INVESTIGATION OF EXPERIMENTAL WARM AND COLD KIDNEY ISCHEMIA-REPERFUSION INJURY IN ANIMAL MODEL

Phd Thesis

by

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1. INTRODUCTION

The disruption of an organ's blood supply is obligatory in clinical transplantation occurring at the time of organ procurement. During this ischaemic period complex pathophysiological changes occur within the organ that leave it primed in a pro-inflammatory state. Although the re-establishment of blood flow is essential to halt ongoing ischaemic damage, the organ incurs additional injury. This paradoxical response to reperfusion is known as ischaemia reperfusion injury (IRI). Renal ischemia-reperfusion (I/R) injury is the major cause of acute renal failure (ARF) in both native and transplanted kidneys. [1]
Renal ischaemia-reperfusion injury (IRI) leads to acute kidney injury (AKI) is not only an invariable consequence of transplantation but also results from vascular surgical procedures requiring suprarenal aortic cross-clamping, sepsis and resuscitation following systemic hypotension. This phenomena exacerbate tissue damage by initiating an inflammatory cascade including reactive oxygen species (ROS), cytokines, chemokines, and leukocytes activation.[2 3] Renal IRI contributes to pathological conditions called acute kidney injury (AKI) that is a clinical syndrome with rapid kidney dysfunction and high mortality rates.[4 5]
The pathophysiology of IRI in kidney is very complex but some pathological pathways such as activation of neutrophils, release of reactive oxygen species and other inflammatory mediators including adhesion molecules and a variety of cytokines are involved.
In the kidney, endothelial cells, tubular epithelial cells and infiltrating leukocytes all demonstrate specific responses during both ischaemic and reperfusion phases of injury, which amplify the tissue damage. Understanding the mechanisms that mediate IRI is a focus of significant scientific endeavour. Therapeutics based on this research would not only improve the utility of transplantation from a finite donor pool by increasing short and long-term outcomes, but may have applications in numerous other clinical settings.

1.1. Renal Ischemia-reperfusion injury

Renal ischemia/reperfusion injury (IRI), results from a generalized or localized impairment of oxygen and nutrient delivery to, and waste product removal from cells of the kidney. [6]
There is mismatch of local tissue oxygen supply and demand and accumulation of waste products of metabolism. As a result of this imbalance, the tubular epithelial cells undergo injury and, if it is severe, death by apoptosis and necrosis (acute tubular necrosis [ATN]), with organ functional impairment of water and electrolyte homeostasis and reduced excretion of waste products of metabolism. There are many pathophysiological states and medications that can contribute to generalized or localized ischemia. [7]

1.1.1. Endothelium in renal ischemia-reperfusion injury

The endothelial and smooth muscle cells of the microcirculation play critical roles in the pathophysiology of AKI. Endothelial cells are important determinants of vascular tone, leukocyte function, and smooth muscle responsiveness. [8]The endothelium is injured, and small arterioles in postischemic kidney vasoconstrict more than do vessels from normal kidney in response to increased tissue levels of endothelin-1, angiotensin II, thromboxane A2 (TXA2), prostaglandin H2 (PGH2), leukotrienes C4 (LTC4) and D4 (LTD4), and adenosine as well as sympathetic nerve stimulation.[9 10 11 12]
There is also decreased vasodilatation in response to acetylcholine, bradykinin, and nitric oxide (NO). [13 14]
Vasoconstriction is amplified due, in part, to reduced production of nitric oxide and other vasodilatory substances by the damaged endothelial cell. These effects on the arterioles are augmented by vasoactive cytokines including TNF-α, IL-1β, IL-6, IL-12, IL-15, IL-18, IL-32, and endothelin, generated as a result of the enhanced leukocyte-endothelial adhesion and leukocyte activation that are characteristic of ischemic injury. [15] The enhanced endothelium-leukocyte interactions due to increased expression of cell adhesion molecules such as ICAM-1 on damaged endothelial cells and increased expression of counterreceptors on leukocytes. [16] This results in activation of the leukocytes, obstruction of capillaries and postcapillary venules, further activation and transmigration of leukocytes, production of cytokines, and a vigorous proinflammatory state. Damage to the endothelium results in loss of the glycocalyx, disruption of the actin cytoskeleton, alteration of endothelial cell-cell contacts, and breakdown of the perivascular matrix, all of which culminate in increased microvascular permeability during AKI and loss of fluid into the interstitium. [17 18]

1.1.2. Inflammation in renal ischemia-reperfusion injury

Both innate and adaptive immune responses are important contributors to the pathology of ischemic injury. The innate component is responsible for the early response to injury in a non-antigen-specific fashion and comprises neutrophils, monocytes/macrophages, dendritic cells (DCs), natural killer cells (NKs), and natural killer T cells (NKTs). The adaptive component, activated by specific antigens, is initiated within hours and lasts over the course of several days after injury. The adaptive response includes DC maturation and antigen presentation, T lymphocyte proliferation and activation, and T to B lymphocyte interactions. Neutrophils attach to the activated endothelium and accumulate in the kidney both in animal models and in human AKI [19 20] particularly in the peritubular capillary network of the outer medulla, as early as 30 minutes after reperfusion. They produce proteases, myeloperoxidase, reactive oxygen species, and cytokines, which leads to increased vascular permeability and reduced tubular epithelial and endothelial cell integrity, aggravating kidney injury . [21]

1.1.3. Complement activation in renal ischemia-reperfusion injury

The complement system is an important contributor to inflammation after IRI, but the kidney is unique in that activation after IRI occurs predominantly, if not exclusively, by the alternative pathway. [22] Complement upregulates expression of endothelial cell adhesion molecules. [23]DCs covalently fix marked amounts of macrophage-derived C3, the most abundant complement protein in the circulation, on their surface. [24] This C3 binding promotes maturation of DCs, which in turn activate T cell responses.

1.2. Ischemia Reperfusion Injury in the Diabetic Kidney

Diabetes mellitus, hypercholesterinaemia and hypertonia all increase the risk of ischemia/ reperfusion injury. [25] For the effect of I/R in all segments of the microvascular apparatus exacerbated dysfunction emerges as a consequence of increased plasma cholesterol levels. According to observations in humans and test animals the endothelium-dependent relaxation in the arteriole is decreased by medium-level hypercholesterinaemia[26], but the process can be prevented by the application of superoxide –dismutase (SOD). The superoxide generation is increased in the endothelium cells of the arterioles leading to NO generation in the cells. The results are supported by the accelerated superoxide generation of endothelial cells of the arteries found in test animals. For the effect of I/R, capillary filtration may increase as well. In the post-ischemic venules of LDL receptor deficient (LDL r-/-) mice and rats an increased rate of “rolling” mechanism, as well as adhesion and migration of neutrophil cells can be observed, along with neutrophil cell aggregation and increased albumin extravasation. The mentioned animals were kept on high cholesterol diet and in each particular case the gained results were compared to the values of animals in the control group. [27]
For the effect of I/R in DM states generated for experimentation significantly increased “rolling”, as well as adhesion and tissue migration of neutrophil cells, along with albumin filtration and the formation of free radicals. [28] The increased rate of „rolling” mechanism is regulated by P-selectin, while the increased adhesion interaction of leucocytes between neutrophils and endothelium cells are regulated by CD11/CD18 and ICAM-1. In diabetic rats the increased leucocyte-endothelial cell adhesion for I/R can be inhibited by PAF receptor antagonist or leukotriene biosynthesis inhibitor. I/R induced increased oxidative stress can be observed in and between the venules of the diabetic test animals. Known data support that particular lipid mediators (generated in the post-ischemic venules of diabetic test animals), are the primary source of free radicals e.g. leucocytes activated by PAF, LTB4. Free radicals generated by xanthine-oxidase form during the first stage of reperfusion, promoting the generation of lipid mediators that contribute to the activation of leukocyte adhesion. Different responses are given in hypercholesterinaemiac and diabetic animals. Regarding the first case, the increased albumin extravasation from post-ischemic venules is closely related to the leukocyte-endothelial cell adhesion. In the latter case, leucocyte independent mechanisms occur to mediate the I/R-induced endothelial barrier dysfunction. [29] The development of endogen adaptation is damaged in hyperlipidemia and diabetes. [30]

2. AIMS

We planned two major investigations. In the first study we performed acut kidney ischemia-reperfusion injury on rat animal model by clamping the left renal artery for 45 minutes. Ischemic phase was followed by reperfusion by removing the microvascular clamp from the renal arteria for 90 minutes. In this study we investigated the possible protecting effects of various PPS (sodium penthosan polysulfate) adminitration against renal ischemia reperfusion injury both in native and diabetic kidneys in rats. We examined the developing oxidative stress and inflammatoric responses and the histological changes of renal structure, and various pro-and antiapoptotic signaling pathways.

Our research looked for answers to the questions:

2.1 Does the long-term preoperative intravenous administration of PPS can reduce the renal ischemia-reperfusion injury in native rats?

2.2 What are the effects of the long-term preoperative intravenous administration of PPS on renal ischemia-reperfusion injury in diabetic rats?

2.3 How can influence the high dose intraoperativ administration of PPS the outcome of ischemia-reperfusion injury in native rats?

2.4 Does have any advantages of high dose intraoperativ administration of PPS against ischemia-reperfusion injury in diabetic rats?

In the second study we performed a renal perfusion system after a removal of kidney from rats.
In transplantation the removed organ has to be perfused with a medium as soon as possible and has to be transported in such conditions that it can keep its ability to function properly. The period of availability has to be carefully defined. By the application of perfusion systems all organs or parts of the body may be analysed that can be isolated from other parts of the body regarding circulation.

2.5 Our research looked for answers to the questions whether what injuries the kidneys have during ischemia, how those processes could be avoided, prevented, thus securing a more perfect early and later functions of the transplant organ during transplantation.

3. PPS REDUCED RENAL ISCHEMIA-REPERFUSION INDUCED OXIDATIVE STRESS AND INFLAMMATORY RESPONSES IN EXPERIMENTAL ANIMAL MODEL.

3.1 Introduction

The ischemia/reperfusion injury is one of the most important cause of acute kidney injury (AKI) in native kidneys and allografts used for transplantation.[31] Acute kidney injury, formerly known as "acute renal failure," has been traditionally described as a rapid (ranging from hours to weeks, to less than 3 months) decrease in kidney function as measured by increases in serum creatinine.[32] The Acute Kidney Injury Network (AKIN) defined it as "An abrupt (within 48 hours) reduction in kidney function," and offered specific laboratory and clinical values to guide diagnosis.[33]

AKI remains to be an independent risk factor for mortality and morbidity by increasing the risk of death in case of patients after several types of vascular surgery such as thoracoabdominal aortic surgery, renal arteries desobliterations or any kind of infrarenal aortic surgery requiring suprarenal aortic clamping. These types of vascular surgery produce renal ischemia/reperfusion injury (IRI), a common cause of AKI[34 35], that mainly results from a generalized or localized impairment of oxygen and nutrient delivery to the cells of the kidney.[36]

Cellular and molecular responses of the kidney to the acute ischemic injury are complex, and not understood in details.[37 38] Several studies have examined - the role of the inflammatory response and intracellular signaling pathways in ishemia/reperfusion injury.[39 40]

Inflammation is now believed to play a major role in the pathophysiology of AKI.[41 42] The vascular endothelium plays a central role in the recruitment and migration of circulating inflammatory cells into sites of inflammation.[43] In addition, several authors have demonstrated the renoprotective effects of various anti-inflammatory therapies, for example , mycophenolate, alpha-melanocyte stimulating hormone targeting the proinflammatory pathways that participate in pathogenesis of AKI.[44]

The ischemia/reperfusion injury besides the inflammation produces cell injury of the affected kidney. The most sensible cells for ischemic injury are the tubular epithelial cells in kidneys. Over the point of no return the ischemic injury causes apoptosis and necrosis of the tubular epithelial cells (acute tubular necrosis/ATN/). [45]

Sodium pentosan polysulfate (PPS) is a mixture of semisynthetic sulfated polyanions that is approved as an oral medication for the treatment of an inflammatory-like disease-interstitial cystitis in the USA. [46] The anti-inflammatory action of the pentosan polysulfate is not clearly understood. It is reported that PPS decreases lipopolysaccharide-mediated NFκB activation, suppresses leukocyte elastase and inhibits complement activity. [47 48 49] It has been demonstrated that PPS decreases tubulointerstitial inflammation and preserve renal function in 5/6 nephrectomized rats.[50] It has also been demonstrated that PPS preserves renal function, reduces albuminuria, and markedly decreases the severity of renal lesions, including tubulointerstitial inflammation in diabetic nephropathy in aging C57B6 mice. [51]

We supposed that the anti-inflammatory effects of PPS could be useful to reduce cell damages in AKI, stabilization of the endothelium of the renal capillary vessels has an advantageous effect to provide the ischemia/ reperfusion injury in kidneys.

3.2. Aim of the study:

The aim of the present study is monitoring the course of renal ischemic and ischemia/reperfusion injury in cellular level, furthermore investigating the efficacy of long-term preoperative and single shot intraoperative administration of PPS to protect renal tissue from acute ischemia/reperfusion injury both in native and diabetic kidneys in rats. We compared the influence of low dose preoperative, and high dose intraoperative administration of PPS to intracellular apoptotic (bax) and antiapoptotic (bcl-2) signal pathways, to oxidative stress followed upon the determination of malondialdehyde (MDA), reduced glutathione (GSH), thiol group (-SH), and superoxide dismutase (SOD) plasma levels. Inflammatory changes are measured by the determination of serum tumor necrosis faktor (TNFα), interleukin 1 (IL-1) levels, and the extent of DNA damage is charaterized by phospho p53 (p-p53) levels. To demonstrate the morphological changes, histological examinations were performed.

3.3. Materials and methods

3.3.1. Animal model

60 male Wistar rats, weighed between 200-250 g were used in the present study from Charles River Breeding Laboratories (Hungary, Isaszeg). The animals were housed in individual cages in a standard temperature (25 ± 2˚C), light controlled (12 hours light-dark cycle) and air-filtered room with free access to food and water. Food was withdrawn 12 hours prior to experiment.

Diabetic animals:
Diabetes is induced in rat by administrating of single shot high dose (50mg/kg) streptozotocin (STZ), a compound that has a preferential toxicity toward pancreatic β cells.

3.3.2. Renal ischaemia-reperfusion model

The animals were anaesthetized with an intraperitoneal injection of ketamine hydrochloride (500 mg / 10 ml) and diazepam (10 mg / 2 ml). The ratio was 1:1 (0.2 ml / 100 g = 5 mg ketamine + 0.5 mg diazepam / 100 g) and the animals were placed on a heated pad. ECG was placed and the carotid artery was catheterized (22 gauge) for blood pressure measurement The skin was disinfected and a midline laparotomy was performed. 2 ml of warm saline was injected into the abdominal cavity to help maintain the fluid balance. The inferior mesenteric vein was catheterized for collecting blood samples, fluid equilibration and supplemental anesthetic. The abdominal aorta and renal vessels at both sides were exposed by gently deflecting the intestine loops to the right.
After fine preparation and isolation of the renal vessels, an atraumatic microvascular clamp was placed on the left renal artery for 45 minutes (ischemic phase=I). The abdomen was provisionally closed and the wound was covered with warm, wet compress to minimize heat

and fluid losses. The duration of left kidney ischemia starts from the time of clamping. Complete ischemia is detected by measurements of blood flow in the periferal part of the left renal artery /CardioMed Flowmeter-CM 2005 Medi-Stim ASA, Oslo, Norway/.
Ischemic phase was followed by reperfusion (reperfusion phase =R) by removing the microvascular clamp from the renal arteria for 90 minutes, which is indicated by the change of kidney color from purple to red, and detectable arterial pulsation with flowmeter.

3.3.3. Administration of PPS

We extrapolated the human drog dose of PPS to rat. [52] During the experiment PPS was administered to animals in two ways.
Long term preoperativ administration: prior to operation both diabetic and non-diabetic groups of animals were treated with intravenous (iv.) low dose administration of PPS for a week (25mg/kg daily).
Single shot intraoperativ administartion: high dose (100mg/kg) intravenous (iv.) PPS was administered to animals intraoperativly at the end of ischemic phase (I) lasting 45 minutes- prior to reperfusion (R).

3.3.4. Experimental groups

Group1: Sham- After median laparotomy abdomen was closed provisionally for 135 min.
Group 2: controll- Ischemia/reperfusion in non-diabetic animals: after median laparotomy 45 min. left kidney ischemia followed by 90 min. reperfusion (I/R)
Group 3: single shot, high dose, iv. administration of PPS intraoperatively at the end of ischemic phase lasting 45 min. prior to 90 min. reperfusion in non diabetic animals (I/PPS/R).
Group 4: long term, low dose, iv. administration of PPS prior to iscehmia/reperfusion injury in non-diabetic animals. Daily iv. administartion of PPS (25mg/kg) for a week prior to 45 min. left kidney ischemia followed by 90 min. reperfusion (PPS/I/R)
Group 5: Sham DM- After median laparotomy abdomen was closed provisionally for 135 min. in diabetic rats.
Group 6: controll -Ischemia/reperfusion in diabetic animals: after median laparotomy 45 min. left kidney ischemia followed by 90 min. reperfusion (DM I/R)
Group 7: single shot, high dose, iv. administration of PPS intraoperatively at the end of ischemic phase lasting 45 min. prior to 90 min. reperfusion in diabetic animals (DM I/PPS/R).
Group 8: long term, low dose, iv. administration of PPS prior to iscehmia/reperfusion injury in diabetic animals. Daily iv. administartion of PPS (25mg/kg) for a week prior to 45 min. left kidney ischemia followed by 90 min. reperfusion (DM PPS/I/R)

3.3.5. Analysis of oxidative stress parameters

Measurement of malondialdehyde (MDA): Malondialdehyde is a marker for the quantification of lipid peroxidation in cell membranes. MDA was determined in anticoagulated whole blood, by photometric method of Placer, Cushman and Johnson. [53]
Measurement of reduced glutathione (GSH) and plasma thiol-groups (-SH): Reduced glutathione is the predominant low-molecular-weight thiol in cells. Because of the cysteine residue GSH is readily oxidized non-enzymatically to glutathione disulfide (GSSG) by electrophilic substances. GSH concentrations reduce markedly in response to protein

malnutrition and oxidative stress. [54] GSH and plasma SH levels were determined in anticoagulated whole blood (ethylenediaminetetraacetic acid (EDTA)) by Ellman's reagent according to the method of Sedlak and Lindsay. [55]
For measuring of superoxide dismutase (SOD) activity in serum we used Superoxide Dismutase Assay Kit (Trevigen Inc., Gaithersburg, USA), following the manufacturer's protocol. This method determines the free i.e. biological active SOD activity.

3.3.6. Serum TNF-alpha and Serum IL-1 quantification

For measuring the TNF-alpha and IL-1 concentration in serum we used Rat TNF-alpha, and IL-1 ELISA kit (R&D Systems, Inc., Minneapolis, USA), following the manufacturer's protocol. This method determines the free i.e. biological active TNF-alpha and IL-1 concentration.

3.3.7. The western-blot analysis of proapoptotic (bax) and antiapoptotic (bcl-2) signaling pathways and extent of DNA damage (p-p53)

Fifty milligrams of left and right kidneys samples were homogenized in ice-cold TRIS buffer (50 mM, pH 8.0), the homogenate was pelleted, and the supernatant was measured by bicinchonicic acid reagent and equalized for 1 mg/ml protein content in Laemmli solution for Western blotting. The samples were harvested in 2X concentrated SDS-polyacrylamide gel electrophoretic sample buffer. Proteins were separated on 12% SDS-polyacrylamide gel and transferred to nitrocellulose membranes. After blocking (2 h with 3% nonfat milk in TRIS-buffered saline) membranes were probed overnight at 4°C with antibodies recognizing the following antigenes: (polyclonal Bax antibody, 1:1000 dilution), pospho-p53 MAPK (Thr^{180}/Tyr^{182}, 1:1000 dilution), (polyclonal bcl-2 antibody, 1:1000 dilution), (Cell Signaling Technology, Danvers, MA, USA). Membranes were washed six times for 5 min in TRIS-buffered saline (pH 7.5) containing 0.2% Tween (TBST) before addition of goat anti-rabbit horseradish peroxidase conjugated secondary antibody (1:3000 dilution; Bio- Rad, Budapest, Hungary). Membranes were washed six times for 5 min in TBST and the antibody-antigen complexes were visualized by means of enhanced chemiluminescence. The results of Western blots were quantified by means of Scion Image Beta 4.02 program. All experiments were repeated four times.

3.3.8. Histological examinations

The animals were terminated at the end of the experiment and biopsy was taken from the left kidneys. The fragments of kidneys did not contain well-identified perirenal fatty tissues or fascia. The definite aim of the biopsy was to register the qualitative differences in changes between the animal groups, firstly the transformations in the kidney tissues. 5-6 paraffin-embedded blocks were made from kidney-pieces, and sample slices were prepared staining by hematoxylin and eosin.

3.3.9. Statistical analysis

All values are expressed as means ± SEM. Differences between the groups were assessed with one-way analysis of variance (ANOVA) and when the terms are passed we used

adequate post-hoc tests (Sidak, Dunnett) for multiple comparisons. T-tests were performed independently to show the differences between the investigated groups. Data were considered significant when p-value was less than 0.05.

3.4. Results

3.4.1. Plasma malondialdehyde levels

The MDA concentration was significantly higher in all groups comparing to the sham both in non-diabetic and diabetic. Significant abatement was measuread in I/PPS/R group (gr.3) comparing to control (gr.2 -I/R) in non-diabetic animals, and in DM PPS/I/R group (gr.8) comparing to control (gr.6 -DM I/R) in diabetic animals.

3.4.2. Reduced glutathione levels (GSH)

The values of reduced glutathione levels were significantly ($p<0.05$) lower in all groups comparing to the sham groups. We found in the PPS/I/R group (gr.4) higher values than in I/R group (gr.2) but these data could not reach the level of significance. At the same time in I/PPS/R group (gr.3) the values were significantly higher than in non- treated control IR group (gr.2).
In treated groups of diabetic animals (gr.7, gr.8) significantly higher values were measured compared to the control DM I/R group (gr.6).

3.4.3. Plasma thiol groups (-SH)

Plasma thiol groups levels were significantly lower in all groups comparing to the sham groups.
We detected in the treated groups (gr.3, gr.4) higher level of –SH comparing to control I/R group (gr.2) in non-diabetic animals, but these data could not reach the level of significance. Also there was no significant difference in –SH level elevation between treated diabetic (gr.7, gr.8) and diabetic control I/R group (gr.6.

3.4.4. Enzyme activity of superoxide dismutase (SOD)

Significantly elevated SOD activity was detected in PPS pretreated non-diabetic group (gr.4) comparing to the control I/R group (gr.2)(564.6 ± 16.62 U/l vs. 642.2 ± 7.41 U/l ($p=0.0007$). In I/PPS/R group (gr.3) we have found non significantly higher values comparing to the control I/R group (gr.2).
We have measured non significantly changes of SOD activity comparing to the control group (gr.6) both in treated diabetic groups (gr.7,gr.8)

3.4.5. Serum TNF-α levels

In the study we measured the TNF-α levels in the groups. The values were significantly lower ($p<0.05$) in one non-diabetic (gr.3) and one diabetic groups (gr.8) than in the control groups.

The protecting effect of PPS administration prior to ischemic reperfusion injury was not marked in non-diabetic rats, while the preoperative long-term administration of PPS significantly decreased the serum TNF-α level in diabetic animals comparing to the control group.

3.4.6. Serum interleukin-1 (IL-1)

I/R caused a significantly elevated level of IL-1 comparing to the sham both in diabetic and non-diabetic animals. The values were significantly lower in I/PPS/R group (gr.3) in non-diabetic, and DM PPS/I/R (gr.8) in diabetic groups than in the control I/R, DM I/R groups (gr.2, gr.5). Long-term preoperative administration of PPS (gr.8) decreased significantly the IL-1 level in diabetic animals comparing to the control group (gr.6), the serum IL-1 level approached the serum IL-1 level in sham group (gr.5), while intraoperative administration of PPS prior to reperfusion did not temper but slightly increased the serum IL-1 level compared to non-treated control group in diabetic animals (gr.7 vs. gr.6).

3.4.7. Western-blot analysis of proapoptotic (bax) and anti apoptotic (bcl-2) signaling pathways, extent of DNS damage (p-p53)

To characterize the expression of proapoptotic (bax) and antiapoptotic (bcl-2) signal proteins we used Western blot analysis to separate and establish them. We also used Western blot analysis to reveal the extent of DNS damage by characterizing the expression and phosphorylation of p53.
We found that the expression of bax was appreciably higher in the PPS preoperative administered group (gr.4) comparing to the control (gr.2) in non-diabetic animals, but it was lower in both PPS administered diabetic groups (gr.7, gr.8) comparing to the diabetic control group (gr.6).
Expression of anti apoptotic bcl-2 was markedly higher in PPS administered groups both in diabetic and non diabetic animals.
Characterizing the extent of DNS damage phosphorilated p53 expression showed diminution in preoperative administration of PPS groups both in diabetic (gr.8) and non-diabetic (gr.4) animals comparing to the nontreated controlls.
This diminution cannot be demonstrated in the intraoperative, single shot PPS administrated non diabetic group (gr.3).

3.4.8. Histological results

In the I/R control group of animals (gr.2) the basic tissue structure is mainly kept in the kidney, there is no fibrosis. Necrosis cannot be defined with absolute certainty, but acidophil cylinders are detected in the tubules. Subsequent upon plasma loosing, painted fibrin can be detected in ischemia/reperfusion injured kidney.
In the I/PPS/R group of animals (gr.3) the basic structure is mainly kept, there is no fibrosis, necrosis, inflammation,and the structures of tubules seem to be intact.
In the PPS/I/R group of animals (gr.4) interstitial haemorrhagic changes can be detected without necrosis or inflammation.

In the DM I/PPS/R group of animals (gr.7) epithelial cells of tubules are gently swelled but interstitial edema or necrosis can not be defined.
In DM PPS/I/R group of animals (gr.8) increased mucus retention and epithelial cell swelling can be detected, without necrosis and damages.

3.5. Discussion

Revascularization procedures performed on ischaemized organs will lead to reperfusion injury which is an integrated response to the restoration of blood flow after ischaemia. Numerous factors could modulate the extent of oxidative stress and generalized inflammatory response. [56,57,58,59] The effect depends on the duration of ischaemia, the ischaemic tissue volume and the general metabolic state of the organism (diabetes, drugs, chronic ischaemia).
Ischemia will lead to decreasing level of intracellular adenosine 5'-triphosphate (ATP) and consecutive elevation of hypoxanthine. Beneficial effects of exogenous nucleotides, including ATP, have been observed in ischemic rat kidneys and in hypoxic isolated rabbit tubules. [60] It was demonstrated that ATP protected renal ischemic injury has been mediated by NF-κB activation via P2Y receptor in proximal tubular cells (PTCs). Profound intracellular ATP depletion and a fall in tissue oxygen content with a concomitant rise in intracellular calcium are the hallmark features of renal ischemia/reperfusion injury. [61]
Ischemic endothelial injury causes changes in the vascular responses to vasoactive substances. [62] Aiming to protect the organs against the effects of I/R, several substances have been used in experimental studies, many of them related to the performance of the nitric oxide (NO). [63] It was shown that some drugs, such as sildenafil induce protective effect in myocardium. [64] It is possible that the vasodilating action of sildenafil contributes to the release of endogenous mediators, such as adenosine and bradykinin from endothelial cells, which triggers a cascade resulting in phosphorylation of nitric oxide syntethase and the release of NO. [65]
Reperfusion injury is a cascade of events initiated by tissue ischemia and the production of oxygen free radicals during the reperfusion process, leading to an active inflammatory response. Free oxygen radicals play a very important role in this process. In the very early moments of reperfusion the oxygen appears in the cell and the xanthine oxidase catalised hypoxanthine-xanthine conversion will produce a mass of superoxide radicals. Through lipid peroxidation the superoxide radicals and other ROI will damage the membrane lipids, proteins and DNA. The endogenous antioxidant system tries to defend the cells and macromolecules against these injuries. [66]
Many tissues and cells can be damaged by free radicals, with red blood cells (RBC) being one of the most susceptible. During ischemia–reperfusion the increased oxidative stress can cause augmented RBC membrane lipid peroxidation with the consequent alteration of cellular deformability. Erythrocyte deformability is of crucial importance for the maintenance of normal circulation: it facilitates the passage of red blood cells through narrow capillaries in the microcirculation and reduces blood viscosity at high shear rates in large blood vessels. [67]
The pathogenesis of microvascular I/R injury involves two pathophysiologically distinct mechanisms, termed *no-reflow* and *reflow paradox.* Whereas reflow paradox comprises reflow-associated injury, including the release of inflammatory mediators, leukocyte activation with interaction and adherence to the microvascular endothelium, and increase of

microvascular leakage , no-reflow primarily represents ischemia-induced failure of capillary reperfusion. [68]Several mechanisms have been proposed to cause capillary no-reflow, including intravascular hemoconcentration and thrombosis, leukocyte plugging, endothelial cell swelling, vasomotor dysfunction, and interstitial edema formation. I/R induces the disruption of the endothelial integrity with loss of fluid to endothelial cells and the interstitial space. As a consequence, these pathological sequelae are associated with intravascular hemoconcentration, endothelial cell swelling and interstitial edema formation, which contribute to capillary lumenal narrowing, increase of hydraulic resistance, and, thus, impairment of perfusion. [69 70]

The aim of our experiments was to study the effects of PPS on a rat in vivo renal ischemic model and find a protective administration method.

This study demonstrated first that the renal IR induced oxidative stress was decreased by intraoperative administration of PPS prior to revascularisation in non-diabetic animals.

Furthermore, long-term, preoperativ administration of PPS decreased the IR induced oxidative stress in diabetic animals.

This positive effect of preoperative administration of PPS in diabetic animals have been detected through the investigation of oxidative parameters, but this positive effect has not been seen in non-diabetic animals.

Inflammatory response has changed the same way. TNF-α , IL-1 showed significant changes due to endothelial dysfunction. This could be the first effect of the inflammatoric response in reperfusion injury. [71] The vascular endothelium regulates vascular permeability and modulates vasomotor, inflammatory, and hemostatic responses. Impairment of these vital endothelial cell functions during and following renal ischemia can contribute to the impairment of renal perfusion, continued renal hypoxia, and the subsequent epithelial cell injury.[72]

It has been demonstrated that PPS has a protective effect for brain endothelium in bacterial infections affecting the blood-brain barrier (BBB)[73], and PPS has an advantage effect in the prevention of atherogenesis by enhancing endothelial regeneration.[74]

Long-term administration of PPS seems to have a benefit against a later date acut renal ischemia/reperfusion injury by the stabilization of endothel function in diabetic animals, while this advantage cannot be seen in non-diabetic animals. On the other hand, PPS administered during ischemic period prior to reperfusion might contributes to survival of the renal cells in non-diabetic animals.

Jin Wu et al. have demonstrated that PPS treatment prevents the progression of nephropathy in streptozotocin-induced diabetes in aging C57B6 mice by decreasing albuminuria, renal macrophage infiltration and TNF-α expression.[75] Our experiment has showed that PPS treatment could be usefull to prevent the ischemia/reperfusion injury by decreasing the inflammatory response (lower serum TNF-α levels compare to the controlls) both in diabetic and non-diabetic animals. While single shot administration of PPS prior to revascularisation decreased the TNF- α level significantly in non-diabetic animals, long term pretreatment has a serum TNF- α decreasing effect in diabetic animals. We assume that sustained inflammatory disorders, oxidative stress caused by diabetes cannot be decreased by single shot injections.

Decreased SOD enzyme activity subsequent upon I/R injury can be explained by the depletion of enzim activity intent to protection against ischemic injury which cannot be increased by single shot administration of PPS prior to revascularisation in non-diabetic rats.

We detected elevated SOD enzim activity after comparing I/R injury to the control in long term PPS administrated non-diabetic group (gr.4)
We have no detected SOD enzim activity changes in diabetic animals after PPS administrations compared to control I/R group. We suppose that SOD enzyme activity cannot be stimulated any further because of deterioration of enzymes caused by sustained diabetic metabolic oxidative stress.
Non-significant changes of serum –SH groups by both way administrations of PPS neither diabetic or non-diabetic animals can be explained by the short time of I/R.

3.6. Conclusion

The ischemia/reperfusion injuries of the kidneys still have a high mortality and morbidity risk during reconstructive vascular surgical procedures requiring a suprarenal aortic clamping. Currently, no therapeutic agents are clinically available specifically for the prevention or treatment of kidney I/R injury. Various pharmacological and non-pharmacological therapies may help reduce I/R injury, but the single established strategy to limit I/R injury is early reperfusion of the ischemic tissue. Non-pharmacological, affective surgical procedures aiming to decrease the ischemia/reperfusion injury (e.g. pre-and postconditioning) lengthen the operation time which has an unprofitable affect to the patient by increasing the operative load. Longer operations have an uneconomic consequences as well. Pharmacological conditioning has the saving grace that it does not elongate the operation time, does not mean extra tasks for the operation stuff. Pentosan is used in clinical practice as an anticoagulant, and as a treatment for interstitial cystitis. Experimental and clinical data suggest that PPS has a therapeutic efficacy in osteoarthritis.[76] Although the mechanism of action may be complex, PPS suppresses inflammation mediated by TNF- α. Our investigation results showed that long term administration of PPS has an advantage to reduce ischemia/reperfusion kidney injury in diabetic rats, while this advantage cannot be seen in non-diabetic animals. On the other hand high dose single shot parenteral administration of PPS prior to revascularisation can reduce the lipid peroxidation, inflammatory response therethrough the ischemia/reperfusion injury, while long term administration of PPS prior to ischemic injury has no benefit in non-diabetic rats.

4. THE EFFECT OF PPAR-GAMMA AGONIST ON ISCHEMIA INJURY IN ISOLATED PERFUSED KIDNEY

4.1. INTRODUCTION

4.1.1. Transplantation, organ conservation

Organ transplantation is the most cost effective treatment in case of end-stage renal dysfunction currently, while regarding the end-stage dysfunction of other organs, such as the liver, the lungs or the heart, this is the only available treatment. In cadaveric donor cases, procedures to conserve the proper operation of organs are necessary to be applied. One of the reasons is that the selection of recipients for the donor organ is not completed before the donation of certain organs (most of all kidneys), since immunological tests are performed on samples from the spleen which is removed from the corpse in parallel with the given organ. Thus, after the removal of the organ several hours are necessary to choose, call and examine the recipient in order to start the implantation. In heart and liver transplantation, the selection of patients is completed before the donation based on blood-type compatibility and body size markers.
The other reason is that the donor and the recipient are at different geographical locations and following the removal of the organ for transplantation has to be transported from the donor hospital to the transplant centre. To maintain the function of removed organs several methods should be applied in unison. The organ has to be refrigerated so that its metabolism is reduced to the minimum between the time of removal and transplantation. The other commonly applied method for organ prevention is the use of conservation solutions. Various solutions may be applied for the preservation of organs, such as the Euro-Collins, the Ross-Marshall and the University of Wisconsin (UW) solutions. The UW solution could be regarded as a “gold-standard” as it is suitable for liver, kidney and pancreas perfusion and produced outstanding results both in clinical and research models. Unfortunately, neither the UW is considered the best resolution. The disadvantages of the liquid include high viscosity and high price moreover it may cause endothelial dysfunction as well. [77] The two methods made the transportation and storage of the organs possible for a few hours, till the actual transplantation. This intermediary period is called cold ischemia during which the organ is in chilled condition and without circulation. The various organs tolerate the mentioned mode of storage for different periods of time and the phenomena is called ischemia tolerance; thus in cold ischemia kidney can be stored between 24-36 hours at 0 °C without deterioration. Hypothermia is a deteriorating factor in itself and is a risk factor regarding the activation of the implanted organ as well as its long-term functioning, hence it is expedient to strive for the shortest possible ischemic period. [78] For kidney transplantations, the 24-hour cold ischemic period is acceptable, since in those cases when the implanted kidney does not start functioning immediately its function can be substituted by dialysis.
The clinical emergence of renal dysfunction in the affected area of the kidney due to ischemic damage depends on the rate of cell mortification. Cell mortification may eventuate as a direct consequence of a hypoxic state during the ischemic period, yet lot of cells perish

right after reperfusion. Investigations by Schumer indicated that apoptosis also contributes to the renal damage emerging during reperfusion which follows ischemia. [79]
The method of removing the organ and its proper conservation are largely accounted for success, as they can essentially influence both the short and long term outcomes of transplantation. [80 81 82 83] With the application of proper techniques ischemic as well as reperfusion damages can be minimized, thus improving the graft's prognosis of longevity and its post function.

4.1.2. The isolated kidney perfusion system

Studies about isolated perfused kidney (IPK) could be traced back to the first decades of the 20th century. [84 85 86] By the application of IPK the study of the kidney from physiological, pathophysiological and pharmacological perspectives all became available. The kidney is probably the easiest to perfuse among organs due to its peculiar anatomy, since there is a single, easily accessible artery leading to the kidney. The IPK is such an experimental model which enables perfusion pressure to be controlled through the kidney, as well as to regulate the concentration of substances administered into the system. [87]During the production of IPK we should aim the preservation of kidney function by helping the organ remain intact. [88] The Krebs-Henseleit solution (KHS) is the typical medium applied for perfusion. [93 88] The ion composition has to map the extracellular milieu both quantitatively and qualitatively particularly regarding the Na^+, K^+ and Ca^{2+} ions. In addition the perfusion solution has to provide proper osmolarity (300 mosmol) and buffer capacity (CO2, HCO3 system) to maintain the physiological 7,40±0,05 pH value. The even temperature of 37°C is provided by a thermostat system. The greatest advantage of the medium is that all the necessary components are available in almost every laboratory, so the solution can be produced cost-effectively.

4.1.3. PPAR- γ agonist

Synthetic PPAR –γ agonists form a group of oral antidiabetics used in the treatment of non-insulin dependent diabetes mellitus. [89]
It is considered a new-fangled evidence that the PPAR –γ activation can regulate inflammatory responses and can inhibit the expression of several pro-inflammatory molecules by receptor dependent transpression. [90] The main source of reactive oxygenic free radicals is the mitochondrium, in particular respiratory chain complex I and III. [91] As it has recently been verified, rosiglitazone and pioglitazone can impede the activity of chain complex I [92] and III [93]. PPAR –γ agonist partially disconnect the respiratory chain having an effect on superoxide production and electron transport as well. Latest research described a mitochondrial target protein on which PPAR –γ agonists can exert their effect (mito-NEET). [94] The mito-NEET has been proven to be related to the components of complex III which may explain the ability of PPAR –γ agonists to bind to mito-NEET and thus block different mitochondrial target molecules selectively. The mentioned effect of PPAR –γ agonists, to influence mitochondrial functions, may provide an explanation for the reduced production of reactive intermediaries.
Besides these processes, several publications appeared about the benevolent effect of PPAR –γ agonists on ischemia-reperfusion injuries with regards to the intestines, [95 96 97] lungs, [98] heart, [99 100 101] kidneys [102] and the brain. [103 104]

4.2. OBJECTIVE

The objective of our experiment is to find out whether cold ischemia tolerance, which has a key role in kidney transplantation as well, can be improved with the continuous, controlled pressure, oxygenized perfusion and with the administration of PPAR-gamma agonist (PPAR –γ Ago).
In the perfusion system basically the Krebs-Henseleit solution is applied with the administration of PPAR –γ agonist and PPAR –γ agonist inhibitor. Following former studies we chose an ischemic period when a minimal damage was observable in the renal tubules, but the majority of the tubule cells were still intact. The referred period of time is 30 minutes by literature [105] and the period perfusion we applied was 60 minutes, therefore we could certainly expect some ischemic injuries in the evaluation of our samples.
We examine the structural injuries with light-microscope, while proteins expressed via the apopotic / antiapopotic transmissions/transductions by Western bolt.

4.3. MATERIALS AND METHODS

4.3.1. Operation technique method

40 male Wistar rats were anaesthetized with an intraperitoneal injection of ketamine hydrochloride and diazepam. The test animal was lied on its back, the mesenteric root was mobilized after laparotomy, and checked whether the kidneys were of similar dimension, as well as identified blood vessels (a. and v. renals) then separated the connective tissue from the infrarenal aorta, thus mobilizing it from vena cava inferior.
Na-heparin was administered to the animals and waited for 60 seconds.
Circulation was shut out both at the level of the aortic bifurcation and the subrenal segment of the aorta by applying an atraumatic microclip. A canulla was installed retrograde and fixed in the lumen.
Having the canulla installed, the right kidney was clamped and the suprarenal aorta was ligatured. The renal a. and v., the cava inferior, the ureters and suprarenale aorta were cut through over the clamp, thus the left kidney could totally be mobilized. As the kidney was removed from the animal, it was immediately immersed in ice water and the perfusion was started through the canulla. Both the temperature of the ice water (0 °C) as well as the pressure of perfusion (125-135 Hgmm) was continually monitored and maintained.

4.3.2. Experimental groups

The animals were classified into four groups according to the medium used for the perfusion and started a 60-minute long perfusion

Ggroup 1: the kidney of the animals were placed in ice water for 60 minutes without applying perfusion (control group)
Group 2: the kidney of the animals were placed in ice water and then perfunded by KHO.
Group 3: the kidneys were placed in ice water and the perfunded medium was composed of KHO and PPAR-γ Ago (1mM) during perfusion.

Group 4: the kidneys were also place in ice water and the perfunded solution was composed of KHO, PPAR-γ Ago (1mM) and PPAR-γ Ago Inh (1mM).

4.3.3. Sampling

Following the 60-minute long perfusion the kidney samples were divided in two sub-groups in each main group. A part of the kidneys were fixated in 10% neutral formalin, while the other part was homogenized.

4.3.4. Light-microscopic examination

According to respective groups the kidneys were fixated in 10% neutral formalin, then were dehydrated in ascending alcohol series and embedded in paraffin. The 5µm thin sections made along the cross-section of the paraffin-embedded kidney were painted by haematoxylin-eosin (HE) and examined under light-microscope.

4.3.5. Protein-expression examination

In the Western-blot examination the detection of proapoptotic (**Bax),** apoptotic (**p53),** and antiapoptotic (**Bcl-2)** proteins was performed.

4.4. RESULTS

4.4.1. The results of the light-microscopic examination

Following the light-microscopic evaluation of the groups we found that mostly the signs of the early reversible ischemia were visible, the structure of the kidneys was partially preserved. There were no serious changes in morphology.
The PPAR-γ Ago treatment could perceptively alleviate the structural injuries. Minimal eosinophilia is observable in tubule epithelial cells along the PPAR-γ treatment, but neither cellular vacuolization nor cellular swelling is observable.

4.4.2. Western-blot

Bax (23kDa)
In case of the Bax protein, in Group 1, where perfusion was not applied, large scale protein expression is observable. In contrast, in Group 3, where PPAR-γ Ago treatment was applied the protein expression decreased significantly. In Groups 2 and 4, protein expression was observable almost to the same extent.

p53 (53kDa)
The expression of apoptotic protein behaved similarly to the previously discussed Bax protein. The dominant appearance of the protein is observable here as well as in Group 1. Similar amount of protein was expressed as in Groups 2 and 4. In Group 3, which received PPAR-γ Ago treatment, the apoptotic protein expression was also of lesser extent.

Bcl-2 (26 kDa)
The antiapoptic Bcl-2 expression did not change according to respective test groups. The amount of protein remained relatively the same during the experiment.

4.5. DISCUSSION

In our animal experiment we studied ischemic injury, a key factor during transplantations, working with an isolated renal perfusion system, on kidneys removed from rats and then perfused. The objective of our experiment was to find how the ischemic tolerance of the kidney could be increased and preserve its intact structure. In the system the Krebs-Henseleit solution developed by Langerdoff [92] was applied for basic perfusion medium to which PPAR-gamma Ago and PPAR-gamma Ago Inh. were administered according to respective groups. Our perfusion was applied throughout 60 minutes.
The only noxa expected was ischemia. After the 60-minute perfusion period, the structural changes caused by ischemia and ischemia indicated prepoptic/apoptic/anti-apoptic protein expressions were validated by light-microscopic and protein-expression examinations.

In the untreated (control) group, where perfusion was not applied, significant differences were found from a histological perspective and more serious injuries were detected. The histological manifestation of early (reversible) ischemia was available on the sections. To the

typical characteristics, the swelling of the tubule epithelial cells and the eosinophilia both contribute.

In the 2nd and 4th groups, milder injuries were observable: increased eosinophilia is present in the cytoplasm of the tubular cells without the swelling of the tubules.

The main reason of reversible cell injury is the lack of oxygen. Mitochondrial activity decreases as a result of the lack of oxygen; there is no ATP generated, and the pumps maintaining membrane potential cease operation in the lack of ATP. The other consequence of the termination of pump operation is cellular swelling as the infiltrating natrium is followed by water too. Increased eosinophilia can be demonstrated by HE-painting. The reason behind is that RNS levels of the cytoplasm decreases and proteins get denaturated.

In the histological image of the group we treated with PPAR-γ agonist (Group 3) shows no serious deviation in comparison with the histological image of the healthy kidney. At some places, one or two eosinophil cells appear among epithelial cells, but there is no sign of oedema or cellular swelling.

In the histological analysis coarse differences, necrotic morphological deviations were not found. During the analysis of histological sections we were explicitly looking for the signs of reversible, early ischemia, since these changes are soundly demonstrable in 30-40 minutes from the start of ischemia. [122]

In the Western-blot test we examined the expression of three different proteins: the proapoptotic Bax, the apoptotic p53 and the anti-apoptotic Bcl2 proteins. The apoptotic cell death plays a key role in the injury indicated by renal ischemia *(Saikumar and Venkatachalam, 2003).* [106] In case of renal ischemia even after 5-10 minutes of the perfusion the different proapoptotic and apoptotic signal transduction pathways are activated (e.g. p38 MAPK and JNK pathways) (Yin et al., 1997). [107]

In the control group (Group 1) the expression of Bax and p53 proteins were dominant. In Groups 2 and 4 these proteins were somewhat less expressed. The antiapoptotic Bcl-2 protein did not show any deviation during the series of experiments. The protein was expressed the same in all the groups. The Bcl-2 expression does not necessarily change immediately after the appearance of ischemia together with the other signal transduction processes.

The Western-blot test of removed kidney samples that were affected by ischemic injury in the isolated perfused system model demonstrated that the PPAR-γ agonist treatment administered to the medium could decrease the ischemia indicated proapoptotic and apoptotic Bax and p53 protein levels.

Referring to our results we could state that the application of the PPAR-γ agonist in the isolated perfused kidney system reduced the ischemia indicated reversible, structural deviations, as well as the ischemia indicated apoptotic signal transduction processes after the development of ischemia.

The administered PPAR-γ agonist did not increase the expression of Bcl-2.

The application of the Krebs-Henseleit solution as basic medium did not influence the structural appearance of early (reversible) ischemic injury significantly in Groups 2 and 4.

4.6. CONCLUSION

The realisation of clinical transplantation may be regarded as one of the most important leaps in 20th century medical science which is due to both the achievements of basic research, as well as to clinical research. Nevertheless, some detrimental side effects of the process should also be considered that significantly influence the success of transplantation. The most important pathophysiological phenomenon is ischemia-reperfusion injury, an inevitable consequences of both live and cadaver donor transplantations. [108 109].

For transplantation, the ischemic injury of the graft is one of the so called non-alloantigenetic dependent risk factor of early and late graft functions according to our current understanding. The organ removed from the donor shows signs of injury despite immediate refrigeration and careful perfusion. The ischemic acute kidney injury consists of interconnected cascade mechanisms that may have acute or chronic consequences. In kidney transplantation several factors can cause the immediate or delayed functioning of the kidney. One of the most significant factors of those is ischemia during which the tubules of the renal cells suffer injury. Ischemia may lead to acute tubular necrosis that is characterised by the acute injury of tubular epithelial cells resulting in their necrosis, and clinical acute renal failure. The functional capacity of the tubular cells significantly contributes to adequate renal functions. Thus efforts have to be made in order to conserve the intactness of tubular cells to be successful in transplantations. [110 111 112] One of the most promising methods to prevent ischemic injuries during organ donations proves to be the perfusion of organs. The solution used in practice has to meet several criteria. The most important of all is the ability to maintain the survival of the organ after isolation. To fulfil this requirement, it has to contain sufficient amount of dissolved oxygen, as well as the necessary ions and nutrition according to the function of the organ.

In our experiment left kidneys from rats were isolated and perfused. We applied the Krebs-Heneleit solution for perfusion [92] which contained PPAR-γ agonist and PPAR-γ inhibitor.

The PPAR-γ is the member of the nuclear receptor superfamily and is such a transcription factor that binds to the peroxisome proliferator responsive elements of the enhancer region in the gene to control. [100] They partake in various processes such as the control of lipid cell differentiation, in controlling insulin sensitivity and inflammatory processes, [101 102] or in the down-regulation of mediator production in proinflammatory macrophages. [103 104 105] The effect of PPAR-γ exerted on ischemic reperfusion injuries in the small bowels [112 113 114], lungs [115] heart [116 117 118], kidney [119] and the brain tissue[120 121], have been reported in literature, along with some positive results found on an in-vivo skeletal muscle lower limb model by our reaserch group recently. (T. Nagy et al, 2015) [113]

Apoptosis has a key role in sustaining balance in the cells of developed organisms. It is a demonstrated fact that apoptosis occurs in the epithelial cells of the renal tubules after ischemia during the reperfusion phase. [122]

In our experiment, on one hand we studied the effect of the PPAR-γ in the isolated kidney reperfusion system, what effect it exerts on structural injuries induced by ischemia, and whether in this way ischemia tolerance of the renal tissue –which has key importance in transplantation- could be enhanced. On the other hand, we wished to prevent the occurrence of apoptosis by administering PPAR-γ agonist into the system.

Based on our results, it could be stated that the application of the PPAR-γ agonist in the isolated perfused kidney system reduced the rate of ischemic injuries. The light-microscopic

experiments confirmed that the PPAR-γ agonist is able to reduce structural injuries of the tissue caused by ischemia. Levels of pro-apoptotic and apoptotic protein expressions reduced along the application of PPAR-γ agonist.

According to the above findings we can conclude that the PPAR-γ agonist exerts renoprotective effects on ischemia induced structural injuries and inhibits apoptotic processes in the isolated perfused kidney system.

5. NOVEL FINDINGS

In the first series of our investigations we observed the effect of different administrations of penthosan polysulfate sodium on kidney ischemia- reperfusion injury in experimental animal (rat) model. In the second phase of our investigation we examined the effects of a non-synthetic PPAR- gamma agonist on kidney cold ischemia- reperfusion damages in isolated perfused system.

We have 5 important observations in the study:

1. We have demonstrated firstly that long term low dose intravenous administration of PPS prior to ischemia has an advantage to reduce ischemia-reperfusion kidney injury in diabetic rats,
2. while this advantage cannot be seen in non-diabetic animals.
3. We described first that high dose single shot parenteral administration of PPS prior to revascularisation can reduce the lipid peroxidation, inflammatory response therethrough the ischemia-reperfusion injury,
4. while long term administration of PPS prior to ischemia-reperfusion injury has no benefit in non-diabetic rats.
5. We have demonstrated firstly that the application of the PPAR-γ agonist in the isolated perfused kidney system reduced the ischemia indicated reversible, structural deviations, as well as the ischemia indicated apoptotic signal transduction processes after the development of ischemia.
6. The application of the Krebs-Henseleit solution as basic medium did not influence the structural appearance of early (reversible) ischemic injury significantly in isolated kidney perfused system

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