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

PhD Thesis

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1. Introduction

Any form of trauma, including surgery, is known to result in oxidative stress. Increased intra-abdominal pressure during pneumoperitoneum and inflation-deflation may cause ischemia reperfusion and, so, oxidative stress may be greater during laparoscopic surgery. During an ischaemic period the duration of ischemia could also be serious, thus after reconstruction we always have to face with reperfusion injury. The aim to reduce the reperfusion injury associated pathways has real clinical importance in laparoscopic surgery.

The pathogenesis of reperfusion injury is a complex process involving numerous mechanisms exerted in the intracellular and extracellular environment. Reperfusion injury is an obligatory response to the restoration of blood flow after ischemia and is initiated at the very early moments of reperfusion, lasting potentially for days. The extent of the oxidative stress and the consecutive generalized inflammatory response depend on the ischemic-time, the ischemic tissue volume, and the general state of the endothelium leukocyte-tissue functional complex. Ischemia/reperfusion (I/R) can induce various forms of cell death, such as programmed cell death, apoptosis, oncosis and necrosis. Free radical formation is increased during abdominal surgery as a result of ischemia-reperfusion, leukocyte activation, and mitochondrial dysfunction. Apoptosis can be caused by both prolonged ischemia/hypoxia and by reperfusion as well. The mechanisms of reperfusion-induced cell death are not completely understood, but it seems that the occurrence of oxidative stress related to the generation of ROS (Reactive Oxygen Species) may play an important role. ROS has downstream effects, that results in the initiation of a highly orchestrated acute inflammatory response through the release of cytokines, activation of vascular endothelial cells and leukocytes with expression of cell surface adhesion molecules, and up-regulation of a program of pro-inflammatory genes, that contribute to the onset and maintenance of post-ischemic inflammation. Free radicals are highly reactive molecules with unpaired electrons, which are continuously produced in the body by mitochondria, leucocytes, and xanthine oxidase. They have important biological functions such as redox signaling and antibacterial defense. However, they can also react with proteins, lipid membranes, DNA (Deoxyribonucleic acid) and cause damage. The human body is endowed with a complex system of enzymatic and non-enzymatic antioxidants to counteract these adverse effects of free radicals. Oxidative stress occurs when there is an imbalance between the production of free radicals and antioxidant levels. Oxidative stress can be quantified by measuring different biomarkers. This can be done by direct measurement of free radicals, the end-products of free radical damage, or the levels of individual and total antioxidants. Different

biomarkers have been used in various clinical settings, and there is not a single biomarker that can truly represent oxidative stress.

The phenomenon of ischemic preconditioning has been recognized as one of the most potent mechanisms to protect against myocardial ischemic injury. In experimental animals and humans, a brief period of ischemia has been shown to protect the heart from more prolonged episodes of ischemia, reducing infarct size, attenuating the incidence, and severity of reperfusion-induced arrhythmias, and preventing endothelial cell dysfunction. Although the exact mechanism of ischemic preconditioning remains obscure, several reports indicate that this phenomenon may be a form of receptor-mediated cardiac protection and that the underlying intracellular signal transduction pathways involve activation of a number of protein kinases, including protein kinase C, and mitochondrial K_{ATP} channels. Among others, oxidant stress can modify some of the cellular activities that have been implicated in vivo as mediators of the IPC phenomenon. It could thus be hypothesized that reperfusion after the initial, “preconditioning” ischemic episode results in the generation of relatively low amounts of oxygen radicals, insufficient to cause cell necrosis, but enough to modify cellular activities and thus induce IPC. Ischemic PC was first identified in 1986 by Murry et al. This group exposed anesthetized open-chest dogs to four periods of 5 minute coronary artery occlusions followed by a 5-minute period of reperfusion before the onset of a 40-minute sustained occlusion of the coronary artery. The control animals had no such period of “ischemic preconditioning” and had much larger infarct sizes compared with the dogs that did. Aksöyek et. al described that preconditioning can reduce ischemic damage in abdominal organs as well. While relatively easy to implement in a controlled, surgical setting such as transplantation or cardiac surgery, ischemic preconditioning (IPC) is not well-suited to emergency settings, as the onset of myocardial or brain infarction cannot be anticipated.

Short periods of ischemia performed just at the time of reperfusion can reduce the infarct size. IPoC was first described by Vinten-Johansen’s group. IPoC can be obtained by different protocols in terms of duration of the periods of reperfusion and ischemia and/or in terms of number of cycles of I/R applied after a sustained ischemia. Virtually in all of the species in which different IPoC algorithms have been tested the proved to be protective, including humans.

Taken together both IPC and IPoC activate the same key pathways, that include phosphatidylinositol 3-kinase-Akt and extracellular signal–regulated kinase (ERK/p42-44). IPC and IPoC influence a variety of endogenous mechanisms that operate at numerous levels and target a broad range of pathological mechanisms.

2. Aims and hypothesis

In all parts our aim was to find a way to reduce the oxidative stress caused by pneumoperitoneum.

In the first part of our investigations we aimed to examine and compare the protective effects of ischemic pre- and postconditioning against oxidative stress caused by pneumoperitoneum in rat animal model. In the literature there are many publications about the effect of IPC in animal models during laparoscopy, but none about IPoC. We used low pressure even at pre- and postconditioning method (5, 10 mmHg), and we compared our findings to normal pneumoperitoneal and sham findings. Furthermore, to confirm the protective effect of the applied ischemic IPC and IPoC we monitored the activation of intracellular anti- and proapoptotic common signaling pathways (Bax, bcl-2, p53) during the early phase of reperfusion.

In the second part we proportioned PPAR-γ (abscisinic acid), a fitohormone in definite times before creating the pneumoperitoneum, or before deflating the abdomen after 60 minutes pneumoperitoneum. We aimed to prove that PPAR-γ may reduce the oxidative stress caused by pneumoperitoneum. We administered the same PPAR-γ doses in certain times before pneumoperitoneum and before deflating the abdomen. We aimed to evaluate the best administration time of the PPARGA in reducing oxidative stress.

In the final third part based on good results concerning laparoscopic preconditioning on rats, we wanted to verify the protective effect of ischemic preconditioning on humans waiting for laparoscopic cholecystectomy. In our pilot study we aimed to establish a clinical model reproducible in any hospital. We compared patients' oxidative stress parameters data before and after operation in a conventional LC group and an IPC group. We measured hospitalization days, and postoperative pain as well.

3. Methods

3.1. Analysis of oxidative stress parameters

MDA, expressing the lipid peroxidation of cell membranes was specified form plasma or diluted blood. The concentration of MDA was determined using a spectrophotometer based on Placer, Cushman and Johnson method. MDA concentration was expressed in nM/ml.

For the determination of GSH and SH- we used anticoagulated blood (EDTA), which was mixed with Ellman-reagent based on Sedlac and Lindsay protocol. The concentrations of GSH and SH- were determined using a spectrophotometer, and expressed in nM/ml.

For the determination of SOD activity, blood was mixed with EDTA, then Hartman's solution was added to the blood sample. A mixture of chloroform and ethanol were added to hemolyzed red blood cells and they were centrifuged. Supernatant was separated thereafter, and adrenalin was added to it. The concentration of SOD was determined by using a spectrophotometer and expressed in U/ml.

For the determination of MPO activity: work solution (10.9 ml Na-citrate, 100 µl *o*-Dianisidin) was mixed with plasma. The compound was incubated then perchloric acid was added to the mixture and centrifuged. MPO concentration was measured using spectrophotometer and expressed in U/l.

To determinate the catalase enzyme level we mixed buffer, 1 ml of peroxide solution and 100 times diluted, washed red blood cell. With spectrophotometer we measured the loss of peroxides. Catalase levels were expressed in BE/ml.

3.2.Serum TNF-alpha and IL-6 quantification

For measuring TNF-alpha and IL-6 concentration in serum we used Rat TNF-alpha and Rat IL-6 ELISA kit (R&D Systems, Inc., Minneapolis, USA), following the manufacturers protocol. These methods determine the free biological active TNF-alpha and IL-6 concentrations.

3.3.Histological analysis

The animals were terminated at the end of the experiment and biopsy was taken from the liver, kidneys, bowels and abdominal muscle. The definite aim of the biopsy was to register the qualitative differences inchanges between the animal groups, firstly the transformations in the

kidney tissues. Paraffin-embedded blocks were made from kidney-pieces, and sample slices were prepared staining by hematoxylin and eosin.

3.4.Western bloth analysis

The Western-blot analysis of proapoptotic (bax) and antiapoptotic (bcl-2) signaling pathways and extent of DNA damage (p-p53). Fifty milligrams of 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, membranes were probed overnight at 4°C with antibodies recognizing the following antigenes: (polyclonal Bax antibody, 1:1000 dilution), pospho-p53MAPK (Thr180/Tyr182, 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.

4. The role of pre- and postconditioning to avoid the ischemia/reperfusion injury caused by pneumoperitoneum

Wistar rats in both sexes, weighed between 200-300 g were used in the study. The animals were acquired from the university animal house and were housed in individual cages in ambient temperature and light-dark cycle-controlled environment with free access to food and water. Pneumoperitoneum was created with Veres, that was transumbilically inserted. For the creation of pneumoperitoneum an automatic insufflator was utilized, applying CO_2 gas at 5 mmHg and 10 mmHg pressures, respectively for 60 min. Animals were divided into seven groups. I. Sham operation, only Veres needle was inserted. In group II. we created pneumoperitoneum with 5 mmHg for 60 min. (5 mmHg). In group III. we used preconditioning (inflation and deflation

for 5 min) with 5 mmHg then pneumoperitoneum was created with 5 mmHg (60 min) (5 mmHg IPC)**.** Group IV.: Pneumoperitoneum with 5 mmHg was created for 60 min. then postconditioning with 5 mmHg (after 60 min. pneumoperitoneum deflation for 5 minutes, then inflation for 5 min (5 mmHg) and at the end deflation was performed (5 mmHg IPoC). Group V. pneumoperitoneum with 10 mmHg for 60 min. (10 mmHg). In group VI. we used preconditioning (inflation and deflation for 5 min) with 10 mmHg, then pneumoperitoneum was created with 10 mmHg (60 min), (10 mmHg IPC). Finally Group VII.: Pneumoperitoneum with 10 mmHg was created for 60 min., then postconditioning with 10 mmHg (after 60 min. pneumoperitoneum deflation for 5 minutes, then inflation for 5 min (10 mmHg) and at the end deflation was performed. (10 mmHg IPoC). After deflation reperfusion time was 120 min. Two hours after the procedure blood samples were taken by heart puncture, and after that before termination of the animal's intraabdominal organs, such as liver, kidneys, small intestines, and muscle tissues as the diaphragm, and abdominal muscle sample were removed, and preserved in formalin and liquid nitrogen under congelation. In order to evaluate the severity of the oxidative stress the lipid peroxidation marker malondialdehyde (MDA), the endogenous antioxidant reduced glutathione (GSH), the concentration of sulfhydryl-group (SH-), as well as antioxidant superoxide-dismutase (SOD) and myeloperoxidase (MPO) activities were determined with upper mentioned methods. For measuring TNF-α and IL-6 concentration in serum we used Rat TNF-α and Rat IL-6 ELISA kit (R&D Systems, Inc., Minneapolis, USA), following the manufacturers protocol. For detection of pro- and antiapoptotic signaling pathways and extent of DNS damage Western blotting was used.

The fresh tissue was fixed in 10% neutral buffered formalin. Sectioning was performed with a sledge microtome from the paraffin-embedded blocks, and staining was carried out with a carousel-type slide stainer with hematoxylin and eosin at University of Pécs, Department of Pathology. To evaluate the histological slices we used the Pannoramic Viewer software (3DHistec Ltd.) and 40x magnification.

Statistical analysis was performed with the SPSS (Ver. 22.0) Statistical Software (SPSS, Chicago, IL, USA) using Independent Samples Kruskal-Wallis test. A p value of less than 0.05 was considered significant.

Results

We measured the values of malondialdehyde plasma-level indicating membrane damage and lipid peroxidation. MDA concentration in blood was significantly higher in all groups compared to Sham group. In 5mmHg IPC and 10 mmHg IPC we noticed lower but not significant

concentrations compared to 5 mmHg and 10 mmHg groups. Contrarily in plasma MDA concentration there was also significantly higher MDA concentration in each group compared to control, there was significant decrease in group IPC 10 mmHg and IPoC 10 mmHg compared to 10 mmHg group. In group 5mmHg IPoC we noticed significantly lower MDA concentrations compared to 5 mmHg group. GSH concentration in blood decreased significantly in all groups compared to Sham group. In groups 10 mmHg IPC and 10 mmHg IPoC we found significantly higher GSH concentrations compared to 10 mmHg group, meaning that preconditioning reduced oxidative stress. We perceived no alteration in SH- groups neither compared to Sham nor comparing groups to each other. In case of SOD activity, we noticed decreased SOD activity levels in groups 5 mmHg, 5 mmHg IPC, 5 mmHg IPoC, 10 mmHg. There was a significantly higher SOD activity in group 10 mmHg IPC compared to 10 mmHg. This data shows that the created pneumoperitoneum caused damage, but we could decrease this by using IPC. Examining MPO levels we noticed significantly lower levels in the not conditioned groups (5mmHg, 10 mmHg). Comparing groups to each other we noticed significantly higher MPO levels in 5mmHg IPC and 5mmHg IPoC compared to 5 mmHg, and the same comparing 10 mmHg group to 10 mmHg IPC and IPoC. TNF-α concentrations were significantly higher in all groups compared to Sham, except in group 10 mmHg IPC. In the 10 IPC group we noticed lower, but not significant alteration in the level of TNF-α compared to the control group. IL-6 concentrations: in groups 5mmHg, 10 mmHg and IPoC we noticed significantly higher IL-6 concentrations compared to Sham. We found lower but not significant concentrations in groups 5 mmHg IPC and 5 mmHg IPoC compared to 5 mmHg, and the same, lower but not significant concentrations in 10 mmHg IPC compared to 10 mmHg. TNF-α, and IL-6 alterations can show us, that pneumoperitoneum can activate the systemic inflammatory response, causes inflammation, and with the application of IPC inflammatory response could be decreased, but only on higher pressure (10 mmHg IPC group-versus 5 mmHg groups).

Histological examinations were most traceable and spectacular in kidney samples. In the Sham group there was no alteration, in the 5 mmHg group we noticed RBCs in chalix, bruising in renal parenchyma, but the ureter, glomeruli and renal capsule were intact. In group 5 mmHg IPC we saw disintegration of glomerular and tubular cells, minimal bruising in parenchyma, and damage of urethral epithelial cells. In 5 mmHg IPoC group we saw damaged cell membranes of glomeruli and tubules, nuclei were swollen, apoptotic, RBCs could be seen in parenchyma and necrosis as well, urethral epithel remained intact. In group 10 mmHg we saw bruising in glomeruli and tubuli as well. In group 10 mmHg IPC we noticed swollen nuclei,

bruising in glomeruli, renal parenchyma, and chalyx. Glomerular tubuli remained intact. And at last in group 10 mmHg IPoC the urethral epithelium and the distal tubuli were torn as well due to pressure. Urethral and parenchymal bleeding and apoptotic cells could be seen.

To characterize the expression of proapoptotic (bax) and antiapoptotic (bcl-2) signal proteins we used Western blot analysis to separate and measure them, and 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 not conditioned groups (5 mmHg, 10 mmHg). Decreased expression was detected in Sham, 5 mmHg IPC, 10 mmHg IPC and 10 mmHg IPoC groups. The expression of (bcl-2) antiapoptotic signal proteins was measured in all groups. Markedly higher expression of antiapoptotic bcl-2 level was measured in 10 mmHg IPC and 10 mmHg IPoC groups. Characterizing the extent of DNS damage phosphorilated p53 expression showed diminution in pre- and postconditioned groups, but significant diminution could be seen in groups 10 mmHg IPC and 10 mmHg IPoC compared to 10 mmHg group. A higher expression of p53 could be seen in Sham, 5mmHg and 10 mmHg groups.

Based on our results we can conclude that pneumoperitoneum associated with an increased intraabdominal pressure has some side-effects. During I/R caused by pneumoperitoneum free radicals accumulate causing oxidative stress. Analyzing oxidative stress parameters, we could measure the extent of injury. Short time pre- as postconditioning could reduce negative effects of pneumoperitoneum. Comparing the methods to each other we found both techniques good enough to reduce surgical harm. This method may also have important clinical implication.

5. The role of PPAR-γ agonist in avoiding the ischemia/reperfusion injury caused by pneumoperitoneum/ Experimental study on rats/

In this experimental study we aimed to investigate the effect of PPAR –γ on oxidative stress in the ischemia-reperfusion injury due to pneumoperitoneum. The peroxisome proliferator-activated receptor-gamma (PPAR –γ) is the member of the nuclear receptor superfamily. The PPARs are ligand dependent transcriptional factors that bind to specific peroxisome proliferator responsive elements in the enhancer region of the gene to be controlled. They play a role in controlling lipid cell differentiation, insulin sensitivity and inflammatory processes, as well as

in down-regulating the generation of pro-inflammatory mediators of the macrophages by blocking the transcription of NF-kB dependent inflammatory genes. We administered PPAR-γ agonist (abscisinic acid) in definite times before creating the pneumoperitoneum, or before deflating the abdomen after 60 minutes pneumoperitoneum. We aimed to prove that PPAR-γ may reduce the oxidative stress caused by pneumoperitoneum. We aimed to evaluate the best administration time of the PPARGA in reducing oxidative stress.

The investigations were performed on 60 Wistar rats (200-300 g). Pneumoperitoneum was created with Veres-needle, that was transumbilically inserted into the abdominal cavity, and the pressure was set to 10 mmHg for 60 minutes. Rats were divided into 6 groups (n=10/group, each): PPARγA (100 μMol) was given to the animals 45, 30 or 5 minutes before insufflation (Groups II-IV. 45 Pre, 30 Pre, 5 Pre), as well as 20 or 5 minutes prior to desufflation (Groups V-VI. 40 Isch, 55 Isch), sham animals were not treated (Group I. Sham). The tail vein was cannulated with a 24G intravenous catheter and PPAR- γ was administered through this catheter. After deflation reperfusion time was 120 min. Two hours after the procedure blood samples were taken by heart puncture, and after that before termination of the animals, intraabdominal organs such as liver, kidneys, small intestines, and muscle tissues as the diaphragm, and abdominal muscle were removed, and preserved in formalin and liquid nitrogen under congelation.

Oxidative stress parameters: malondialdehyde (MDA), reduced glutathione (GSH), sulfhydryl group (-SH) concentrations, superoxide-dismutase enzyme (SOD) activities were determined with the same method as mentioned in the introduction part. Using ELISA kit (IL-6 ELISA kit, TNF-α ELISA kit, both R&D Systems, Inc., Minneapolis, USA) we measured TNF-α and IL-6 cytokine concentrations following the manufacturer's protocol. In the Western-blot examination the detection of proapoptotic (Bax), apoptotic (p53), and antiapoptotic (Bcl-2) proteins was performed.

Statistical analysis was performed with the SPSS (Ver. 22.0) Statistical Software (SPSS, Chicago, IL, USA) using Independent Samples Kruskal-Wallis test. A p value of less than 0.05 was considered significant.

Results

We detected higher TNF-α leveles in each group compared to Sham. In group 40 Isch we found significantly lower levels compared to 5 Pre and 30 Pre-groups. Examining IL-6, we found elevated IL-6 leveles in all groups compared to Sham group. Comparing groups to each other we found the mentioned data: in 30 Pre, 40 Isch, 55 Isch groups we found significantly lower

IL-6 levels compared to 5 Pre-group. In SOD activity we found that 5 Pre-group data was significantly lower compared to Sham, 40 Isch and 30 Pre-groups. In group 45 Pre-group there was a significant decrease compared to 40 Isch and 30 Pre-groups. We also found significant decrease in group 55 Isch compared to 30 Pre-group. The mean of Sham, 30 Pre, 40 Isch groups remained at almost the same level showing that in group 30 Pre and 40 Isch PPARGA had a protective effect. We detected significantly lower GSH concentrations in all groups compared to Sham group. 5 Pre-group's data was significantly lower compared to 30 Pre group data. Data in 55 Isch group was significantly lower compared to 30 Pre and 40 Isch groups. While examining MDA blood levels we found, that in all groups MDA blood levels were significantly higher compared to Sham. There was no statistical difference between groups while comparing them to each other. Examining MDA plasma levels, we found that in all groups there was an increase compared to Sham, but we found significant alterations only in groups 45 Pre, 5 Pre and 55 Isch. 30 Pre-group was significantly lower compared to groups 45 Pre, 5 Pre and 55 Isch. In group 40 Isch we found significantly lower data compared to groups 5 Pre and 55 Isch. We also wanted to characterize the expression of proapoptotic (bax) and antiapoptotic (bcl-2) signal proteins, so we used Western blot analysis to separate and measure them, and 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 Sham, 45 Pre and 5 Pre-groups. Decreased expression was detected in groups 40 Isch, 55 Isch and even more in 30 Pre-group. The expression of antiapoptotic (bcl-2) signal proteins was measured in all PPARGA administered groups. Markedly higher expression of antiapoptotic bcl-2 level was measured in 40 -, and 55 Isch groups. Characterizing the extent of DNS damage phosphorilated p53 expression showed diminution in 30' Pre-group. There was no significant difference between groups in p53 expression.

Elevated intraabdominal pressure due to pneumoperitoneum triggers oxidative stress. Administration of PPARGA may reduce the harmful effect. Further experiments required to find the optimal timing of the injection.

6. Preconditioning, that may reduce the negative side effects caused by carbon-dioxide pneumoperitoneum /Clinical pilot study/

Based on our promising results in rat study, we aimed to investigate the protective effects of ischemic preconditioning (IPC) during laparoscopic cholecystectomies (LC).

This pilot study was conducted from February 2013 to June 2014 at the Surgery Clinic and at the Department of Surgical Research and Techniques, University of Pécs, Hungary. Informed consent was obtained from patients before the procedures. This study was carried out in accordance with the Code of Ethics of the Declaration of Helsinki. The study protocol was authorized by The Hungarian Committee of Ethics (No. ad.774/PI/2012; ad.50760/2012/EKU). At random a total of thirty patients waiting for laparoscopic cholecystectomy were enrolled for this prospective blinded clinical study. 15 patients were submitted to IPC before the operation, and 15 were operated on with a routine laparoscopic procedure. Patients aged between 18 and 70 could participate. Information sheets were given about the procedure, and patients signed an Informed Consent prior to the operation. Exclusion criteria included any known malignancy, morbid obesity, any disorder of the immune system, autoimmune disease, uremia, massive hypoproteinemia, icterus, chronic decompensated hepatic disorder, and refusal to participate. Laparoscopic operations were performed in the Surgical Clinic of University Pécs (Hungary); analysis of blood samples, and all statistical calculations were performed at the Department of Surgical Research and Techniques and the Institute of Bioanalysis, University of Pécs. All patients were operated on under general anesthesia. Antibiotics and low molecular weight heparin were not administered preoperatively. A skin incision was made in the umbilical region, and in the IPC phase pneumoperitoneum was created by CO_2 insufflation using a Veres needle. After trocars were inserted, the intra-abdominal pressure was set at 15 mmHg.

Preconditioning

Before starting the operation a 5-minutes interval was kept with constant 15 mmHg intraabdominal pressure, followed by another 5 minutes with complete desufflation of the abdomen. After this procedure a routine laparoscopic cholecystectomy was performed.

Venous blood samples were collected from patients on four occasions: before hospitalization (BH), after induction of anesthesia (A), after operation (AO) and on 1st postoperative day (Post). Lipid peroxidation marker malondialdehyde (MDA) concentration, endogenous antioxidant reduced glutathione (GSH), and sulfhydryl-group (SH-) concentrations, antioxidant superoxide-dismutase (SOD) and catalase (KAT) activities were measured from whole blood

for detection of the magnitude of the oxidative stress. Plasma malondialdehyde (MDA) concentration, and myeloperoxidase (MPO) activity was also measured. We also checked liver enzyme changes. Pain was evaluated with a Visual Analog Scale on the day of the operation, and 24 hours later. We tracked the hospitalization days. Size and state of wounds as well as adverse reactions were also evaluated.

The Statistical Package for Social Sciences (SPSS; SPSS Inc., Chicago, IL) version 22.0 was used for statistical analysis. Serum MDA, GSH, SH, SOD and plasma MDA, MPO levels were analyzed by Mann-Whitney or Wilcoxon signed – rank test. Statistical significance was set at $p \leq 0.05$.

Results

In total 30 patients were enrolled in the study. The mean age was 38.3±6.4 years and the length of procedure was 25–80 mins. Distribution ratio between male-female was 6:24. Average hospitalization was 2.37 days but in IPC group we noticed a shorter hospitalization by 10%. Comparing the two groups, the mean hospitalization was 2.67 days in the LC group, and 2.29 days in the IPC group.

Data was very heterogeneous therefore no statistical analysis was carried out. During control (C) we measured almost the same SOD activity in both groups. According to the B.S data it can be concluded that anesthesia causes decrease in SOD activity in both groups, but this decrease was not significant. In case of MDA concentration heterogeneity was also detected. There was no significant difference between LC and IPC groups. In case of GSH concentrations we also noticed heterogeneity, but in the IPC group a significantly higher concentration was measured postoperatively compared to the LC group. No significant changes were detected in SH-concentration and catalase levels. In case of plasma MPO activity and MDA concentration we noticed no significant changes.

Pain measured with Visual Analog Scale

A significant difference was noted in the IPC group, both after the operation and also 24 hours later. Pain in IPC group was about 2–2.5 units lower at both times. One day advantage was detected in pain level due to preconditioning.

Laparoscopic surgery causes systemic ischemia and this ischemic effect can be confirmed by measuring serum antioxidant levels. In our pilot study we established a clinical model

reproducible in any hospital. Based on the findings of our pilot study a larger scale multicenter trial can be initiated. For adequate result a more homogeneous patient population has to be selected (minimal comorbidity, no chronic medication, normal BMI etc.) Based on our findings it can be stated that preconditioning made laparoscopic operations 10 minutes longer, but shortened hospitalization, and decreased pain. Considering cost-benefit aspects, preconditioning might be introduced into everyday clinical practice.

7. Novel findings

In the first series of our investigations we observed the effects of IPC and IPoC methods during laparoscopic pneumoperitoneum in an animal (rat) model.

We have three important observations in the study. Both IPC and IPoC can reduce oxidative stress caused by pneumoperitoneum but based on our findings in group 10 mmHg IPC was more effective. To our knowledge no study was carried out neither with laparoscopic postconditioning nor with comparing laparoscopic pre- and postconditioning.

In the second part of our investigation we examined the effect of a non-synthetic PPAR-γ on I/R injury caused by constant peritoneal pressure. We found that the administered PPAR-γ had a protective effect during laparoscopic procedures, but this protective effect depended on the time of administration. Based on our findings PPAR-γ was more effective when it was administered 30 minutes before the procedure or after 40 minutes of ischemia and 20 minutes before the deflation of the abdomen.

In the third part of our investigation in a clinical pilot study we aimed to evaluate the protective effects of preconditioning during laparoscopic cholecystectomies. To our knowledge we were the first group to study the role of preconditioning during laparoscopic procedures. Our results showed that IPC during LC could reduce the oxidative stress caused by anaesthesia, pneumoperitoneum and surgical harm. We could reduce hospitalization by using IPC and we also could decrease postoperative pain as well. IPC seems to be a beneficial and simple surgical method in laparoscopic surgery.

8. Publications and presentations

Publications related to the thesis

1. Kürthy Mária, Miklós Zsanett, Kovács Dóra, Degrell Péter, Rantzinger Eszter, Arató Endre, Sínay László, Nagy Tímea, Hardi Péter, Kovács Viktória, Jávor Szaniszló, Veres Gyöngyvér Tünde, Rőth Erzsébet, Lantos János, Jancsó Gábor: A posztkondicionálás hatása az iszkémia/ reperfúziós károsodásra hiperlipidémiás patkánymodellen, MAGYAR SEBÉSZET (ISSN: 0025-0295) 66: (2), pp. 95-96 type of document: Journal paper/Review paper impact factor: 0.120

2. T Nagy, V Kovács, P Hardi, T Gy Veres, I Takács, G Jancsó, L Sinay, G Fazekas, Ö Pintér, E Arató. 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. JOURNAL OF VASCULAR RESEARCH 52:(1) pp. 53-61. (2015)
IF: 2.90

3.T.Gy.Veres, I.Takács, T.Nagy, G.Jancsó, A. Kondor, L.Pótó, A. Vereczkei *Pneuomperitoneum induced ischaemia-reperfusion injury of the peritoneum /*Preconditioning may reduce the negative side-effects caused by carbon-dioxide pneumoperitoneum/ Pilot study Clinical Hemorheology and Microcirculation vol. 69, no. 4, pp. 481-488, 2018, DOI 10.3233/CH-170336 IOS Press; **IF: 1,914**

4. T.Gy. Veres, T. Nagy,L. Petrovics, K. Sárvári, A. Vereczkei, G. Jancsó, I. Takács The effect of laparoscopic pre- and postconditioning on pneumoperitoneum induced injury of peritoneum Clinical Hemorheology and Microcirculation – accepted for publication in Clinical Hemorheology and Microcirculation, IF: …

Abstract

1. Veres Gyöngyvér Tünde, Jávor Szaniszló, Wéber György: Az alacsony nyomáson történő prekondicionálás csökkenti a pneumoperitoneum okozta szisztémás, káros oxidatív hatásokat

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List of presentations related to the thesis

1.T Gy Veres, Sz Javor, Gy Weber: PRECONDITIONING EVEN USING LOW PRESSURE CAN REDUCE SURGICAL STRESS FOLLOWING LAPAROSCOPIC PROCEDURES 47th Annual Congress ESSR 2012.06.06-09. Lille- France

2. Veres Gyöngyvér Tünde, Jávor Szaniszló, Wéber György Az alacsony nyomáson történő prekondicionálás csökkenti a pneumoperitoneum okozta szisztémás, káros oxidatív hatásokatA Magyar Sebész Társaság 61. Kongresszusa, Szeged, 2012. szeptember 13–15.

3.Veres Gyöngyvér Tünde, Nagy Tibor, Kovács Viktória, Hardi Péter, Jávor Szaniszló, Kürthy Mária, Dézsi László, Vereczkei András, Rőth Erzsébet, Jancsó Gábor, Wéber György: A pre- és posztkondicionálás szerepe a pneumoperitoneum által kiváltott iszkémia-reperfúzió indukáltaoxidatív stressz kivédésében A Magyar Sebész Társaság Kísérletes Sebészeti Szekciójának XXIV. Kongresszusa, Debrecen, 2013. június 13-15.

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