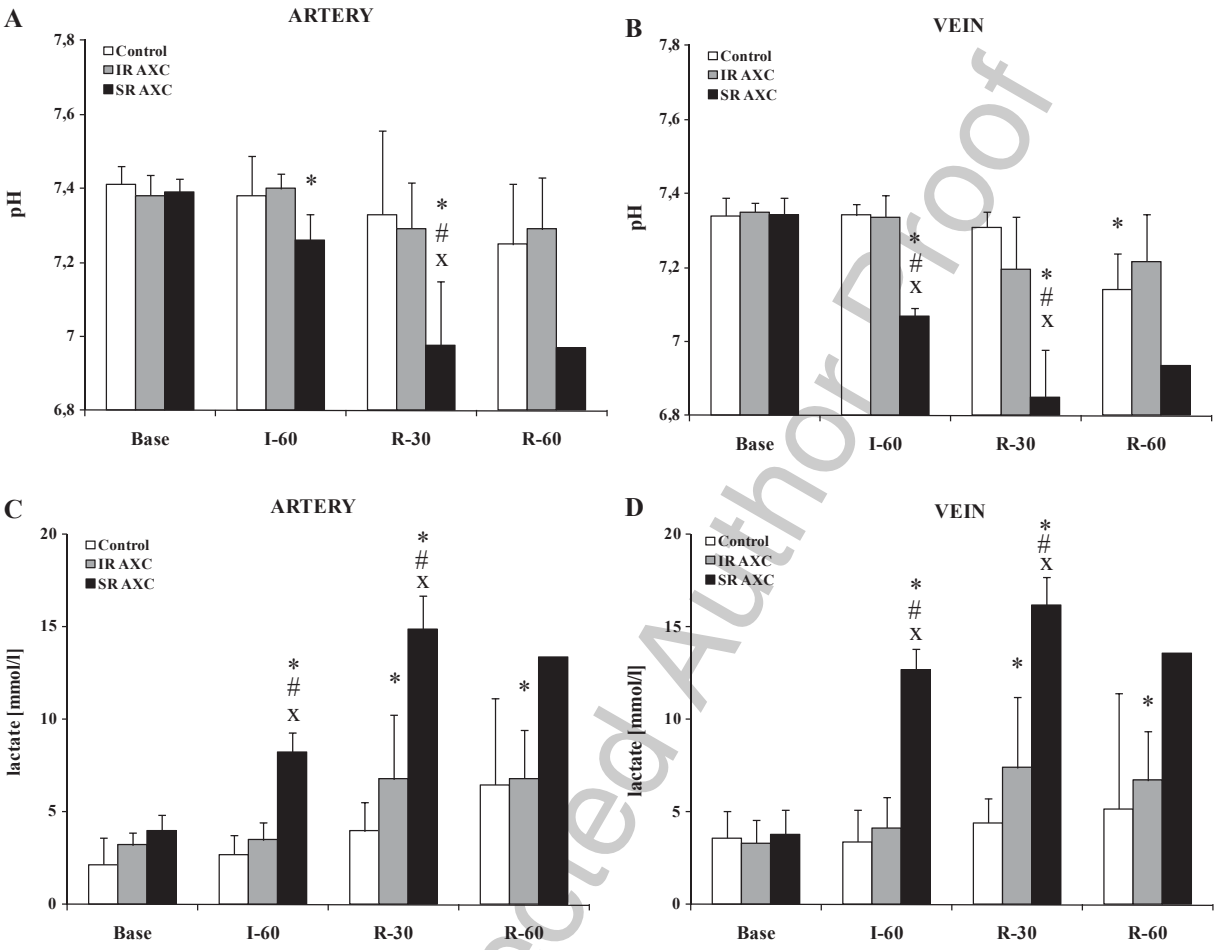

Fig. 3. Changes of blood pH in arterial (A) and venous (B) blood samples and the alterations in lactate concentration [mmol/l] in arterial (C) and venous (D) blood samples of the Control, the Infrarenal Aortic Cross-Clamping (IR AXC) and the Infrarenal Aortic Cross-Clamping (IR AXC) groups. means ± S.D.; Base = before ischemia; I-60 = the end of the 60-minute ischemia; R-30 = the 30th minute of the reperfusion; R-60 = the 60th minute of the reperfusion. *$p < 0.05$ vs. Base; # vs. Control; X vs. IR AXC.

Investigating the osmotic gradient ektacytometry (osmoscan) parameters, we found that the maximal measurable elongation index at 30 Pa showed only moderate decrease by the end of the ischemic period in arterial blood samples of both aortic cross-clamping groups, while in venous blood the decrease was well observable dominantly in SR AXC group over the reperfusion period. The osmolarity values at maximal elongation index after a minimal decrease by the end of ischemia showed differences only by the 60th minutes of the reperfusion. In venous blood samples this stepwise difference was visible from the 30th minutes of the reperfusion. However, these differences did not reach the significance level (Table 1).

3.6. Red blood cell aggregation

Aggregation index values showed colorful but contradictory results (Table 2). In general, Control group presented relatively stable M and M1 values at 5 seconds, while at 10 seconds it showed moderate

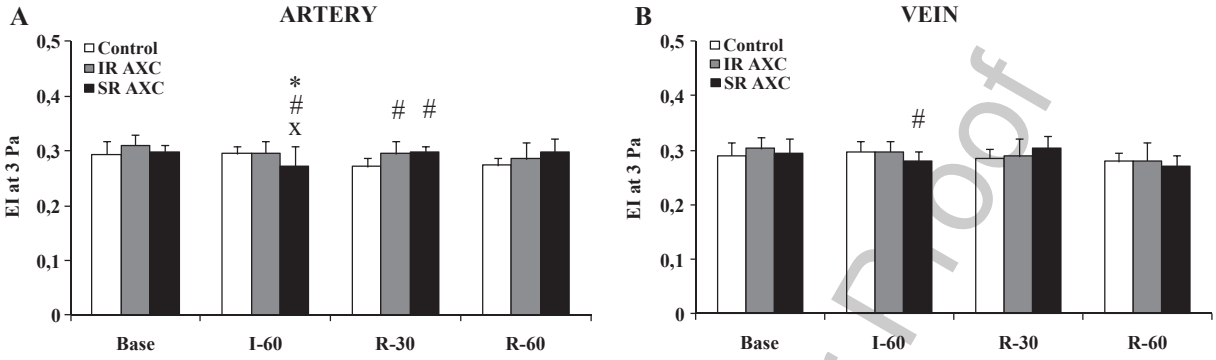

Fig. 4. Changes of elongation index (EI) measured at shear stress of 3 Pa in arterial (A) and venous (B) blood samples of the Control, the Infrarenal Aortic Cross-Clamping (IR AXC) and the Infrarenal Aortic Cross-Clamping (IR AXC) groups. means ± S.D.; Base = before ischemia; I-60 = the end of the 60-minute ischemia; R-30 = the 30th minute of the reperfusion; R-60 = the 60th minute of the reperfusion. *$p < 0.05$ vs. Base; # vs. Control; X vs. IR AXC.

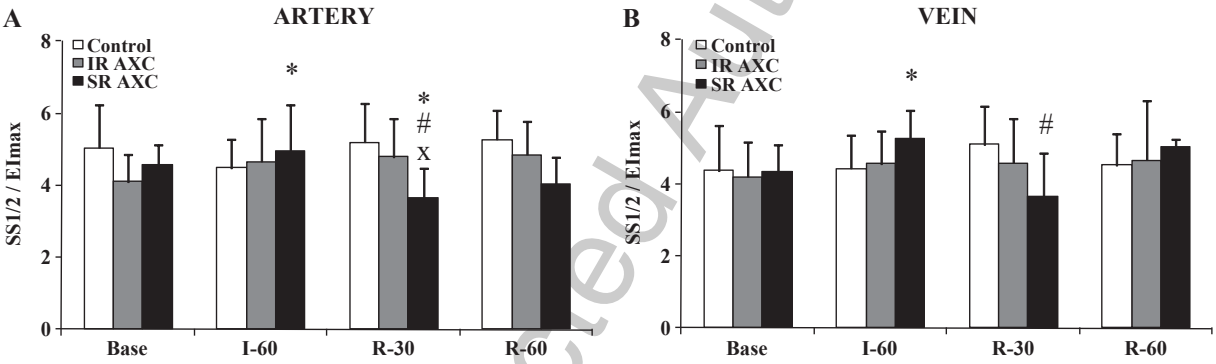

Fig. 5. Alterations in the ratio of shear stress at half maximal elongation index ($SS_{1/2}$ [Pa]) and maximal elongation index (EI_{max}) in arterial (A) and venous (B) blood samples of the Control, the Infrarenal Aortic Cross-Clamping (IR AXC) and the Infrarenal Aortic Cross-Clamping (IR AXC) groups. means ± S.D.; Base = before ischemia; I-60 = the end of the 60-minute ischemia; R-30 = the 30th minute of the reperfusion; R-60 = the 60th minute of the reperfusion * $p < 0.05$ vs. Base; # vs. Control; *X* vs. IR AXC.

fluctuation and resulted in very low or even immeasurable aggregation index (M1 at 10 sec). The high deviation of data and often the presence of zero values were experienced in all groups, thus informative and statistically significant differences could not be found.

What was generally observable: in IR AXC group M values at 5 sec showed moderate decrease till the end of reperfusion period, while M1 values were mildly elevated at the 60th minutes of the ischemia in venous, and at the 60th minutes of the reperfusion in arterial blood samples. The SR AXC group showed increased values of M 5 sec by the 30th minutes of the reperfusion in venous, and at the 60th minutes of the reperfusion in arterial blood samples. The tendency was similar in case of M1 values.

Aggregation index M at 10 sec showed very low values by the end of the ischemia in the SR AXC group compared to the Control (artery: $p = 0.048$; vein: $p = 0.022$) and IR AXC groups (artery: $p = 0.018$; vein: n.s.), and increased in the reperfusion period both in arterial and venous samples (at R-30 in venous blood: $p = 0.002$ vs. base and $p = 0.003$ vs. IR AXC). Unfortunately, in case of M1 values at 10 sec we could not get informative results because many samples showed zero (0.0) values.

Table 1
Changes of selected osmoscan variables in arterial (A) and venous (V) blood samples of Control, Infrarenal- (IR AXC) and Suprarenal Aortic Cross-Clamping (SR AXC) groups

Variable	Group	Sample-type	Base	I-60	R-30	R-60
Maximal EI	Control	A	0.480 ± 0.011	0.472 ± 0.023	0.462 ± 0.016	0.473 ± 0.022
		V	*0.475 ± 0.024*	*0.493 ± 0.014*	*0.479 ± 0.017*	*0.459 ± 0.014*
	IR AXC	A	0.471 ± 0.02	0.472 ± 0.019	0.466 ± 0.016	0.465 ± 0.022
		V	*0.474 ± 0.016*	*0.477 ± 0.022*	*0.465 ± 0.02*	*0.467 ± 0.011*
	SR AXC	A	0.452 ± 0.028	0.467 ± 0.021	0.481 ± 0.02	–
		V	*0.457 ± 0.027*	*0.447 ± 0.032*	*0.399 ± 0.129*	–
Osmolarity at maximal EI [mOsm/kg]	Control	A	327.2 ± 16.3	324.4 ± 8.8	326.5 ± 9.4	306.3 ± 7.6
		V	*328.2 ± 10.1*	*319.6 ± 15.2*	*322.2 ± 3.1*	*320 ± 14.1*
	IR AXC	A	349.8 ± 12.3	343.4 ± 18.6	336.3 ± 22.2	341.3 ± 37.9
		V	*361.8 ± 15.8*	*339.8 ± 18.8*	*345.8 ± 12.7*	*340 ± 24.7*
	SR AXC	A	347 ± 34.1	323 ± 7.1	332 ± 5.7	–
		V	*344.7 ± 29.3*	*332 ± 16.9*	*358 ± 11.2*	–

means±S.D.; A = artery, *V* = *vein*. Base = before ischemia; I-60 = the end of the 60-minute ischemia; R-30 = the 30th minute of the reperfusion; R-60 = the 60th minute of the reperfusion. $*p < 0.05$ vs. Base; $^{\#}$ vs. Control; ^{X} vs. IR AXC.

4. Discussion

Depending on the level of the vascular disease, malformation injury, the temporary clamping of the abdominal aorta can be performed at various sites during the vascular surgical procedures. According to the necessity, aortic clamping can be positioned on the infrarenal part, on suprarenal position or even at supracoeliac level [8, 23, 27, 52, 57]. Obviously, all, but mostly the suprarenal cross-clampings mean bigger surgical challenges and increased risk for intra- and post-operative complications, including organ failure of ischemically injured territories or even in remote organs [1, 14, 16, 23, 27, 29, 37, 50].

Suprarenal clamping of the aorta can be necessary in several cases in vascular surgery. Obviously the clamping time is a key factor dominantly in relation with the renal function. Wahlberg et al. reported clinical comparative analysis of elective operations of infrarenal vascular disease in which they conclude that suprarenal aortic clamping less than 50 minutes can be still well tolerable, however the risk for transient renal dysfunction is ten-fold higher when the clamping time was greater than 50 minutes, compared to the situation with the clamping time of 30 minutes or less [56].

Chong et al. also reported in their clinical comparison with high case number, how the outcome is related with the position of the aortic cross-clamping. In this comparison infrarenal and suprarenal clampings with or without renal revascularization procedures were analyzed [8].

There are very useful methods to reduce the risk of renal dysfunction after suprarenal clamping of the aorta. Pichlmaier et al. reported a venous renal perfusion during the suprarenal clamping [45]. Renal perfusion via the venous system provides good opportunity even for local hypothermia, for which experimental and clinical data are also available [34].

In the literature, describing animal models, wide range of aortic clamping time can be found. Haithcock et al. in porcine model investigated 60 versus 30 minutes of supracoeliac aortic cross-clamping. They found that coagulation time parameters (prothrombin time, partial thromboplastine time) and platelet count did not show significant difference, however, tissue plasminogen activator increased mostly

Table 2
Changes of aggregation index values in arterial (A) and venous (V) blood samples of Control, Infrarenal- (IR AXC) and Suprarenal Aortic Cross-Clamping (SR AXC) groups

Variable	Group	Sample-type	Base	I-60	R-30	R-60
M 5 s	Control	A	0.92 ± 0.49	1.34 ± 0.85	0.75 ± 0.5	1.07 ± 0.26
		V	*0.85 ± 0.86*	*0.96 ± 0.62*	*1.37 ± 1.5*	*0.66 ± 0.28*
	IR AXC	A	0.47 ± 0.34	0.76 ± 0.51	0.63 ± 0.23	0.75 ± 0.34
		V	*0.41 ± 0.23*	*0.85 ± 0.44*	*0.64 ± 0.34*	*0.4 ± 0.15*
	SR AXC	A	0.5 ± 0.31	0.56 ± 0.26	0.75 ± 0.34	1.12 ± 0.29
		V	*0.54 ± 0.19*	*0.54 ± 0.16*	*1.27 ± 0.48*	*0.6 ± 0.14*
M1 5 s	Control	A	1.01 ± 1.36	1.14 ± 0.65	0.82 ± 0.29	0.6 ± 0.1
		V	*1.22 ± 1.08*	*1.27 ± 0.92*	*1.45 ± 1.21*	*0.56 ± 0.46*
	IR AXC	A	1.51 ± 0.89	0.68 ± 0.5	0.57 ± 0.27	1.13 ± 1.85
		V	*0.84 ± 0.67*	*1.48 ± 1.21*	*0.5 ± 0.31*	*0.76 ± 0.71*
	SR AXC	A	1.16 ± 1.24	0.41 ± 0.09	0.72 ± 0.26	0.87 ± 0.22
		V	*1.03 ± 0.56*	*0.44 ± 0.13*	*1.22 ± 0.56*	*0.2 ± 0.1*
M 10 s	Control	A	1.82 ± 0.64	3.36 ± 2.26	1.87 ± 1.2	3.35 ± 1.04
		V	*3.21 ± 1.74*	*3.9 ± 2.17*	*3.02 ± 2.67*	*0.76 ± 0.23*
	IR AXC	A	1.41 ± 0.94	2.37 ± 2.19	1.56 ± 1.06	1 ± 0.57
		V	*2.81 ± 1.89*	*2.7 ± 2.64*	*1.45 ± 0.61*	*1.2 ± 0.53*
	SR AXC	A	0.4 ± 0.56	$0.46 \pm 0.4^{\#X}$	2.8 ± 0.82	2.92 ± 0.61
		V	*1.37 ± 1.03*	1.32 ± 0.59^{X}	$3.42 \pm 1.64^{*X}$	*1.75 ± .031*
M1 10 s	Control	A	0.7 ± 0.98	3.37 ± 1.3	0.65 ± 0.91	0.75 ± 1.06
		V	*3.72 ± 2.29*	*3.61 ± 1.29*	*4.67 ± 0.75*	–
	IR AXC	A	3.21 ± 2.44	3.17 ± 0.86	1.67 ± 0.73	–
		V	*3.4 ± 1.99*	*4.1 ± 1.49*	*2.44 ± 0.93*	–
	SR AXC	A	0.82 ± 0.53	–	1.72 ± 0.55	1.35 ± 0.49
		V	*2.2 ± 1.13*	–	*1.87 ± 0.98*	–

means±S.D.; A = artery, *V* = *vein*. Base = before ischemia; I-60 = the end of the 60-minute ischemia; R-30 = the 30th minute of the reperfusion; R-60 = the 60th minute of the reperfusion. $*p < 0.05$ vs. Base; $^{\#}$ vs. Control; ^{X} vs. IR AXC.

after the 60-minute cross-clamping. They also concluded that 30 and 60 minutes of supracoeliac aortic cross-clampings may result in the similar magnitude of fibrinogen depletion and degree of intravascular thrombotic events [14].

Yeung et al. used a rat model of 45-minute suprarenal aortic clamping, in which study they used a group with additional clamping of the infrarenal part for 20 minutes. The additional infrarenal aborting clamping caused more expressed renal damage and oxidative stress, supposedly due to the increased renal perfusion and arterial pressure [62].

Anagnostopulos et al. in their porcine model also studied the hemostatic consequences of aortic cross-clamping at supracoeliac level. They used 30 minutes clamping time. Blood samples were taken before clamping, just before unclamping and in the 5th, 30th and 60th minutes of the reperfusion period. The platelet count decreased in suprarenal clamping group by the 30 minutes of the reperfusion, accompanied by gradually decreasing of fibrinogen concentration and with initial rise in thrombin-antithrombin complex and tissue plasminogen activator [1].

Wu et al. used 30-minute supracoeliac aortic cross-clamping in rats and investigated hemodynamic and metabolic parameters. They observed decrease in pH shortly after unclamping which was significantly lower compared to the base-line over 180 minutes of reperfusion, while the lactate concentration increased significantly. The lactate concentration was more expressed in portal venous blood samples. The mean arterial pressure continuously decreased over the examined reperfusion period [61]. Our results show similar tendency in suprarenal clamping group.

Concerning the time of infrarenal cross-clamping, several further examples can be found in the literature using various animal models. In rats, Liang et al. used 30 minutes [30], Song et al. 45 minutes of infrarenal clamping in renal ischemia [53]. In rabbit model, Izumi et al. [17] and Watanabe et al. [59] used 15 minutes, Kakimoto et al. applied a 17-minute clamping [21], Huang et al. used 20 minutes [16], Kazanci et al. completed 25 minutes of infrarenal aortic occlusion [25] in their models.

It is well-known that ischemia and reperfusion may affect hemorheological and microcirculatory properties and parameters [3, 18, 28, 39, 41, 42, 55, 58, 60]. The magnitude of changes can be influenced by the ischemic time (e.g., clamping of the vessels in surgery or in surgical research models), the temperature (e.g., normothermia, hypothermia), the type of the affected tissue or organ (ischemic tolerance, extension of the endothelial injury) [3, 32, 39]. The mechanisms that cause altered blood rheology during and after ischemia and reperfusion includes free radical reactions, inflammatory processes, changes in acid-base parameters, in lactate concentration, in oxygenation and in micro-environmental osmolarity, presence of mechanical stress (magnitude and duration), hemoconcentration, altered fluid distribution, increased fibrinogen concentration (part of acute phase reaction), increased blood viscosity and its effect on endothelial function – all being combined in various manner and well-discussed in the literature [2, 3, 9–11, 18, 19, 22, 35, 36, 39, 42–44, 51, 55, 60].

In this study our main issue was trying to explore the magnitude of simultaneous changes, which were found to be different. At various time points when hemodynamic changes were prominent, microcirculatory or hemorheological parameters did not show such large differences. And in turn, not all the micro-rheological changes were detected together with deterioration of microcirculatory blood flux data. However, every parameter changed in various manners, showing more or less differences between infra- or suprarenal cross-clamped conditions.

The possible explanations of these alterations must include the consideration of limitations or technical properties of this model. First of all, the general stress caused by the anesthesia, immobilization and the surgical interventions (preparations, cannulations, laparotomy, blood samplings) cannot be neglected. Also, the additive blood sampling volume was significant during the entire experimental period. However, the same conditions and sampling protocol was applied in the Control group, too.

In our current model we faced contradictory results, mostly in the red blood cell aggregation data, compared to our other, previous ischemia-reperfusion studies [39]. In this model we used sodium-heparin systematically (∼100 U/kg), which was a difference versus our previous models. It has been demonstrated, that sodium-heparin may alter micro-rheological parameters [7, 38]. The other limitation of this model is the lack of intensive therapeutic controls and interventions. In the clinical practice the operations are under controlled anesthesia, including metabolic, acid-base and hemostaseological control, as well as intensive therapeutic interventions according to the necessity. These compensatory interventions are dominantly missing from the experimental models.

Other issue is the anatomy of the collaterals. Interestingly, Haacke et al. in their study reported that pigs' vascular system with providing sufficient collateral support may allow complete infrarenal aortic occlusion without serious humbling ischemia [13]. It is supposed, that it may be different in animal species, and thus determining and affecting the expected alterations during and after ischemia and reperfusion.

5. Conlcusions

Summarizing our findings, we can conclude that the magnitude of hemodynamic, microcirculatory, acid-base and hemorheological changes was not the same in this model. Although the largest deviations and changes were observable in suprarenal aortic cross-clamping group, the acid-base and hemodynamic alterations were much more expressed than the micro-rheological ones. It is known that ischemia and reperfusion result in composite inflammatory, free radical mediated processes, showing further alterations with the reperfusion time as well as during the early postoperative days [3, 22, 24, 36, 39]. It is also suggested that the acute, transient changes in hemodynamic parameters and microcirculatory conditions together with the deterioration of acid-base balance *in vivo* may have more important effects than the *ex vivo* detectable changes of micro-rheological parameters in the blood samples. The reversibility-irreversibility border of the changes in micro-rheological parameters as well as local/regional versus systemic alterations are still very interesting and important questions to be answered, also in relation of the red blood cells' morphological alterations along the stomatocyte-discocyte-echinocyte sequence [48].

Further investigations of *in vivo* relations-correlations of changes in hemodynamic, microcirculatory, metabolic and hemorheological factors need further studies providing simultaneous examinations and monitoring possibilities in various induced models.

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