Cellular and molecular background of the prevention of tissue ischemia/reperfusion injury - experimental and clinical investigations

The work done in this study describes and discovers in details the background of the ischemia-reperfusion injury and its possible opportunities to reduce the extent and severity of these injuries. We have planned on one hand in vivo experiments in animal models and on the other hand clinical experiments to investigate and discover the possible pathways playing crucial role in decreasing the injury of tissues, reduce the phosphorilation of proapoptotic enzymes and diminish the developing inflammatoric response.

Our study describes in details the effect of used drugs, ischemic postconditioning, controlled reperfusion, enzyme agonist on ischemia-reperfusion injury after cardiovascular interventions.

In this study 9 articles have been accepted by different international journals with IF.

As a result of this study 3 researcher/clinician colleagues have taken the PhD degree. (Viktória Kovács, Tibor Nagy, Péter Hardi) Another 5 colleagues have written their dissertations and they are now under consideration. (Gyöngyvér Tünde Veres, Gábor Fazekas, Örs Pintér, Laura Petrovics, Laura Bognár)

As a result of work have done in this study, one of our colleague has been nominated as a full-professor at the University of Pécs, Medical School. (Endre Arató)

The effects of penthosan polysulfate natrium on ischaemia-reperfusion injury

Introduction: There is a real risk of ischemic and reperfusion injury during vascular surgery affecting different arteries such as renal arteries. The elongated ischemia followed by reperfusion causes a cellular oxidative stress, local and systemic inflammatoric response.

Aims: We studied the effects of penthosan polysulfate natrium (PPSN) on ischaemia-reperfusion injury of the kidneys. We aimed to examine the oxidative stress parameters (OSP), the inflammatoric response and the activation of proapoptotic signaling proteins (PSP) after revascularization surgery in normal and diabetic rats.

Methods: 60 Wistar rats in six groups underwent a 45 min left renal arteric cross clamping. The half of the animals suffered from diabetic disease, induced by an intravenous administration of streptozotocine three month earlier. The ischaemic phase was followed by a 90 min of reperfusion. In two groups the animals were treated with high dose intravenous PPSN administered prior to the reperfusion. In two groups of animals were treated with intraperitoneus PPSN administered daily for a week before the operation. Blood samples and biopsy from left kidneys were collected. Plasma malondialdehyde, reduced glutathione, -SH-

groups, TNF-alpha, IL-1 concentrations and superoxide dismutase enzyme activity were measured. Apoptotis and proapoptotic signal pathways were detected.

Results: The levels of OSP and the inflammatoric proteins have shown no changes when PPSN was administered before the iscemic injury in non diabetic rats. The administration of PPSN prior to the revascularisation can reduces the levels of OSP and inflammatoric proteins in non diabetic rats. The ratio of phosphorylated proapoptotic proteins was lower in the PPSN administered prior to reperfusion group in non diabetic rats.

The levels of inflammatoric proteins were non significantly lower in the case of prolonged administration of PPSN before ischemic injury, but have no changes when PPSN was administered prior to revascularisation in diabetic rats.

Conclusions: The administration of high dose PPSN during the ischemic injury has a benefit for non diabetic rats, but has no effect in diabetic rats. The prolonged, daily administration of PPSN is worth considering by reducing the inflammatoric response followed by an ischemic injury in diabetic rats.

The role of pre- and postconditioning to avoid the ischaemia-reperfusion injury caused by pneumoperitoneum

Objective: Laparoscopy is more beneficial technique in surgery than conventional open technique, but the used pneumoperitoneum has a lot ischemic side effect. Pneumoperitoneum causes hypoperfusion of abdominal organs, wich leeds to formation reactive oxigen specimens, and activation of leucocytes. Aim of our investigation was to compare and evaluate the beneficial effect of pre- and postconditioning

Methods: 70 female Wistar rats were used for the experiment. Animals were divided into 7 groups (each n=10): I. group sham (only anesthesia), II. group pneumoperitoneum with 5 mmHg for 60 min., III. group preconditioning with 5 mmHg (5 min. insuffletion and 5 minutes desuflation), and following 60 min. pneumoperitoneum (5mmHg), IV. group pneumoteritoneum with 5 mmHg, then postconditioning (5 minutes desufflation, 5 min. insufflation and finally desufflation), V. group pneumoperitoneum with 10 mmHg for 60 min., VI. group: preconditioning with 10 Hgmm then pneumoperitoneum for 60 min, VII. group pneumoteritoneum with 10 mmHg, then postconditioning (5 minutes desufflation, 5 min. insufflation and finally desufflation).

Pneumoperitoneum was created by Veres needle inserted into the abdominal cavity. Blood samples were taken 120 minutes after procedure. Within oxidative stress markers superoxide-dismutase (SOD) activity, reduced glutathione (GSH), sulfhydryl groups (SH-) and malondialdehid (MDA) concentration were measured.

Results: Such a sin pre and post conditioned groups the GSH activity was significantly lower, MDA activity was higher compared to sham group. SOD activity, GSH concentration was decreased in group comparing to control and 2xID+PP and ID+PP group. There was no

difference between the groups in SH- and MDA concentrations. In ID+PP group was the mildest surgical stress.

Conclusion: Based on our results we can declerate that pneumoperitoneum such as an increased intraabdominal pressusre has some side-effects. as short time pre- as postconditioning can reduce negative effects of pneumoperitoneum. This method has also important clinical implication.

Ischaemic postconditioning reduces reperfusion induced oxidative stress and inflammatory responses after human aortic surgery

OBJECTIVE: Abdominal aortic surgery subjects the lower extremities to ischemia and reperfusion. Although it is not extensive or prolonged, ischemia-reperfusion (IR) injury and reactive oxygen species (ROI) production of the lower extremities after aortic cross-clamping is gradually and steadily induced.

We studied the protective effects of ischaemic postconditioning (IP) on ischemia-reperfusion injury of the lower extremities in a clinical model of abdominal aortic intervention. We aimed to examine the evoked oxidative stress after the revascularisation surgery.

METHODS: In the prospective randomised study 30 patients were examined. The indications of the vascular-surgical intervention were aortic or iliacal chronic occlusion and abdominal aorta aneurism. The patients underwent an aorto-bifemoral bypass (ABB) surgery, where the aortic ischaemia lasted for 40 ±5 min. Patients were divided in two groups: lege artis ABB surgery and ABB surgery with 2x2min IP.

Peripherial blood sample collection was before the operation (G1), and after the reperfusion in the 2nd (G2) and 24th (G3) hours, and on the 7th day (G4).

We measured the rate and the speed of the free radical production of the leukocytes, and detected the activity of superoxide-dismutase (SOD) and the concentration of reduced glutathion (GSH). The degree of lipidperoxidation was marked with the quantity of malondialdehyde (MDA). The expressions of the adhesion molecules were measured with flowcytometry. Furthermore we examined the effectivity of postconditioning adaptation in the late (one month) clinical outcome.

RESULTS: Our results showed a time-dependently elevation in the oxidative stress parameters (SOD, GSH, MDA) in both group after declamping the aorta. In the postconditioned group the elevation was significantly lower in the very early reperfusion (G2) than in control group. The late clinical results did not showed difference between the groups.

CONCLUSIONS: Postconditioning could reduce the ROI production after IR in the early reperfusion period, thus limiting the ROI mediated tissue lesion, cytokine-leukocyte activation, and inflammatory responses. Postconditioning seems to be an effective tool in vascular surgery to reduce reperfusion injuries after revascularization interventions.

Polymorphisms in glutathione S-transferase are risk factors for perioperative acut myocardial infarction after cardiac surgery

Objectives: In the present study we explored glutathione S-transferase (GST) polymorphisms in selected patients who experienced accelerated myocardial injury following open heart surgery and compared these to a control group of patients without postoperative complications.

Methods: 750 patients records and biobank blood samples were screened of patients subjected to open heart surgery. Ultimately 132 patients (89 male and 43 female, mean age 59,84 years) were selected retrospectively to the trial. Patients were devided the following groups: Group I: control patients (n=78) and Group II.: study patients (n=54) with or without evidence of acute myocardial infarction in 24 hours following open heart surgery based on myocardial enzyme release criteria. Genotyping for GSTP1 A, B and C alleles was performed by using RT-PCR.

Results: The frequency of AA, AB and BC alleles was similar between control and study groups. However, the heterozygous AC allele was nearly three times elevated (18.5 vs 7.7%) in the patients who suffered postoperative myocardial infarction compared to controls. Contrary, we found allele frequency of 14.1% for homozygous BB allele in the control group whereas no such allele combination was present in the study group. In the multiple logistic regression analysis we found a significant difference in disease risk and in the carriers of the A allele combination.

Conclusions: These preliminary results may suggest some protective role for the B and C alleles during myocardial oxidative stress whereas the A allele may represent predisposing risk for cellular injury in patients undergoing cardiac surgery.

Controlled reperfusion of infrarenal aorta decreased ischaemic-reperfusion injuries after aortic clamping in vascular surgery

INTRODUCTION: Long aortic clamping in vascular surgery induces reperfusion injury accompained with serious oxidative stress and inflammatory responses. The hypothesis of this study was that the aortic occlusion followed by controlled reperfusion (CR) can reduce the biochemical changes that occur with ischaemia-reperfusion and the systemic and local inflammatoric response induced by oxydative stress.

MATERIALS AND METHOD: Animal model was used. Control group: animals undervent a 4-hour infrarenal aortic occlusion followed by continuous reperfusion. Treated group: animals were treated with CR: after a 4-hour infrarenal aortic occlusion we made CR for 30 minutes with the crystalloid reperfusion solution (blood:crystalloid solution ratio 1:1) on pressure 60 Hgmm. Blood samples were collected different times. The developing oxydative stress was detected by the plasma levels of malondialdehyde, reduced glutathion, thiol groups and superoxide dismutase. The inflammatoric response was measured by phorbol myristate acetate-induced leukocyte reactiv oxygene species production and detection of change in myeloperoxidase levels. We examined the TNF-alpha and HIF1-alpha tissue changing. The

animals were anaesthetised one week after terminating ligation and biopsy was taken from quadriceps muscle and large parenchymal organs.

RESULTS: CR significantly reduced the postischaemic oxydative stress and inflammatoric responses in early reperfusion period. Pathohistological results: The rate of affected muscle fibers by degeneration was significantly higher in the untreated animal group. The infiltration of leukocytes in muscle and parenchymal tissues was significantly lower in the treated group.

CONCLUSION: CR can improve outcome after acute lower-limb ischaemia. The results confirm, that CR might be also a potential therapeutic approach in vascular surgery against reperfusion injury in acute limb ischaemia.

Relationship between GST genetic polymorphism and oxidative stress parameter changes and clinical complication rates in patients undergoing coronary artery surgery or intervention.

Introduction: Reperfusion, following coronary artery surgery or intervention, induces oxidative stress and inflammatory responses. The extent of these responses substantially influences the rate and the course of the clinical complications. The GST antioxidant enzyme plays a key role in cellular protection against reperfusion induced oxidative stress. Genetic variant of GST can significantly determine the effectiveness of defence against oxidative stress.

Objective: We wanted to study the relationship between GST polymorphism, the extent of oxidative stress induced by cardiac interventions and the consequent clinical complications.

Patients and methods: Patients undergoing isolated coronary artery bypass operation or coronary artery intervention were involved into this prospective, randomized study. The GSTP1 allel pair genetic variations were determined by RT-PCR method. Oxidative stress was examined by malondialdehyde, reduced glutathione, thiol-group and superoxide dismutase plasma levels. The inflammatory response was measured by phorbol myristate acetate – induced radical production in leukocytes and changes in the myeloperoxidase plasma levels. Blood samples were taken a day before surgery, 1 and 24 hours and 7 days after surgery. All cardiopulmonary complications and necro-enzyme changes were recorded.

Results: Our results show that in GSTP1 homozygous BB genotype patients the oxidative stress parameters, the inflammatory markers and perioperative complication rates were significantly lower.

Conclusions: GST enzyme polymorphism examination is suitable to estimate the perioperative risk in cardiovascular interventions.

Explicit role of a peroxisome proliferatoractivated receptor gamma agonist against renal ischemic failure in isolated perfused kidney system in rats

Background: Approximately 65-75% of all cases of acute renal failure caused by ischemic renal failure (IRF) still requiring symptomatic treatment. IRF as one of the most important risk factors of kidney transplantation also plays a determinative role in the manifestation of early and late graft failure in kidney transplantation. These processes can cause structural manifestations leading to functional failure of the kidney.

Objectives: The aim of our study was to protect renal survival against IRF by ameliorating renal ischemic tolerance with peroxisome proliferator-activated receptor gamma agonist (PPAR-γ Ago) in isolated perfused rat kidney model.

Material/Patients and Methods: Animal model was used (n=28). After the removal of kidneys from rats, kidneys were placed into a bottle filled with ice and water. After an hour perfusion with special solutions the animals were divided into four groups. Group 1 (control): no perfusion of the kidneys. Group 2: perfused solution was Krebs-Henseleitsolution (KH). Group 3: perfused solution contained KH and PPAR-γ Ago. Group 4: perfused solution consisted of KH, PPAR-γ Ago and PPAR-γ Ago inhibitor. The structure of kidneys and apoptotic/anti-apoptotic protein expression were analyzed in light microscope and Western-blot.

Results: PPAR-γ Ago treatment reduced the ischemic failure in Group 3. In the control group the tubular lumen dilatation was remarkably larger and apoptotic protein overexpression was shown. There was no significant difference between the control and KH-perfused groups.

Conclusion: The results confirm, that PPAR-γ Ago can protect against ischemic failure in kidney.

Ischemic preconditioning may reduce oxidative stress following laparoscopic cholecystectomies – Clinical trial

Background: Laparoscopy is morebeneficial technique than conventional open technique, but the used pneumoperitoneum has a lot ischemic side effect.

Objective: Aim of our investigation was to evaluate the potential protective effects of preconditioning during laparoscopic cholecystectomies (LC). Based on our rat experiments preconditioning has a beneficial effect.

Methods: 30 patients undergoing laparoscopic cholecystectomy were randomized to 2 groups: I. PREC (preconditioning: 5 min. insuflation, 5 min. desuflation, followed by conventional LC), II: LC (conventional LC). Blood samples were taken before surgery (C=control), before surgery, but after anaesthesia (B. S.), after surgery (A. S.) and 24 hours after the procedure (24h). Measured parameters: malondialdehyde (MDA), reduced glutathione (GSH), sulfhydril groups (-SH), superoxidedismutase (SOD), catalase (CAT), myeloperoxidase (MPO), AST, ALT, GGT, length of hospitalization and pain (VAS=visual analogue scale).

Results: The change of SOD's activity and MDA levels there were not remarkable compared with the B. S. results. GSH concentrations were near significantly increased in the PREC group after operation. SH-, MPO, CAT and liver function enzymes were not significantly different.

Hospitalization was shorter in PREC group. Based on the VAS patients had less pain in the PREC group.

Conclusion: Significant differences concerning PREC objects were only found in the case of GSH. It is remarkable that in PRECgrouppain was decreased with 2-2,5 scale units following the procedure and 24 hours after surgery. In our previous experiences in rats the potential protective effect of preconditioning seemed to be proven, but in humans the already analysed data have not shown remarkable differences.

Inhibition of Glutathione S-transferase by ethacrynic acid augments the ischaemia-reperfusion damages and apoptosis and attenuates the positive effect of ischaemic postconditioning in bilateral acut hindlimb ischaemia rat model

Aims: We studied the effects of inhibition of the endogene antioxidant glutathione S-transferase (GST) by ethacrynic acid (EA) on ischaemia-reperfusion injury and postconditioning of the lower extremities. We aimed to examine the oxidative stress parameters (OSP), the inflammatoric response and the activation of proapoptotic signaling proteins (PSP) after revascularization surgery.

Methods: 60 Wistar rats in six groups (control, IR, PC, EA-control, IR/EA, PC/EA). IR, PC, IR/EA and PC/EA rats underwent a 60 min infrarenal aortic cross clamping. After the ischaemia in PC and PC/EA groups ischaemic postconditioning was performed. In three signed groups the animals were treated with EA (EA-control, IR/EA, PC/EA) as well. The ischaemic phase was followed by a 120 min of reperfusion. Blood samples and biopsy from quadriceps muscle were collected. Plasma malondialdehyde, reduced glutathione, -SH-groups, TNF-alpha, IL-6 concentrations and superoxide dismutase enzyme activity were measured.

Results: The levels of OSP and the inflammatoric proteins were significantly higher in the EA administered groups. The ratio of phosphorylated proapoptotic proteins was higher in the EA administered groups and the protective effect of postconditioning did not develop.

Conclusions: Inhibition of GST by EA augments the ischaemia-reperfusion damages. GST inhibition was associated with different activation of mitogen activated protein kinases and PSP regulating these pathways in the process of apoptosis and postconditioning.

Controlled reperfusion reduces hemorheological alterations in a porcine infrarenal aortic-clamping ischemia-reperfusion model

INTRODUCTION:

Restoration of blood flow after prolonged acute ischemia causes further injury to tissues. The role of increased oxidative stress is emphasized in the pathogenesis, and impairment of hemorheological factors may also hinder proper microcirculation. Controlled reperfusion at lowered pressure with diluted blood may help to decrease reperfusion injury.

METHODS:

Four-hour infrarenal aortic clamping was performed in 16 Yorkshire pigs. In 8 animals blood flow was restored subsequently (full reperfusion, FR), in the other 8 animals clamping was followed by an initial 30 minutes of controlled reperfusion (CR) at 60 mmHg pressure with a 1 : 1 ratio mixture of blood and reperfusion solution. Blood samples were taken before the intervention, at the end of ischemia, 15 minutes, 60 minutes, 1 day and 1 week after the start of reperfusion. Hemorheological parameters were measured.

RESULTS:

Hematocrit, plasma and whole blood viscosity decreased significantly during CR, these attenuated at 1 day. At 1 week whole blood and plasma viscosities were elevated in the FR group. Erythrocyte deformability did not change significantly at any measurements. Erythrocyte aggregation decreased during CR but not in FR, and was found elevated in both groups at 1 week.

CONCLUSION:

Our results suggest slightly improved hemorheological properties in case of controlled reperfusion compared to full reperfusion, which may help to reduce tissue damage.

Pentosan polysulfate sodium (PPS) reduced renal ischemic-reperfusion induced oxydative stress and inflammatory responses in experimental animal modell.

Acute kidney injury (AKI) remains to be an indipendent risk factor for mortality and morbidity after vascular surgery affecting the renal arteries, or aortic surgery requireing suprarenal aortic clamping. These types of vascular surgery produce renal ischemia/reperfusion injury (IRI), a common cause of AKI.

The present studys are aimed at monitoring the course of renal ischemic and IRI in cellular level furthermore investigating the efficacy of longterm preoperativ and single shot intraoperativ administration of PPS to protect renal tissue from acute ischemic/reperfusion injury both in native and diabetic kidneys in rats.

Western-blot analysis of proapoptotic (bax), antiapoptotic (bcl-2) signal pathways,and extent of DNS damage(p-p53) were performed. Oxidative stress followed upon the termination of malondialdehyde (MDA), reduced glutation (GSH), tiol group (-SH), and superoxide dismutase (SOD) plasma levels. Inflammatoric changes are mesured by determination of serum tumor necrosis faktor (TNFα), interleukin 1 (IL-1) levels. Morfological changes were detected by hystological examinations.

Our investigation results showed that long term administration of PPS has an advanatge to reduce ischemic/reperfusion kidney injury in diabetic rats, while high dose single shot parenteral administration of PPS prior to revascularisation might be usefull in nondiabetic rats.

Oxidative stress and leukocyte activation after lower limb revascularization surgery

The examination aimed to study of oxidative stress and thrombocyte function in the perioperative interval following the revascularization surgery of lower limb. The prospective randomised study involved 10 patients whose surgical interventions were indicated by lower limb embolism, thrombosis or abdominal aorta aneurysm, and 10 healthy volunteers were also involved in the study. Peripheral blood samples were collected before, and after the surgery (2, 24 hours and one week). The maximal free radical production and lag time of the free radical production of activated leukocytes were measured, and leukocyte adhesion molecules (CD11a and CD18) signing leucocyte activation were determined as well. Endogenous antioxidant defence status, reduced glutathione (GSH), total thiol-groups (-SH), SOD activity and thrombocyte function were investigated in platelet rich plasma and in whole blood. White blood cell count and free radical production was significantly higher in patients group before surgery than in healthy group (in case of the free radical production the difference proved to be 10 times ($p < 0.01$)) and elevated continuously during the observation time. The CD11a and CD18 expression of the granulocytes significantly decreased right after the revascularization, but with a gradual elevation, until the 7th day they exceed the ischaemic value. GSH concentration decreased significantly 2 and 24 hours after surgery and total thiol groups (-SH) followed the same kinetics. SOD activity was significantly lower in patients group haemolysates before surgery when it was measured in healthy groups ($p < 0.01$) and decreased further significantly 24 hours after the surgery ($p < 0.01$ vs. before surgery). Suppressed thrombocyte aggregation was detected in platelet rich plasma and in whole blood.

Pentoxifylline attenuates the local and systemic inflammatory response after infrarenal abdominal aortic ischemia-reperfusion.

AIMS:

We studied the new anti-inflammatory effects of non-specific phosphodiesterase (PDE) inhibitor pentoxifylline (PTX) on ischaemia-reperfusion injury and postconditioning of the lower extremities. We aimed to examine the oxidative stress parameters (OSP), the inflammatory response and the changes in structure of skeletal muscle after revascularization surgery.

METHODS:

50 Wistar rats in five groups underwent a 60 min infrarenal aortic cross clamping. After the ischaemia in IR+PC group ischemic postconditioning was performed, intermittent 15 seconds reperfusion, 15 seconds ischaemic periods were applied four times. The ischemic phase was followed by a 120 min of reperfusion. In IR+PTX group the animals were treated with PTX. In IR+PC+PTX group both ischemic postconditioning and PTX treatment were performed. Blood samples and biopsy from quadriceps muscle were collected. Plasma malondialdehyde, reduced glutathione, -SH-groups, TNF-alpha, IL-6 concentrations and superoxide dismutase enzyme activity were measured.

RESULTS:

The levels of OSP and the inflammatory proteins were significantly higher in the IR group. PTX treatment and PC could significantly decrease the levels of OSP and inflammatory proteins. When the animals were co-treated with PTX and PC the results were even better.

CONCLUSIONS:

Inhibition of PDE by PTX could markedly decrease the inflammatory response and moderate the ischaemia-reperfusion damages after lower limb ischemia and reperfusion. Administration of PTX could potentiate the beneficial effects of PC.

Controlled reperfusion decreased reperfusion induced oxidative stress and evoked inflammatory response in experimental aortic-clamping animal model.

Revascularization after long term aortic ischaemia in vascular surgery induces reperfusion injury accompanied with oxidative stress and inflammatory responses. The hypothesis of this study was that the aortic occlusion followed by controlled reperfusion (CR) can reduce the ischaemia-reperfusion injury, the systemic and local inflammatory response induced by oxidative stress. Animal model was used.

CONTROL GROUP:

animals underwent a 4-hour infrarenal aortic occlusion followed by continuous reperfusion. Treated group: animals were treated with CR: after a 4-hour infrarenal aortic occlusion we made CR for 30 minutes with the crystalloid reperfusion solution (blood: crystalloid solution ratio 1:1) on pressure 60 Hgmm. Blood samples were collected different times. The developing oxidative stress was detected by the plasma levels of malondialdehyde, reduced glutathion, thiol groups and superoxide dismutase. The inflammatory response was measured by phorbol myristate acetate-induced leukocyte reactive oxygen species production and detection of change in myeloperoxidase levels. The animals were anaesthetized one week after terminating ligation and biopsy was taken from quadriceps muscle and large parenchymal organs.CR significantly reduced the postischaemic oxydative stress and inflammatory responses in early reperfusion period. Pathophysiological results: The rate of affected muscle fibers by degeneration was significantly higher in the untreated animal group. The infiltration of leukocytes in muscle and parenchymal tissues was significantly lower in the treatedgroup.CR can improve outcome after acute lower-limb ischaemia. The results confirm that CR might be also a potential therapeutic approach in vascular surgery against reperfusion injury in acute limb ischaemia. Supported by OTKA K108596.

Effects of a PPARG agonist on ischemia-reperfusion injury in bilateral hindlimb ischaemia rat model

The peroxisome proliferator-activated receptor-γ (PPARG) is a member of the nuclear receptor superfamily. PPARs are ligand-dependent transcription factors that bind to specific peroxisome proliferators response elements at the enhancer sites of regulated genes. They

are implicated in adipocyte differentiation, insulin sensitivity and inflammatory processes and also down-regulate proinflammatory mediators in macrophages.

AIMS: The aim of the study was to investigate the effects of novel therapies on bilateral hind limb ischemia reperfusion injury following a temporal infra-renal aortic occlusion and reperfusion. A PPARGA was applied in rats. Two experiments were planned: time course experiment, and the dosage experiment. Oxidative stress was followed upon the determination of malondialdehyde (MDA), reduced glutathione (GSH), thiol group (-SH), and superoxide dismutase (SOD) plasma levels. In addition the expression patterns of SOD-mRNA (messenger ribonucleotic acid) and PPARG-mRNA were measured with RNA extraction, complementary deoxyribonucleotic acid (cDNA) synthesis and semi-quantitative reverse transcription polymerase chain reaction (PCR) analysis.

METHODS: A PPARGA solution was injected in an appropriate concentration (e.g., 10, 50, 100, or 500 μM at a final concentration) into the superior mesenteric vein at different time points (e.g., 20, 40, or 60 minutes before reperfusion of the ischemic period).

Time course experiment: The rats were divided randomly into five groups. The first group was a group of operated rats that underwent ischemia followed by an hour-long reperfusion without being treated with PPARGA (operated untreated control). The other four groups included operated rats that underwent ischemia followed by an hour-long reperfusion with aPPARGA treatment (intravenously; final concentration of 100 μM) either 0 minute, 20 minutes, 40 minutes, or 60 minutes before reperfusion.

Dosage experiments: Rats were randomly divided into five groups. The first group was an operated untreated control. The other four groups included operated rats that underwent ischemia followed by an hour-long reperfusion with an intravenous PPARGA treatment 20 minutes before reperfusion at a final concentration of either 10 μM, 50 μM, 100 μM, or 500 μM of PPARGA.

In another dosage experiment, rats were randomly divided into five groups. The first group was an operated untreated control. The other four groups included operated rats that underwent ischemia followed by an hour-long reperfusion with an intravenous PPARGA treatment 40 minutes before reperfusion at a final concentration of either 10 μM, 50 μM, 100 μM, or 500 μM of PPARGA.

Results of the time course experiment:

The PPARGA treatment efficiently diminished the level of MDA at all time-points tested. We have observed increases in reduced GSH levels when PPARGA was administered 40 or 60 minutes prior to reperfusion and the observed increases in SOD activity at all timepoints tested.

Results of dosage experiments I.

The PPARGA treatment efficiently diminished the level of MDA when injected to deliver a final concentration within the rats of 10, 50, 100, and 500 μM. In addition, administration of PPARGA at 100 and 500 μM resulted in an increase in SOD activity. Administration of PPARGA

at 50, 100, and 500 μM 20 minutes before reperfusion resulted in a decrease in the levels of reduced GSH levels.

Results of dosage experiments II.

The PPARGA treatment efficiently diminished the level of MDA when injected to deliver a final concentration within the rats of 100 and 500 μM. In addition, administration of PPARGA at 50, 100, and 500 μM resulted in an increase in the levels of reduced GSH levels. Administration of PPARGA at 10, 100, and 500 μM also resulted in an increase in SOD activity. Further, the gene expression pattern of SODcorrelated with the SOD activity results as samples for gene expression analysis taken from renal tissue exhibited increased levels of SOD mRNA expression as the amount of PPARGA injected 40 minutes before reperfusion increased. Administration of PPARGA at 10, 50, 100, and 500 μM resulted in an increase in the levels of SH. Thiols can help aerobic cells maintain their reducing state in an oxidizing environment. Thus, higher total thiol levels can indicate a greater reducing state of the cell. Administration of PPARGA 40 minutes before reperfusion also resulted in increased expression of PPARG mRNA.

DISCUSSION: We used in our investigations a non-synthetic PPARGA. We found that the administration of PPARGA can reduce severity of the ischemia reperfusion injury through decreasing the systemic inflammatory response. In the time course experiments results also demonstrate that PPARGA is effective when administered at the time of reperfusion or when administered before reperfusion (e.g., 20, 40, or 60 minutes before reperfusion). In the first dosage experiment results demonstrate that PPARGA may be used more effectively as an inhibitor of oxidative stress during the ischemia-reperfusion process when administered more than 20 minutes prior to reperfusion. In the second dosage experiment results confirm that PPARGA is an effective inhibitor of oxidative stress during the ischemia reperfusion process at levels as low as 10 to 50 μM.

Pneumoperitoneum induced ischemia-reperfusion injury of the peritoneum - Preconditioning may reduce the negative side-effects caused by carbon-dioxide pneumoperitoneum - Pilot study.

INTRODUCTION:

Laparoscopy is more beneficial than the conventional open technique, however the pneumoperitoneum created may have an ischemic side effect.

OBJECTIVE:

Our aim was to evaluate the protective effects of preconditioning during laparoscopic cholecystectomies (LC).

METHODS:

30 patients were randomized into 2 groups: I. PreC (preconditioning: 5 min. inflation, 5 min. deflation, followed by conventional LC), II: LC (conventional LC). Blood samples were taken before hospitalization (C = control), before surgery, after anaesthesia (B.S.), after surgery (A.S.) and 24 hours after the procedure (24 h). Measured parameters were: malondialdehyde (MDA), reduced glutathione (GSH), sulfhydril groups (-SH), superoxide-dismutase (SOD), catalase (CAT), myeloperoxidase (MPO), length of hospitalization and pain (VAS = visual analogue scale).

RESULTS:

Compared to the BS levels, no significant changes were detected in SOD's activity and MDA levels. GSH concentrations were significantly increased in the PreC group after operation. SH, MPO, CAT and liver function enzymes were not significantly different. Hospitalization was shorter in the PreC group. Based on the VAS score patients had less pain in the PreC group.

CONCLUSION:

Significant differences concerning PreC group were found in GSH values. In the PreC group pain decreased by 2-2.5 units following the procedure, 24 h after surgery, and hospitalisation was also significantly shorter. In our pilot study the potential protective effect of preconditioning could be defined.

The effect of trimetazidine in reducing the ischemia-reperfusion injury in rat epigastric skin flaps.

BACKGROUND:

Ischemia-reperfusion injury may lead to insufficient microcirculation and results in partial flap loss during the free flap surgeries.

OBJECTIVE:

This study aimed to investigate the effect of trimetazidine (TMZ) on oxidative stress, inflammation and histopathological changes, using the epigastric skin flap model in rats.

METHODS:

40 male Wistar rats were used, that were divided into four groups. Control group, non-treated ischemic (I/R)-group and two trimetazidine treated groups (preischemically, postischemically) were established. To create ischemia in the skin flap, the superficial epigastric vessels were clamped for six hours, followed by twenty-four hours of reperfusion. Blood samples and biopsies from skin flaps were collected at the end of the reperfusion period. The inflammatory

response, the degree of oxidative stress (by measuring the plasma level of malondialdehyde (MDA), reduced glutathione (GSH); sulfhydryl (-SH) groups) and histopathological changes were evaluated.

RESULTS:

Inflammatory response, and oxidative stress were significantly attenuated in the trimetazidine treated groups, compared to the non-treated ischemic group. Histopathological findings were also correlated with the biochemical results.

CONCLUSION:

In our study trimetazidine could reduce the ischaemia-reperfusion injury, even after an unexpected ischemic period, so it is a promising drug during free tissue transfer, replantation or during revascularization procedures in the future.

The results of this study are based on a valuable collaboration between the Department of Surgical Research and Techniques, the Clinic of Vascular Surgery, the Clinic of Surgery and the Clinic of Cardiac Surgery at the University of Pécs, Medical School.