THESES FOR DEGREE OF DOCTOR OF PHILOSOPHY (Ph.D.)

Analysis of Functional and Morphological Changes in Vessel Wall and Renal Parenchyma Following Temporary Clamping of the Renal Artery

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1. INTRODUCTION

Cessation of blood supply to the organs and tissues for any reason results in ischemia, which causes damage to tissue metabolism in a short time and the changes may become irreversible. Paradoxically, the restoration of blood flow launches a series of events which further engrave the condition of the organ or tissue in question. This is known as ischemia-reperfusion injury.

In clinical practice, renal ischemia-reperfusion injury can be an inevitable consequence of several diseases and surgical interventions. It may result from the restoration of circulation following general hypoperfusion in association with resuscitation because of cardiac arrest, and shock and local hypoperfusion in abdominal traumata. The injury may occur during any intervention in which the full lumen of the aorta has to be clamped off above the root of the renal artery.

Hot and cold ischemic and reperfusion injuries can also be expected in kidney transplantations. The chances of early and late ischemic reperfusion injury have to be considered in association with different renal resections (uniplanar or wedge-shaped resections, longitudinal nephrotomy), on removing renal tumor and in other urological interventions when the renal artery has to be clamped off temporarily for more or less time (e.g. traumatic injury and operation on the cavital system of the kidneys). It has been an established fact that the renal artery can be clamped off for maximum 30 minutes to avoid the irreversible damage of the kidneys. In certain types of surgery, longer clamping time is required which, in the absence of proper protection, makes the operations impossible.

In this type of operations it is not only the *renal parenchyma* that may suffer reversible or even irreversible damage depending on the duration of clamping off, but similar changes may develop in the wall of the *clamped artery*. Such changes have an unfavorable effect on the contraction-relaxation capacity of the renal artery.

In addition to the morphological and functional condition of the tissues, the rheological characteristics of the circulating blood may also play an important role in the various processes. Blood of changed rheology may have an effect on the whole of the body, even far away from the injured region. The reactions of free radicals, released mediators, local or systemic hemodynamic changes, change in the pH, rearrangement of fluid spaces and their pathological changes can all influence the hemorheological condition. Therefore, the investigation of hemorheological parameters can provide important and valuable information about the changes emerging during ischemia-reperfusion.

Several experimental models have been developed to reveal the pathomechanism of renal ischemia-reperfusion injury over the past decades. They have confirmed the causative role of free radicals. Simultaneously, there has been a continuous increase in possibilities, biological and chemical substances (natural and synthetic antioxidants, radical scavengers, inhibitors of different location of effect), which can be used in the prevention or decrease of injury.

One of them is allopurinol, which is the inhibitor of xanthine-oxidase playing a key-role in the formation of free radicals whose favorable effect on ischemia-reperfusion injury has been confirmed in several experiments. However, we have not found specific references in the literature on data related to clamped vessels, including the renal artery, in connection with ischemia-reperfusion changes emerging in the vascular wall due to temporary clamping, its possible prevention – after pretreatment or posttreatment with allopurinol.

Therefore we planned to develop a surgical model, in which ischemia-reperfusion injuries caused by the temporary clamping of the renal artery can be detected using different techniques and reduced by the use of relevant methods, not only in the renal tissue, but also in the vascular wall, at the site

of clamping, and in connection with the systemic changes in the whole of the body.

Our final goal was to suggest applicable procedures for use in clinical practice in the analysis of data obtained during the experiments. The application of such surgical techniques may improve surgical safety.

2. GOALS

1. Elaboration of a suitable model on the dog's renal artery to examine ischemic reperfusion after the temporary clamping of the full lumen of the renal artery.
2. Using various functional and morphological measurement techniques (in 1-week follow-up) in a vascular model, we wanted to justify the finding that it is not only the renal parenchyma but also the renal artery that get damaged due to changes, well-known from the literature, after the clamping and releasing of the renal artery.
3. Vascular reactivity investigations to prove the supposed protective effect of allopurinol, a xanthine-oxidase inhibitor, against injuries of the vascular wall possibly developing after the clamping of the renal artery.
4. Proving the allegedly protective effect of allopurinol not only on the kidney but also in association with the morphological changes of the renal artery during 45-minute ischemia and subsequent reperfusion.
5. Detection of systemic changes emerging after 45 minutes of ischemia and subsequent reperfusion, using hematological and hemorheological examinations and specifying antioxidant and endothelin levels.
6. In addition to the detection of the most commonly applied investigations into routine renal functions (serum urea and creatinine) in the above model, measurement of urinary N-acetyl-β-D-glucosaminidase activity, a technique which has not been used in the detection of ischemia-reperfusion injuries until now.

3. MATERIAL AND METHODS

3.1. Experimental animals, surgical technique, experimental groups

Experimental animals

The experiments were made on 82 mongrel dogs – with no regard to age and sex –, whose weight was 21±3.2 kg. The experiments were performed with due consideration of the ruling in Act XXVIII of 1998 and in the possession of permissions issued by the Committee of Animals Research at University of Debrecen (25/1996 ÁTEB, 15/2000 DEMÁB, 6/2001 DEMÁB).

Anesthesia

Anesthesia was induced by the intramuscular administration of SBH-Ketamin (10% ketaminum hydrochloricum, 10 mg/bwt) and Primazin (2% xilazinum hydrochloricum, 1 mg/bwt) in combination. With regard to vascular reactivity examinations, unlike in the usual protocol, we did not use Atropin in premedication, Lidocaine infiltration during the preparation of the renal hilus, or anticoagulant treatment since they may have influenced the results.

Surgical technique

At the beginning of the operation, in each animal, we prepared and cannulated the left external jugular vein through which physiological saline solution or allopurinol, dissolved in physiological saline solution, were administered. For the laboratory tests during the operation, we also drew blood via the cannula. Next, the left kidney was explored after upper-middle median laparotomy. To prepare the structures in the renal hilus, we used infiltration with physiological saline solution. The renal artery was prepared and a thread was introduced underneath the vessel. Next, using Blalock clamps, we clamped off the vessel softly.

The experiments were performed in two major series:

In the first series of experiments, we examined vessel reactivity changes in the renal artery in the 60-minute reperfusion process following ischemia for 45 minutes, in the following groups:

I. *Ischemia-reperfusion group (I/R, n=8)*:

The left renal artery was clamped. (In the case of a double artery, both branches were clamped.) In the meantime, the abdominal organs were covered with wet gauze wipes of body temperature. For 20 minutes prior to the clamping of the renal artery, the animals were infused with 200 ml of physiological saline solution via the cannulated external jugular vein. The left renal artery was excised for vascular reactivity tests following the 60 minutes of reperfusion after clamping had been lifted. The animals were exterminated by overnarcotization.

II. *Ischemia-reperfusion group pretreated with allopurinol (AP+I/R, n=8)*

For 20 minutes prior to the clamping of the left renal artery, the animals were given allopurinol in a dose of 100 mg/bwkg, via the cannulated external jugular vein. The allopurinol was dissolved in 200 ml physiological saline solution and, in the interest of the best possible dissolution, it was alkalinized with NaOH (eventual pH: 8.6). Sampling of the renal artery was performed after 60 minutes' of reperfusion after clamping was lifted and the experimental animals were exterminated.

III. *Sham-operated group (SH, n=6)*

For 20 minutes after the opening of the abdomen, the animals were only given physiological saline solution via the cannula introduced into the external jugular vein. After we had waited for 45 and 60 minutes (periods corresponding to clamping and reperfusion, respectively) we excised the left renal artery and exterminated the animals.

IV. The right renal arteries in the allopurinol-pretreated ischemia-reperfusion group served as allopurinol-treated controls (C+AP).

In the *second series of experiments*, we examined the consequences of ischemia-reperfusion with respect to the functional and structural damage to the kidney according to the protocol of the first series, after 7 days of follow-up:

I. *Ischemia-reperfusion group (I/R, n = 20):*

In this group, the treatment of animals with 200 ml physiological saline solution and clamping of the left renal artery were done according to the protocol in the first experiment. After the occlusion was stopped, we closed the abdominal wall.

II. *Allopurinol-pretreated ischemia-reperfusion group (AP+I/R, n=22):*

In this group, the animals were pretreated with allopurinol given in 200 ml infusion (100 mg/bwkg) and the clamping of the renal artery was done as in the first experiment. We closed the abdominal wall after lifting clamping.

III. *Sham-operated group (FO, n=18):* after the opening of the abdomen, the animals were given 200 ml physiological saline, as in the first experiment, and the abdominal wall was closed after 45 minutes.

After surgery, the animals were given pain killers (Demalgonil®), but they did not receive anticoagulant treatment.

Protocol of sampling

In the *first series of experiments*, sampling for vessel reactivity tests was done after the 60 minutes' reperfusion period, following the 45 minutes of clamping.

In all of the laboratory tests in the *second series,* blood was drawn to assess the suitability of experimental animals in the preoperative days. Blood then was drawn on the morning of the operation (basic), at the beginning of reperfusion (R0), in the 30^{th}, 60^{th} and 120^{th} minutes of reperfusion (R30, R60, R120) and on the 1^{st}, 2^{nd}, 3^{rd}, 5^{th} and 7^{th} postoperative days. Prior to the

operation, blood was drawn from the cephalic vein, during the operation from the prepared external jugular vein and after the operation from the cephalic vein again. Urine sampling was done via bladder catheterization. Histological samples were obtained on the 3rd and 7th postoperative days.

3.2. Functional and morphological examination of ischemia-reperfusion injuries in the vessel walls

3.2.1. Vascular reactivity examinations

The aim of our experiment was the detection of concentration-dependent relaxing effect of acetylcholine, adenosine and nitroglycerin on the ring segments of the renal artery *in vitro*.

Before the start of the examinations, the preparations were kept in oxygenated Krebs solution. The tunica adventitia was carefully removed from the surface of the excised renal artery. Ring segments of 2 mm were prepared from the artery, fixed with capillarity-free suture material in a double-wall tissue chamber arranged vertically, thermostatted at 37°C and containing Krebs solution.

The culture solution was oxygenated using a mixture of 95% O_2 and 5% CO_2, thus the pH of the solution became 7.4 on average. One of the threads was fixed to the steel hook, the other one was in touch with the sensor of the isometric mechanoelectric transducer.

The mechanical changes of vascular smooth muscle were registered by a polygraph. After adequate equilibration time, using noradrenaline, cumulative concentration effect curves were registered. After arterial precontraction induced by 1 μM noradrenalin, acetylcholin (muscarin agonist) was added to the Krebs solution in increasing concentration (10 nmol/1-100 umol/l), then, until equilibrium was reached, continuous washing was done. After that, P_1 purinergic receptor activating adenosine was added to the solution, whose concentration ranged between 1 umol/l and 1 mmol/l. When the next state of

equilibrium was achieved, we examined the concentration-dependent relaxing effect of nitroglycerin at a concentration of 1 pmol/l-10umol/l (exogenous NO donor). At the end of each pharmacological experiment, potassium chloride was added to the solution; dose-effect curves were recorded in the concentration range of 10 mmol/l and 120 mmol/l.

The concentration-effect curves were fitted by means of a least-square iterative algorithm to the following formula:

$$E = \frac{E_{\max}[A]^S}{[IC_{50}]^S + [A]^S}$$

in which E denotes the effect, E_{max} is effect maximum, [A] is the concentration of the agonist, IC_{50} is the concentration required to induce half-maximum response, S means the parameter of the steepness of the curve (Hill coefficient). The IC_{50} values are expressed as their negative base 10 logarithms ($pD_{2)}$.

3.2.2. Light microscopic histological investigation of the clamped renal artery

Samples for histological investigations were taken from the experimental animals on two occasions, on the 3rd and 7th postoperative days, including 4 animals in 1 examination period each.

The excised renal artery was fixed in formalin and embedded in paraffin, sections were made and stained with hematoxylin eosin (HE).

3.2.3. Apoptosis investigation of the clamped renal artery

Five-micrometre-thick sections were prepared from the samples of the renal artery taken on the 3rd and 7th postoperative days, after fixation in 10% formaldehyde and embedding in paraffin. Apoptotic cells were made visible using the Apoptag Plus Peroxidase in situ apoptosis detection kit (Biomarker

Ltd) and applying TUNEL technique. The investigation was made according to the instructions of the manufacturer.

During apoptosis, endonucleases activated in the nucleus can cause the break of one of the helices (nick) by breaking up the DNA matrix. TdT-mediated X-dUTP nick end labeling (TUNEL) technique means the labeling of the 3'OH end of the DNA molecule using digoxigenin-labeled nucleotides.

3.3. Investigation of functional and morphological changes following ischemia-reperfusion injury after the clamping of the renal artery, affecting the kidney

3.3.1. Routine renal function investigations

Serum creatinin and urea-nitrogen concentrations were determined from blood treated with anticoagulant sodium citrate. The measurements were made using colorimetric technique, in a Praxislab photometer (reagent: Fabio Kft, 470 nm) Serum urea and creatinin changes were compared to the values before the operation and given as relative values.

3.3.2. Urinary N-acetyl-β-D-glucosaminidase

Measurements were carried out using Horak's colorimetric technique modified by Pócsi et al, using VRGA-GlcNAc substrate (PPR Diagnostics, London, UK). Having stopped the enzymatic process, we measured the absorbance of the resulting colored product at 505 nm in a SPECOL-1000 spectrophotometer (Jena-Zeiss). NAGase activity was calculated on the basis of absorbance measured against the reagent blind value. To rule out enzyme release fluctuation at different times during the day we used the NAG index (NAG_i), i.e. the ratio of NAG activity and urinary creatinin.

3.3.3. Light microscopic histological investigation of renal parenchyma

Histological investigations in the different groups were performed on the 3rd and 7th postoperative days, usually including 4 animals in 1 period of time each.

Sampling was done during relaparotomy in general anesthesia, which was followed by the extermination of the animals via overnarcotization. The excised renal tissue was fixed in 10% formalin and embedded in paraffin. The sections were stained using hematoxylin-eosin (HE).

3.4. Investigation of systemic ischemia-reperfusion injury after the clamping of the renal artery

3.4.1. Measurement of hematological parameters

The investigations were made using K_3-EDTA anticoagulated blood, which was drawn by Sysmex F-800 hematological apparatus (TOA Medical Electronics Co., Ltd., Japan).

The device is capable of doing erythrocyte, leukocyte and thrombocyte counts, provide hemoglobin and hematocrit values, in addition to specifying MCV (mean corpuscular value), MCH (mean corpuscular hemoglobin), MCHC (mean corpuscular hemoglobin concentration), MPV (mean platelet volume) and the proportion of monocytes, granulocytes and lymphocytes expressed as percentages.

3.4. Hemorheological parameters

3.4.1.1. Measurement of erythrocyte deformability

Erythrocyte deformability was measured in a Carat FT-1 filtrometer (Carat Diagnostic Kft., Budapest), which was based on the working principle of St. George's Blood Filtrometer and developed by Dormándy et al. The Na-heparin anticoagulated blood was centrifuged for 10 minutes (2500 g) and the plasma and 'buffy coat' were removed. The cell suspension was washed twice in

a phosphate buffer solution. After the final centrifugation, the supernatant was removed, and the erythrocyte suspension was diluted to 5% using PBS. After that, it was flown through a polycarbonate filter with a mean pore diameter of 5 μm (Nuclepore® , Whatman Inc.) at a constant (negative) flow pressure (4 cm water). "Knowing" the filtration velocity of the liquid column and hematocrit of the erythrocyte suspension, the software can determine parameters such as the initial relative filtration rate (IRFR) and relative cell transit time (RCTT).

3.4.2.2. Whole blood and plasma viscosity

The tests were carried out in a Hungarian-developed Hevimet-40 capillary viscosimeter (Hemorex Kft., Budapest), which is also widely applied in clinical laboratories. The measurements were made from sodium-heparin anticoagulated blood. According to national and international conventions, whole blood viscosity values, measured at 90 s^{-1} rate-gradient, were compared. Since whole blood viscosity is greatly dependent on hematocrit, we have also given blood viscosity figures corrected for 40% hematocrit values using the mathematical formula suggested by Mátrai et al

$$TVV_{40\%}/PV = (TVV_{Htc}/PV)^{40\%/Htc}$$

in which $TVV_{40\%}$ stands for the viscosity of whole blood corrected for 40% hematocrit, TVV_{Htc} means the whole blood viscosity of the sample of a given hematocrit value; PV is the plasma viscosity of the sample and Htc represents the original hematocrit of the sample.

3.4.1.2. Fibrinogen concentration

Plasma fibrinogen concentration (Fbg, g/L) was determined from sodium citrate anticoagulated blood, using Clauss' principle, in a Sysmex CA-500 automatic coagulometer (TOA Medical Electronics Co., Ltd., Japan).

3.4.3. Serum antioxidant activity

Serum antioxiant activity was determined using the modified technique of Stocks et al. Serum added to bovine brain homogenizate inhibits lipid peroxidation taking place in a certain period of time, which we followed photometrically, applying thiobarbituric acid color reaction. The quantity of the resulting TBA-reactive aldehides (e.g. MDA or malondialdehide) is in reverse ratio with serum antioxidant capacity. In the control sample, the serum is substituted with distilled water and its auto-oxidation is taken as 100% compared to the initial MDA values (Abs. blind).

Residual auto-oxidation (RAO)=(Abs. sample-Abs. blind)/(Abs. control-Abs. blind)

Antioxidant activity (AOA)% = (1-MAO) x 100

The antioxidant activity values were given as percentages (relative antioxidant activity), compared to the preoperative values.

3.4.4. Plasma endothelin level

Endothelin was determined using the enzyme-linked immunoassay (ELISA, Biomedica) technique. Preparing the sample, we added 150 µl Heparin (Inj. Heparibene-Na 5000 IU/ml) and 150 µl GORDOX (Trasylol 10000 IU/ml) to 3 ml of plain blood. It was stored in ice until use and centrifuged for 10 minutes (2500 g).

An antigen-specific bond develops between the endothelin of the sample and the specific polyclonal antibody at the bottom of the microtitration plate. The quantity of the binding component can be visualized and photometrically measured using an enzyme (peroxidase)-conjugating second antibody and the relevant enzyme substrate (tetramethyl benzidine, TMB). Color reaction (measured at 450 and 620 nm in a Shimadzu Spectrophotometer) is in proportion with the endothelin quantity of the sample.

3.5. Statistical analysis

The results are provided as mean ± deviation (SD) or standard error of the mean (SEM). SigmaStat for Windows software (Jandel Scientific Co. 1992-1994. Erkhrath, Germany) was used to prepare the statistical analysis. During the variance analysis of the parameters (ANOVA), Dunnett's test and Kruskal-Wallis test were used to make comparisons within the groups and between the groups, respectively. In the vessel reactivity tests, the effects obtained for the different experimental groups were compared using the Newman-Keuls post hoc test. Statistically significant deviance was accepted at $p<0.05$.

4. RESULTS AND CONCLUSIONS

4.1. Results of functional and morphological investigations of ischaemia-reperfusion injuries following the clamping of the renal artery

4.1.1. Results of vascular reactivity investigations

	noradrenaline		acetylcholine		adenosine	nitroglycerin	
	E_{max} (mN/mm^2)	pD_2	E_{max} %	pD_2	-log(IC_{50})	E_{max} %	pD_2
I/R+AP	13,6±1,3	6,3±0,2	75±15	$7.4±0.1^{*}$	$4.2±0.1^{++}$	107±10	8.7±0.2
I/R	12,4±1,4	6,3±0,2	50±15	$5.8±0.2^{+}$	$3.7±0.1^{\#}$	83±7	8.4±0.2
C+AP	12,5±1,0	6,2±0,2	48±14	6.5±0.3	$3.5±0.1^{\#}$	99±11	$9.0±0.2^{+}$
FO	14,8±1,3	6,3±00,1	70±17	7.1±0.4	3.6±0.1	93±6	8.2±0.2

mean±S.D.; p<0,05: * vs I/R; + vs FO; p<0,01: ++ vs FO; # vs I/R+AP

Analyzing the noradrenaline concentration effect curves we found that, following ischemia-reperfusion, the α-adrenergic receptor-mediated responses had not been injured and there were no significant differences in the dose-effect curves among the individual groups. Allopurinol did not influence α-adrenergic receptor sensitivity in either normoxia or following ischemia-reperfusion.

Relaxation induced by acetylcholine is an important marker of the vessel's endothelial function. It is typical of the relaxation by acetylcholine

(muscarin receptor agonist) that acetylcholine sensitivity decreases significantly in the I/R group following clamping – compared to the sham-operated group, the decrease was almost two orders of magnitude (50±15; 70±17) – which, apparently, could be avoided by allopurinol pretreatment, since practically identical parameters were found in the AP+I/R group (75±17).

Compared to the intact control, adenosine receptor (P_1 purinoceptor activator) sensitivity did not change significantly after either clamping or following allopurinol treatment; surprisingly enough, however, allopurinol significantly increased P_1 purinoceptor sensitivity (3.6±0.1; 4.2±0.1). It could be regarded as an ischemia-specific phenomenon, i.e. allopurinol had this effect in only hypoxia but not normoxia.

In contrast with adenosine, allopurinol only had an influence on the NO-induced relaxation under normoxic conditions (4.2±0.1) in the case of relaxation induced by nitroglycerin (exogenous NO donor). There was a shift to the left in the dose-effect curve of the C+AP group, parallel to the rise in pD_2 values.

4.1.2. Results of the light microscopic histological investigation of the clamped renal artery

Examining the renal artery in the samples of the I/R group taken on the 3rd postoperative day, endothelial cells of different sizes, flattening of the lamina elastica and lamina interna, even their break at intervals, and subendothelial fibrosis – at some sites even necrosis – were detected which did not appear or appeared in a milder form in the group having received allopurinol treatment.

In the samples of the I/R group, taken on the 7th postoperative day, leukocyte margination and, plain muscle and endothelial cell proliferation were detected, while there were no changes worth mentioning in the AP+I/R group.

4.1.3. Results of the apoptosis investigations in the renal artery

The apoptosis of endothelial cells in the vessels of the tunica adventitia and of the plain muscle cells could be regarded pathological.

It was as early as the *3rd postoperative day*, that 1-2 apoptotic dead cells appeared in the tunica media, some vascular endothelial cells emerged in the tunica adventitia in the I/R group. The number of apoptotic cells grew slightly on the *7th postoperative day*. In the allopurinol-pretreated group, there were no apoptotic plain muscle cells or adventitial endothelial cells on the 3rd postoperative day, but they could be seen scattered on the 7th day. In the sham-operated group, apoptosis of the physiological range could be detected in the sections. There was a significant difference in the quantity of apoptotic cells per visual field between the I/R and AP+I/R groups on the 3rd postoperative day.

4.2. Functional and morphological changes in the kidney due to ischemia-reperfusion injury after clamping the renal artery

4.2.1. Results of routine renal function tests

Changes have been given as relative values compared to the preoperative ones.

Compared to the basic ones (155±68%), the serum creatinine values in the I/R group increased significantly in the 30th (193±90%) and 60th minutes of reperfusion, then they started to decrease and reached the initial level a week after the operation. In the AP+I/R group, except for the first day, these figures were always lower than in the I/R group and, practically speaking, they were identical to those of the sham-operated group. The serum creatinine level was significantly higher (149±64%) on the day after the operation. In the sham-operated group, slow, steady, not significant elevation could be observed until the first day after reperfusion and, after gradual decrease, they got back to the initial values one week after surgery.

After nonsignificant decrease noted in the early reperfusion period, compared to the initial value (150±66%), the *serum urea* concentration in the

I/R group rose significantly on the first postoperative day, but after that, decrease could be observed on the coming postoperative days, which was expressed on the 2nd, 5th and 7th days. In the AP+I/R group, the serum urea level remained near the basic level in the early reperfusion period and on the 1st postoperative day, but on the 2nd and 3rd postoperative days, significantly lower values were measured (65±11% and 61±13%). In the sham-operated group, continuously higher levels were measured from the start of reperfusion until the 2nd postoperative day, and, from the 3rd postoperative day on, decrease could be observed, which became significant on the 5th day, compared to the basic value (59±22%).

4.2.2. Changes of urinary N-acetyl-β-D-glucosaminidase activity

In the I/R group, significant NAG_i increase was experienced at the end of the ischemic period (8.13±1.87), which was at its highest in the second hour of reperfusion (11.19±1.56), then it started to go down and, on the 2nd postoperative day, it was near the basic value again (2.63±0.31), and on the 5th postoperative day it decreased to half of the initial level (1.11±0.02). Compared to the initial NAG_i, the change was significant ($p<0.05$) at the time the clamping of the renal artery was lifted, also in the first and second hour of reperfusion and on the first postoperative day.

A similar tendency was noted in the animals of AP+I/R, but the elevation was to a lesser degree and reached its maximum at the beginning of reperfusion (6.67±0.98). Compared to the basic value, a more significant change was observed at the beginning of reperfusion and in its first and second hours.

In the sham-operated group, compared to the basic value, no significant change could be seen at any time.

Compared to the sham-operated group, significantly higher NAG_i was found in the I/R group, at the start of reperfusion, in its first and second hours,

but in the group treated with allopurinol, similar observations were only made at the start of reperfusion. Comparing the figures in the I/R and AP+I/R groups, significantly higher values were measured in the I/R group in the first and second hours of reperfusion.

4.2.3. Histological changes in renal parenchyma

In the light microscopic investigations, no significant changes could be detected in the sham-operated or AP+I/R groups.

In the AP+I/R group, interstitial fibrosis was spotted and dilated cortical tubules containing tissue debris could be seen in the sections made on the 7^{th} postoperative day.

4.3. Systemic ischemia-reperfusion injury following the clamping of the renal artery

4.3.1. Changes in hematological parameters

4.3.2.1. Changes in erythrocyte deformability

Preoperative RCTT levels were nearly identical in the experimental groups, while in the I/R and AP+I/R groups they were significantly higher than the basic ones (7.51±1.75 and 8.51±1.12; $p=0.015$ and $p<0.001$) in the 30^{th} minute of reperfusion. Values returned close to the preoperative level in the first one and half hour of reperfusion and after continuous but not significant increase in the AP+I/R group on the 1^{st} – 3^{rd} postoperative days, they decreased to the level before the operation by the 7^{th} postoperative day. In the I/R group, maximum values were measured on the 2^{nd} postoperative day, after significant increase. The difference appeared to be significant on the first and second postoperative days (Day 1: 8.66±1.21; Day 2: 10.5±2.83) compared to the basic figures ($p=0.015$ and $p=0.011$), sham-operated group ($p=0.001$ and $p=0.002$), as well as the AP+I/R group ($p=0.019$ and $p<0.001$). In each group, similar values were obtained on the 3^{rd}-7^{th} postoperative days, except for a moderate, not

significant decrease in the sham-operated group on the 5th and 7th postoperative days.

At the start of reperfusion, compared to the basic figures, IRFR was significantly lower in the AP+I/R group (p=0.006), while in the 30th minute of reperfusion, the values in the I/R and AP+I/R groups were significantly lower than the basic ones (p<0.001 in both cases). On the first and second postoperative days, a small, not significant decrease of the IRFR values was observed, which was not the case with RCTT values.

4.3.2.2. *Viscosity changes in whole blood and plasma*

At 90 s^{-1} cutting tension, whole blood viscosity exhibited a mildly elevating tendency and it remained significantly higher in the I/R group on the first two postoperative days, compared to the AP+I/R group (7.21±1.13 mPas, p=0.033, and 7.17±1.33 mPas, p=0.036). In all of the groups, whole blood viscosity values decreased moderately, although not significantly on the 3rd-7th postoperative days, which was accompanied by a similar change in hematocrit values. Whole blood viscosity values corrected for 40% hematocrit and, also, plasma viscosity values rose on the 1st – 7th postoperative days.

4.3.2.3. *Changes in fibrinogen concentration*

Measuring fibrinogen concentration, we got the highest values on the 1st postoperative day in all of the three groups. In the case of the I/R group, it remained constantly and significantly higher on the 2nd-7th postoperative days (p<0.002), compared to the basic values.

4.3.3. Changes in serum antioxidant activity

Changes in serum antioxidant activity were given in comparison with the preoperative values, expressed as its percentage.

In the I/R group, antioxidant activity was significantly lower (69±27% vs 10%) at the 30th minute and 120th minute (65±20% vs 100%) of reperfusion than before surgery. Then, after transitory, not significant elevation, it came close to the original value by the 3rd postoperative day. In the 120th minute of reperfusion, compared to the AP+I/R group, the antioxidant activity was significantly lower (83±25% vs 65±20%).

In the case if the AP+I/R group, antioxidant activity in the 120th minute of reperfusion decreased to approximately 70% of the original level, but by the 5th postoperative day, it practically returned to the level measured before surgery. There was no significant difference compared to the values measured before the operation.

There was no significant change in the sham-operated group.

4.3.4. Changes in plasma endothelin levels

In the I/R group, the plasma endothelin level was significantly higher than in the sham-operated and AP+I/R groups at the beginning of reperfusion (0.88±0.23 vs 0.45±0.6 and vs 0.32±0.3). Compared to values obtained before the operation, significantly higher plasma endothelin levels were obtained in both ischemic groups on the 1st postoperative day (I/R: 1.98±1.24 vs 0.67±0.45; I/R+AP: 1.9±1.53 vs 0.50±0.32). In the I/R group it remained high until the end, while in the I/R+AP group it remained significantly higher compared to the basic value, except for the 5th postoperative day. On the 5th and 7th postoperative days, the plasma endothelin level of AP+I/R group was significantly lower than that of the I/R group (0.75±0.9 vs 1.47±0.48 and 1.25±0.38 vs 2.23±0.17). No significant change was experienced in the sham-operated group.

5. SUMMARY OF IMPORTANT RESULTS AND CONCLUSIONS

1. Elaborating the vascular model, we concluded that the administration of certain drugs may fake the results of vessel reactivity tests, therefore, differently from the usual protocol, we must not give anticoagulant treatment or use Atropin premedication. It is also contraindicated to use Lidocaine solution, despite the fact that this has been a clinically established technique in renal surgery.
2. Examining the consequences of unilateral clamping of the renal artery for 45 minutes and the subsequent reperfusion in mongrel dogs, we were the first to demonstrate that ischemia-reperfusion-related functional and morphological changes developed not only in the renal tissue but also in the clamped vessel.
3. This way, the contraction-relaxation capacity of the clamped vessel was injured: acetylcholine-induced relaxation – one of the important markers of endothelial function – was significantly weakened, similarly to adenosine-induced (endothelium-dependent) relaxation. Nitroglycerin-induced (endothelium-independent) relaxation did not actually change.
4. The morphological investigation of renal arteries could reveal minimal changes in sections prepared with hematoxylin-eosin staining. Apoptosis tests could detect expressed and only minimum apoptotic activities in the vascular wall on the 3^{rd} and 7^{th} postoperative days, respectively.
5. We could establish, that among the renal function tests, urinary N-acetyl-β-D-glucosaminidase level measurements – which we were the first to use to mark the ischemia-reperfusion injuries in the kidneys – appeared to be an early, sensitive and non-invasive technique in detecting renal damage and proving the protective effect of allopurinol.
6. Examining systemic changes, ischemia for 45 minutes and the subsequent reperfusion caused characteristic and significant changes in several hemorheological parameters. Erythrocyte deformability and fibrinogen

concentration were of predictive force. Some of the changes could be associated with the damage caused by free radicals, others were traced back to the surgical trauma resulting from the interventions.

7. Plasma antioxidant activity and plasma endothelin level changes among the other parameters for the detection of systemic effects were of indicative force in the detection of ischemia-reperfusion damage and, also, the justification of the protective effect of allopurinol.
8. Although xanthine-oxidase inhibiting allopurinol, given in a dose of 100 mg/bwt for systemic ischemia prophylaxis, could not completely prevent the harmful effects of 45-minute ischemia and the subsequent reperfusion – which also refers to the role of factors other than free radicals – , but could significantly decrease them in both vascular reactivity and renal function, let alone the whole of the body. We were the first to call attention to the fact that the use of allopurinol could become a useful technique in the protection of the kidneys and vessels in operations requiring the clamping of the renal artery.

PUBLICATIONS

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