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

PhD Thesis

Tünde Gyöngyvér Veres, M.D.

Director of Doctoral School: Professor Gábor L. Kovács, M.D., PhD.

Leader of PhD. Program: Gábor Jancsó, M.D., PhD.

Supervisor: Professor András Vereczkei, M.D., PhD.

Ildikó Takács, M.D., PhD.

University of Pécs, Faculty of Medicine

Department of Surgical Research and Techniques

Pécs

-2019-

Contents

1. Abreviations

24 h- 24 hours

A.- after induction of anesthesia

A.O.- after operation

A.S.- after surgery

ABA- abscisic acid = PPARγ

A1 -adenosine acting on A1 receptors.

Bax- apoptotic signal proteine

Bcl-2- antiapoptotic signal protein

BH.- before hospitalization

BMI- body mass index

B.S. before surgery

C- controll

CGRP- calcitonine gene related peptide

CO_2- carbon dioxide

COX-1- cyclooxygenase-1

COX-2- cyclooxygenase-2

CRP- C-reactive protein

DNA- Deoxiribonucleic acid

EDTA- ethylenediaminetetraacetic acid

ELISA- enzyme-linked immunosorbent assay

eNOS- endothelial nitric oxide synthase

GSH- reduced glutathione

HE- haematoxylin-eosin

I/R- Ischemia/Reperfusion

IL-6- interleukin-6

iNOS- inducible nitric oxide synthase

IPC- ischemic preconditioning

IPoC- ischemic postconditioning

K_{ATP} -ATP-sensitive potassium channel

LC- laparoscopic cholecystectomy

LIPoC- local ischaemic postconditioning

MDA- malondialdehide

mmHg- milimeter of mercury

MPO- myeloperoxidase

mRNA- messenger ribonucleic acid

NAC- N-acetylcysteine

NF-kB- nuclear factor kappa-light-chain-enhancer of activated B cells

NO- nitric oxide

p53- cellular tumor antigen 53= tumor antigen 53= phosphoprotein p53

PG- prostaglandin

Post -1st postoperative day

PPARγ- peroxisome proliferator-activated receptor-gamma

PTX- pentoxifylline

RIPC- remote ischemic preconditioning

RIPoC- remote ischemic postconditioning

ROS Reactive Oxigen Species

RT-PCR real-time polymerase chain reaction (Real-Time PCR)

SD- standard deviation

SDS-sodium dodecy sulphate

SEM- standard error of mean

SH- sulfhydryl-group6 thiol group

SIRS- systemic inflammatory response syndrome

SOD- superoxide-disumatse

SPSS- Statistical Package for the Social Sciences

SWOP- second window of protection

TNF-α- tumor necrosis factor alpha

TOS- total oxidant status

TRIS- buffer

Tween (TBST)- mixture of tris-buffered saline (TBS) and Polysorbate 20

2. Introduction

2.1. The ischemic/reperfusion injury

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.[1] During an ischemic 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.[2] Free radical formation is increased during abdominal surgery as a result of ischemia-reperfusion, leukocyte activation, and mitochondrial dysfunction.[3,4]

2.2. Oxidative stress in ischemia-reperfusion injury

Apoptosis can be caused by both prolonged ischemia/hypoxia and by reperfusion as well.[5] 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.[6] ROS has downstream effects, which 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.[7] Free radicals are highly reactive molecules with unpaired electrons, that are continuously produced in the body by mitochondria, leucocytes, and xanthine oxidase.[8,9] They have important biological

functions such as redox signaling and antibacterial defense.[10,11] 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 nonenzymatic 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. It is said to be one of the drivers of the systemic inflammatory response syndrome (SIRS) and can cause distant organ damage.[12] 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.[13,14]

2.3. Ischemic preconditioning /IPC/

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. Apoptosis, a genetically programmed form of cell death, has been associated with cardiomyocyte cell loss in a variety of cardiac pathologies, including cardiac failure and those related to I/R injury.[15] Several mechanisms might explain the effects of oxygen radicals. Recently, it has become appreciated that exposure of cells to mild oxidative stress can reversibly modify several cellular activities, in the absence of cell damage, but secondary to changes in the activity of various enzymes and other cell components.[16] Among others, oxidative 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. [17] 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. It appears that there is a bimodal distribution of protection; the initial phase described by Murry, Reimer and Jennings lasts around one to three hours, depending on species and model, whilst a delayed preconditioning or “second window of protection” (SWOP) originally identified and described in 1993 [18], exists between 12 and 72 hours following the initial ischemic insult. Since the underlying pathophysiology and mechanisms of these two phases of endogenous cardioprotection may be different, it is important to make critical distinctions between the two. Accordingly we refer to the early phase of protection as “classic preconditioning”. Fig No. 1. Aksöyek et. al [19] 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.

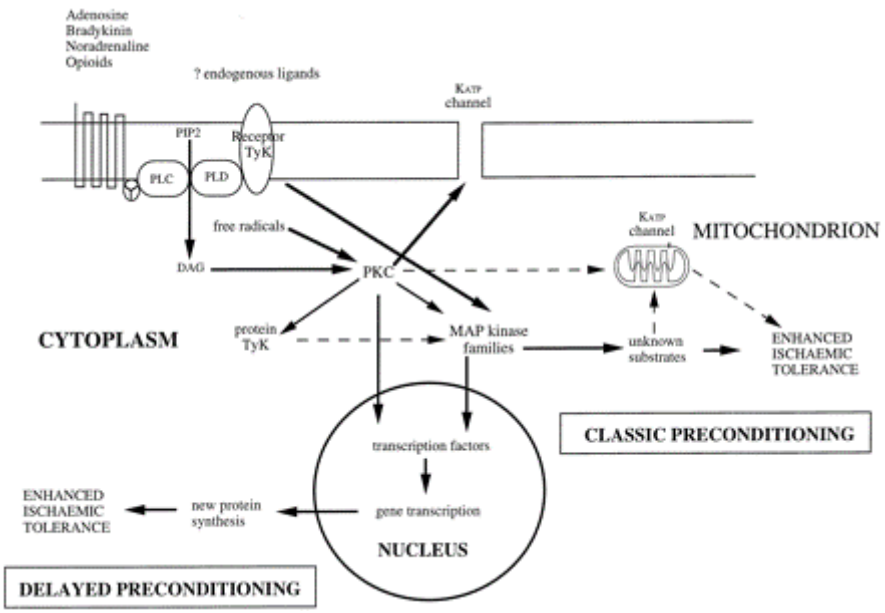


*Fig. No 1.*Cellular events following a preconditioning stimulus. Source: Oxford University Press

Polish group working at the University in Cracow studied this phenomenon in the gastric mucosa subjected to brief 2-5 episodes of short ischemic preconditioning followed by prolonged ischemia-reperfusion that within 3 h causes gross and microscopic erosions in the

stomach. It was found for the first time that few short ischemic episodes protects the gastric mucosa from the damage induced by prolonged ischemia-reperfusion via mechanism involving endogenous prostaglandins (PG) derived from cyclooxygenase (COX1 and COX2), nitric oxide (NO) due to overexpression of inducible nitric oxide synthase (iNOS) and adenosine acting on A1 receptors. Moreover, using molecular techniques of real time-polymerase chain reaction (RT-PCR) and Western Blot, they showed directly COX-2 overexpression in the preconditioned gastric mucosa, at the levels of both, messenger RNA (mRNA) and protein, while signals for mRNA and protein of COX-1 were unchanged.[20]

2.4. Ischemic postconditioning /IPoC/

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.[21] Fig. No 2. 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 it proved to be protective, including humans. [22]

Taken together both IPC and IPoC activate the same key pathways, which include phosphatidylinositol 3-kinase-Akt and extracellular signal–regulated kinase (ERK/p42-44).[23,24] There may be an upstream activation of G-protein coupled receptors, and the many downstream events include key phosphorylation of endothelial nitric oxide synthase (eNOS) and inhibition of the apoptosis promoters. IPC and IPoC influence a variety of endogenous mechanisms that operate at numerous levels and target a broad range of pathological mechanisms.

Hypothetical scheme postulating the possible mechanisms of protection induced by ischemic Postcond

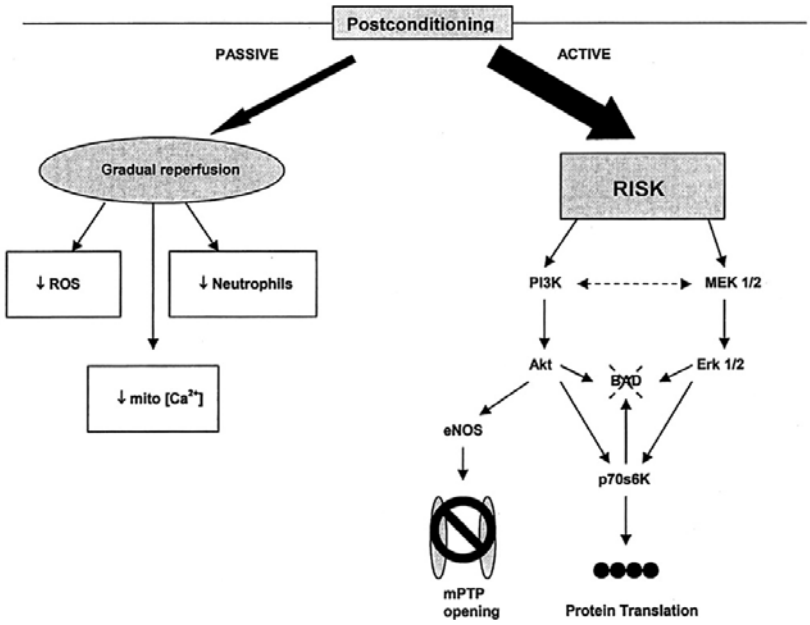


Fig. No.2. J Vinten-Johansen, D Yellon, L Opie. Circulation.2005.Oct.2085-

2.5. Oxidative stress markers

There are a lots of oxidative stress markers used to detect the magnitude of the caused stress. Different biomarkers have been used in various clinical settings, and there is not a single biomarker that can truly represent oxidative stress. Of the many biological targets of oxidative stress, lipids are the most involved class of biomolecules. Lipid oxidation gives rise to a number of secondary products. Malondialdehyde (MDA) is the principal and most studied product of polyunsaturated fatty acid peroxidation. This aldehyde is a toxic molecule and should be considered as more than just a marker of lipid peroxidation. Its interaction with DNA and proteins has often been referred to as potentially mutagenic and atherogenic. Because blood glutathione concentrations may reflect glutathione status in other, less accessible tissues, measurement of both reduced glutathione (GSH) in blood has been considered essential as an index of whole-body GSH status and useful indicator of oxidative stress status. It has been well established that a decrease in GSH concentration may be associated with aging and the pathogenesis of many diseases. [25] The prime physiological function of superoxide dismutase is the protection of oxygen-metabolizing organisms against the potentially detrimental effects of the superoxide free radical, a biologically produced

intermediate resulting from the univalent reduction of molecular oxygen. In our Institute we measured MDA, endogenous antioxidant reduced glutathione (GSH), sulfhydryl-group (SH-), antioxidant superoxide-dismutase (SOD) and catalase (KAT) activity. From blood plasma we measured malondialdehyde (MDA), and myeloperoxidase (MPO).

2.6. Methods

Detection of malondialdehyde (MDA) concentration

A mixture of 4.5 ml TBA (thiobarbituric acid) and TCA (trichloroacetic acid) were added to 0.5 ml plasma or diluted blood. Samples were incubated for 20 minutes at 100 ℃ then cooled to 0 ℃. Blood was centrifuged in a cooled centrifuge at 4000 rpm for 15 min. The concentration of MDA was determined using a spectrophotometer at 532 nm and expressed in nM/ml.[26]

Detection of reduced glutathione (GSH) and sulfhydryl-group (SH-) concentration

For the determination of GSH and SH- a mixture of one ml quintuple blood sample and 4 ml trichloroacetic acid (TCA) were used. The mixture was centrifuged at 4000 rpm for 15 min. The supernatant was added to 4 ml TRIS puffer (0,4 M, pH:8,7) 2 ml and 100 µl DTNB (5.5'-ditio-bis-2-nitro-benzoe acid) was added to the mixture immediately before measurement. The concentrations of GSH and SH- were determined using a spectrophotometer at 412 nm and expressed in nM/ml.[27]

Detection of superoxide-dismutase (SOD) activity

For the determination of SOD activity 1 ml blood was mixed with EDTA, then 9 ml Hartman's solution was added to the blood sample. The mixture was centrifuged at 2000 rpm for 5 min. After discarding the supernatant the washing procedure was repeated. A mixture (2:1) of 1 ml chloroform and ethanol were added to 1 ml hemolyzed red blood cells and they were centrifuged at 17000 rpm for 4 min. Supernatant was separated thereafter, and adrenalin (16.488 mg adrenalin diluted in 10 ml 0.1N hydrochloric acid) was added to it. The

concentration of SOD was determined by using a spectrophotometer at 480 nm and expressed in U/ml.[28]

Detection of myeloperoxidase activity (MPO)

For the determination of MPO activity 1 ml work solution (10.9 ml Na-citrate, 100 µl *o*-Dianisidin) was mixed with 200 µl plasma. The compound was incubated at 37 °C for 5 min, then 1 ml of 35% perchloric acid was added to the mixture and centrifuged at 2500 rpm for 10 min. MPO concentration was measured using spectrophotometer at 560 nm and expressed in U/l.

Detection of catalase levels (KAT)

To determinate the catalase enzyme level we mixed 2 ml of buffer, 1 ml of peroxide solution and 100 times diluted, washed red blood cells. With spectrophotometer we measured the loss of peroxides at 240 nm. Catalase levels were expressed in BE/ml.

3. Aims and hypothesis

In each part 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 a 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) 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 in a conventional LC group and an IPC group. We measured hospitalization days and pain as well.

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

4.1. Introduction

Laparoscopic technique is beneficial compared with conventional open surgical techniques, because after laparoscopic operations hospitalization is getting shorter and patients suffer from less postoperative pain among others. However, experts are concerned about the adverse effects of laparoscopic surgery. Today, laparoscopic abdominal surgery in general surgery departments is the basis of all abdominal surgical interventions. Disadvantages include longer surgery time and higher equipment costs. Laparoscopic surgery is mostly associated with systemic and splanchnic hemodynamic alterations. Inadequate splanchnic perfusion in critically ill patients is associated with increased morbidity and mortality. The underlying pathophysiological mechanisms are still not well understood.[29] Pneumoperitoneum, created for better visualization and for enlarged working place results in an increased intraabdominal pressure (12-15 mmHg), which decreases perfusion of the splanchnic area.[30] By hypoperfusion, I/R injury, and accumulation of ROS as well as inflammatory cytokines occur. The extent of the injury depends on the magnitude of intra-abdominal pressure and the length of its application.[31] To perform safe and effective laparoscopic surgery it is extremely important to know the normal physiology and pathophysiology of local and systemic effects of pneumoperitoneum. Murry et al. [17] noted, that short repeated ischemia-reperfusion cycles, the so called preconditioning (early, classical preconditioning) before a long ischemic insult can reduce oxidative stress of the myocardium. Aksöyek et. al [18] 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, IPC is not well-suited to emergency settings, as the onset of myocardial or brain infarction cannot be anticipated. Thus the interest in the IPoC phenomenon, which was described subsequently to IPC. Sequential clamping and de-clamping of organ vessels after the ischemic insult can also confer some degree of protection, or higher repair potential to organs. Both local (LIPoC) and remote (RIPoC) IPoC have been described.[32] 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 [20]. These authors were using a canine model of one-hour coronary occlusion and 3 hours of reperfusion. The IPoC algorithm was 30 sec of reperfusion followed by a 30 sec coronary occlusion, which were repeated three times at the onset of reperfusion, which lead to protection against reperfusion injury. In our Institute Javor et al. have proved in rats that there is no difference between pneumoperitoneum created via transvaginal approach and the conventional method. They have also found that preconditioning can reduce adverse effects of pneumoperitoneum using the standard pressure (10-15 mmHg).[33] Schilling MK et al. in their study measured gastric, duodenal, jejunal, colonic, hepatic, and peritoneal blood flow with a custom-made laser Doppler flow probe at an intra-abdominal pressure of 0, 10, and 15 mm Hg. They found that intra-abdominal pressure elevation from 10 mm Hg to 15 mm Hg significantly decreased the blood flow in the stomach by 40 percent to 54 percent, the jejunum by 32 percent, the colon by 44 percent, the liver by 39 percent, the parietal peritoneum by 60 percent, and the duodenum by 11 percent. Splanchnic blood flow decreased with operative time at a constant intra-arterial pressure ($r = 0.88$, $p < 0.0001$). [34]

4.2.Aims

The aim of our study was to investigate in rat animal model, whether IPC or even IPoC can reduce the injury of splanchnic circulation elicited by pneumoperitoneum during laparoscopic operations.

4.3.Materials and Methods

We used seventy Wistar rats (both male-female) weighted between 200-300 g, divided into seven groups. Pneumoperitoneum was created using Veres needle, that was transumbilically inserted into the abdominal cavity. Blood samples were taken 2 hours after the procedure, and at the end animals were terminated.

Groups

I.	**Sham**	Sham operation, only Veres needle was inserted
II.	**5 mmHg**	Pneumoperitoneum with 5 mmHg for 60 min 
III.	**5 mmHg Pre**	Preconditioning (inflation and deflation for 5 min) with 5 mmHg then pneumoperitoneum with 5 mmHg (60 min) 
IV.	**5 mmHg Post**	Pneumoperitoneum with 5 mmHg for 60 min Postconditioning with 5 mmHg (after 60 min. pneumoperitoneum deflation for 5 minutes, than inflation for 5 min (5 mmHg) and deflation at the end 
V.	**10 mmHg**	Pneumoperitoneum with 10 mmHg for 60 min 
VI.	**10 mmHg Pre**	Pneumoperitoneum with 10 mmHg for 60 min Preconditioning (inflation and deflation for 5 min) with 10 mmHg and then pneumoperitoneum with 10 mmHg (60 min) 
VII.	**10 mmHg Post**	Postconditioning with 10 mmHg (after 60 min pneumoperitoneum deflation for 5 minutes, then inflation for 5 min (10 mmHg) and deflation at the end 

4.3.1. Animal model and operation

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. The present study conforms to the Guide for the Care and Use of Laboratory Animals published by the US National Institutes of Health (NIH Publication No. 85-23, revised 1996) and was approved by the local institutional Committee on Animal Research of Pécs University (BA02/2000-29/2001). Rats were fasted 24 hour prior to operation, water was given ad libitum. Animals were anaesthetized with a combination of 37,5 mg/kg ketamine and 3,75 mg/kg diazepam intraperitoneally, then a Veres needle was inserted. For the creation of pneumoperitoneum an automatic insufflator (Karl Storz GmbH&Co.KG, Tuttlingen, Germany) was utilized, applying CO_2 gas at 5 mmHg and 10 mmHg pressures, respectively. Both 5 minutes and 60 minutes pneumoperitoneum was maintained at constant pressure. After deflation reperfusion time was 120 min. Two hours after the procedure blood samples were taken by heart puncture, and after that before the termination of the animals, 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.

4.3.2. Analysis

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 the upper mentioned methods.
For measuring TNF-α and IL-6 concentrations in serum we used Rat TNF-α and Rat IL-6 ELISA kit (R&D Systems, Inc., Minneapolis, USA), following the manufacturers protocol. These methods determine the free i.e. biological active TNF-α and IL-6 concentrations.
For detection of pro- and antiapoptotic signaling pathways and extent of DNS damage fifty milligrams of left and right kidney-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 antigens: Bcl-2 (1:1000 dilution), Bax (1:1000 dilution), p-53 (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. Detection of density was performed with Scion Beta 4.02 software. All experiments were repeated three times.

The animals were terminated at the end of the experiment and biopsy was taken from liver, kidney, small intestine, diaphragm and abdominal muscle samples from each group. The definite aim of the biopsy was to register the qualitative differences in changes between the animal groups. 5-6 paraffin-embedded blocks were made and sample slices were prepared staining by HE. The biopsies were made with the following method:

The fresh tissue was fixed in 10% neutral buffered formalin. Sample preparation was performed with a tissue processor equipment (Thermo Shandon Path center, Thermo Fisher Scientific Inc. Waltham, MA, USA). Sectioning was performed with a sledge microtome (5 μm, Reichert Optische Werke AG, Vienne, Austria) from the paraffin-embedded blocks, and staining was carried out with a carousel-type slide stainer (Thermo Varistain 24-4, Thermo Fisher Scientific Inc., Waltham, MA, USA) with hematoxylin and eosin at the Medical School University of Pécs, Department of Pathology, Pécs, Hungary. To evaluate the histological slices we used the Pannoramic Viewer software (3DHistec Ltd.) and 40x magnification.

4.3.3. Statistical analysis

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.

4.4. Results

We perceived no complications during operations. Bleeding through Veres needle evolved in 3 rats. 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. (Sham 69,67 ± 2,38 nM/ml; 5 mmHg 76,75 ± 3,61 nM/ml; 5 IPC 74,208 ± 2,35 nM/ml; 5 IPoC 75,385 ± 4,72 nM/ml; 10 mmHg 77,283 ± 5,25 nM/ml; 10 IPC 73,42 ± 3,11 nM/ml; 10 IPoC 75,225 ± 5,008 nM/ml) Fig. No. 3. Contrarily in plasma MDA concentration there was also a 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. (Sham 1,18 ± 0,10 nM/ml; 5 mmHg 1,58 ± 0,12 nM/ml; 5 IPC 1,33 ± 0,22 nM/ml; 5 IPoC 1,24 ± 0,11 nM/ml; 10 mmHg 1,62 ± 0,07 nM/ml; 10 IPC 1,24 ± 0,07 nM/ml; 10 IPoC 1,27 ± 0,08 nM/ml) Figure No.4.

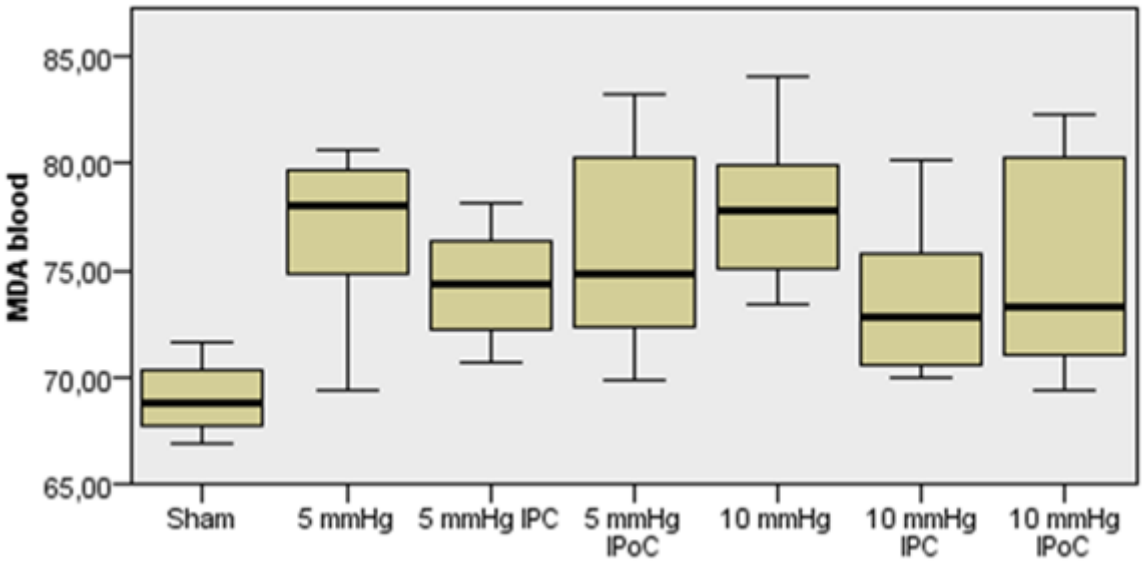


Fig. No. 3. MDA concentration in blood. MDA mean concentration ± SEM in blood was significantly higher in all groups compared to the Sham group. In 5mmHg IPC and 10 mmHg IPC we noticed lower but not significant concentrations compared to the 5 mmHg and 10 mmHg groups.

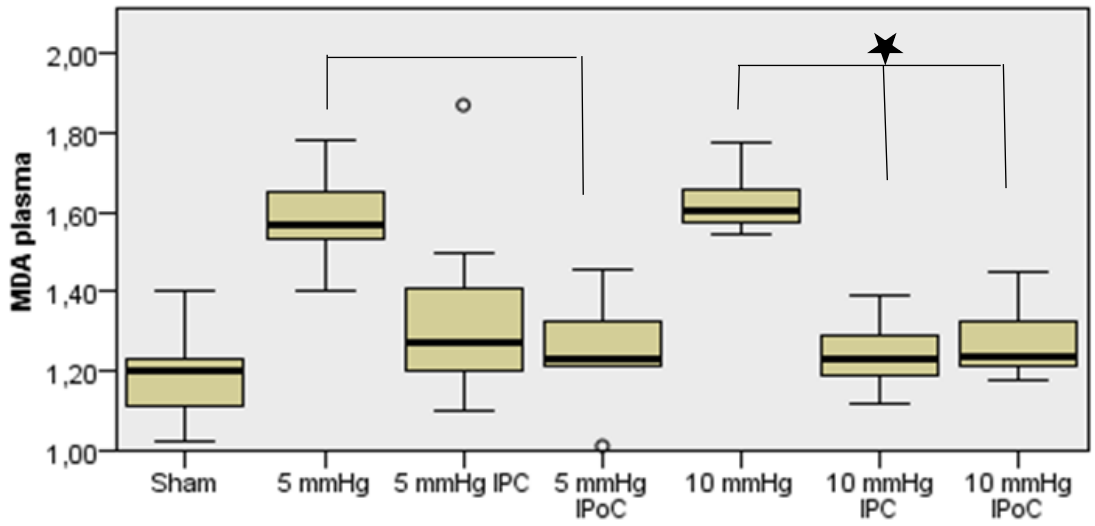


Fig. No. 4. Plasma MDA concentration mean ± SEM. We noticed significantly higher MDA concentration in each group compared to the Sham group. There was a significant decrease in group IPC 10 mmHg and IPoC 10 mmHg compared to the 10 mmHg group. In group 5mmHg IPoC, we noticed significantly lower MDA concentrations compared to the 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. (Sham 1153,96 ± 55,35 nM/ml; 5 mmHg 881,69 ± 126,62 nM/ml; 5 IPC 983,58 ± 67,59 nM/ml; 5 IPoC 972 ± 42,18 nM/ml; 10 mmHg 907,51 ± 21,69 nM/ml; 10 IPC 1031,18 ± 22,89 nM/ml; 10 IPoC 1019,59 ± 41,80 nM/ml) (Figure No. 5.)

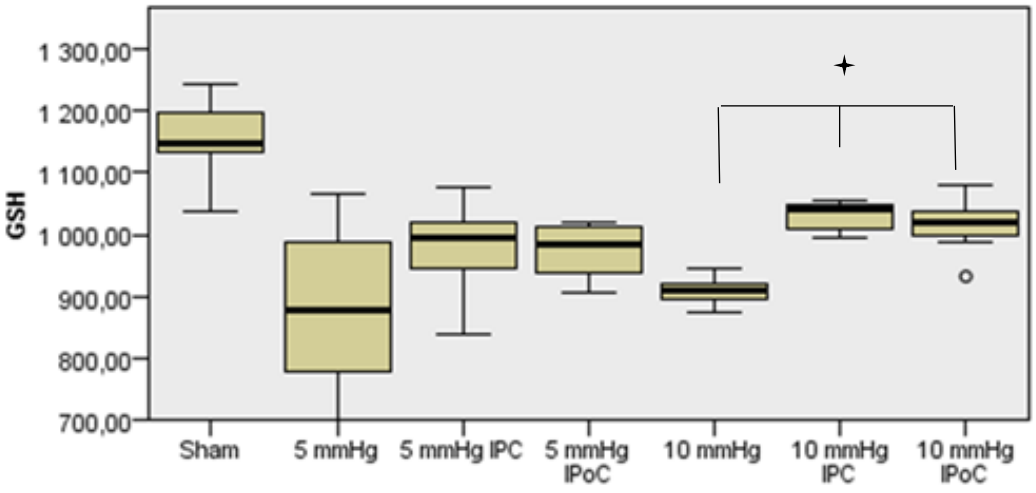


Figure No. 5. GSH concentration mean ± SEM in blood. In groups 10 mmHg IPC and 10 mmHg IPoC we found significantly higher GSH concentrations compared to the 10 mmHg group, meaning that both procedures reduced oxidative stress.

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. (Sham 1022,34 ± 69,86 U/ml; 5 mmHg 884,12 ± 90,89 U/ml; 5 IPC 901,93 ± 137,40 U/ml; 5 IPoC 896,82 ± 108 U/ml; 10 mmHg 852,06 ± 177,38 U/ml; 10 IPC 1104,6 ± 71,62 U/ml; 10 IPoC 1036,26 ± 138,56 U/ml) This data shows that the created pneumoperitoneum caused damage, but we could decrease this by using IPC. Fig No.6.

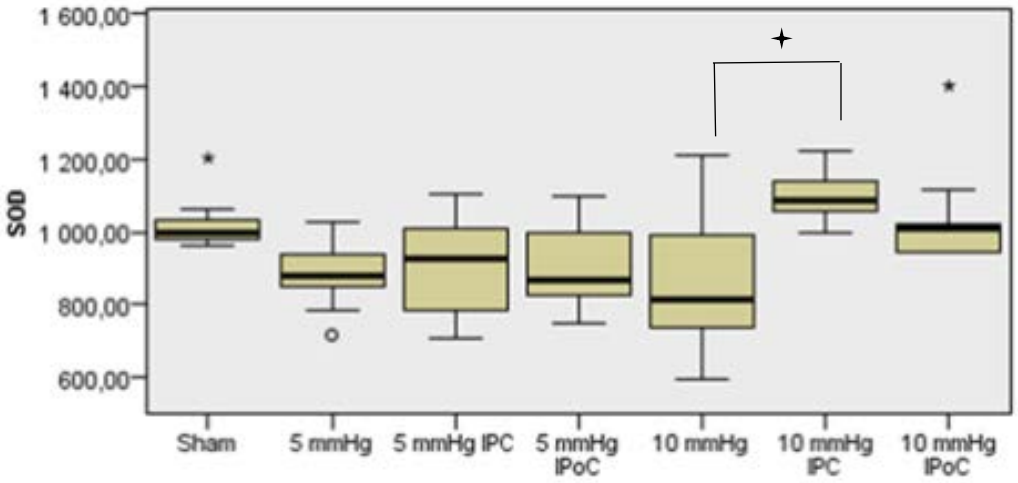


Figure No. 6. SOD activity mean ± SEM in blood. SOD enzyme activity decreased in groups 5 mmHg, 5 mmHg IPC, 5 mmHg IPoC, 10 mmHg. There was a significantly higher SOD activity in group 10 mmHg IPC compared to the 10 mmHg group.

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. (Sham 1,86 ± 0,07 U/ml; 5 mmHg 1,105 ± 0,04 U/ml; 5 IPC 1,314 ± 0,12 U/ml; 5 IPoC 1,24 ± 0,089 U/ml; 10 mmHg 1,09 ± 0,06 U/ml; 10 IPC 1,61 ± 0,163 U/ml; 10 IPoC 1,579 ± 0,152 U/ml) Fig No. 7.

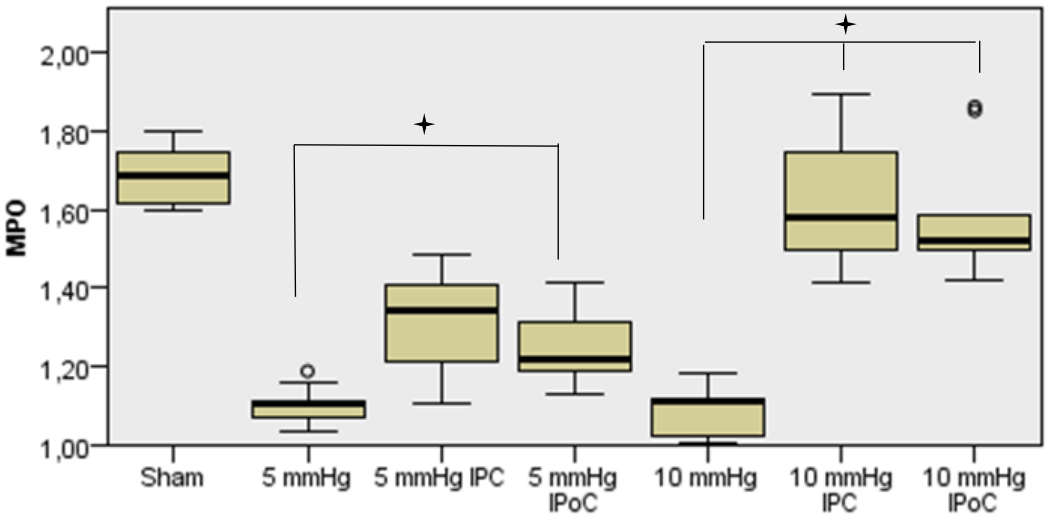


Fig. No. 7. MPO level mean ±SEM in blood. We noticed significantly lower levels in the non-conditioned groups (5mmHg, 10 mmHg). Comparing groups to each other we noticed significantly higher MPO levels in 5mmHg IPC and 5mmHg IPoC compared to the 5 mmHg group, and the same comparing 10 mmHg group to 10 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. (Sham 17,79 ± 0,57 pg/ml; 5 mmHg 24,00 ± 2,55 pg/ml; 5 IPC 24,19 ± 2,70 pg/ml; 5 IPoC 24,47 ± 4, 09 pg/ml; 10 mmHg 25,16 ± 3,81 pg/ml; 10 IPC 22,56 ± 1,62 pg/ml; 10 IPoC 23,78 ± 1,15 pg/ml) (Figure No 8.) 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 (Sham 108 ± 6,68 pg/ml; 5 mmHg 131 ± 22,69 pg/ml; 5 IPC 119 ± 6,01 pg/ml; 5 IPoC 120 ± 7,30 pg/ml; 10 mmHg 149 ± 16,93 pg/ml; 10 IPC 123 ±15,29 pg/ml; 10 IPoC 128 ± 7,42 pg/ml) Figure No. 9. 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).

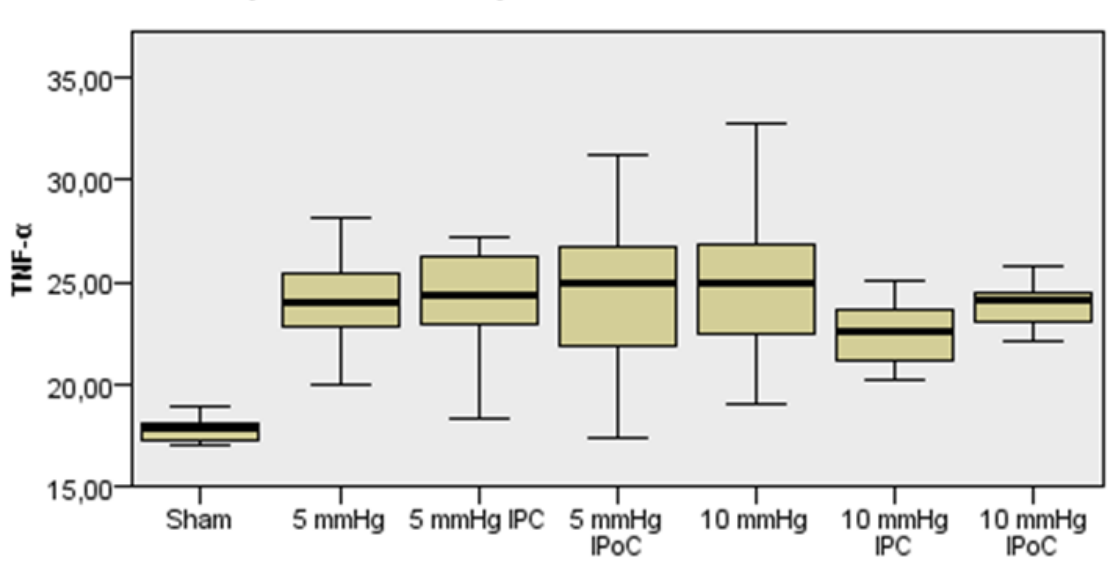


Fig. No. 8. Serum TNF-α concentration mean ± SEM. In the 10 mmHg IPC group we noticed lower, but not significant alteration in the level of TNF-α compared to the Sham group.

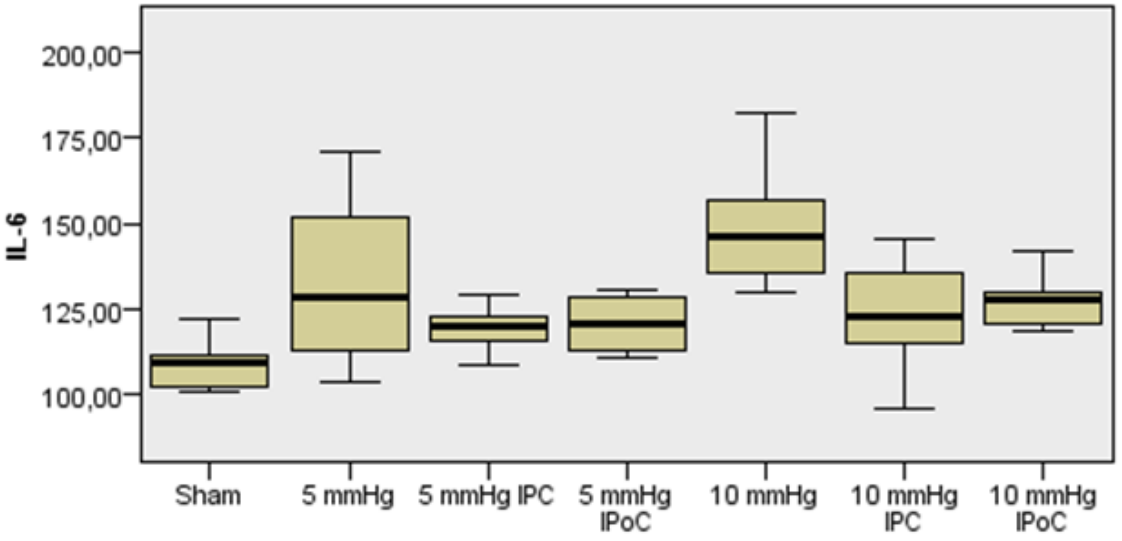


Fig. No. 9. Serum IL-6 concentration mean ± SEM. In groups 5mmHg, 10 mmHg and IPoC we noticed significantly higher IL-6 concentrations compared to the Sham group. We found lower but not significant concentrations in groups 5 mmHg IPC and 5 mmHg IPoC compared to 5 mmHg, and the same, lower but not significant concentrations in 10 mmHg IPC compared to 10 mmHg.

Histological examinations were most traceable and spectacular in kidney samples. Examining the diaphragm samples we noticed intact striated muscle in the Sham group, in the 5 mmHg group the striated feature was lost, fibers were torn, connective tissue was expanded, nuclei were inflated, apoptotic. Bruising could be noticed. In 5 mmHg IPC and IPoC groups we

noticed transversely torn fibers, there were neither apoptotic cells, nor bruising. In the 10 mmHg group there was expanded connective tissue, red blood cells (RBC) in muscle and the nuclei were apoptotic, inflated. In group 10 mmHg IPC there were extravasal RBCs, the striated muscle was intact, there was no apoptosis. In 10 mmHg IPoC there was a longitudinal tear in muscle cells, and a transverse expansion, we also noticed apoptosis as well. In the Sham group the abdominal muscle was intact, in group 5 mmHg we noticed longitudinal and transverse fraying, but in group 5 mmHg IPC in some parts we also noticed longitudinal fraying due to inflation. The abdominal muscles in groups 5 mmHg IPoC and 10mmHg lost their longitudinal and transverse striates, cells were apoptotic. In group 10mmHg IPC there was little bruising, but the muscle was intact, in contrast to group 10 mmHg IPoC the abdominal muscle lost its striated muscle structure, but there was neither apoptosis, nor bruising. In bowel samples we noticed no structural changes except in group 10 mmHg, where the smooth muscle of the bowel was partially torn, and in some parts we noticed opened cell membranes as well. As mentioned above we noticed the most characteristic histological results in kidney samples. In the Sham group there was no alteration, in group 5 mmHg we noticed few RBCs in chalix, and renal parenchyma, but the ureter, glomeruli and renal capsule were intact. Fig.No. 10.

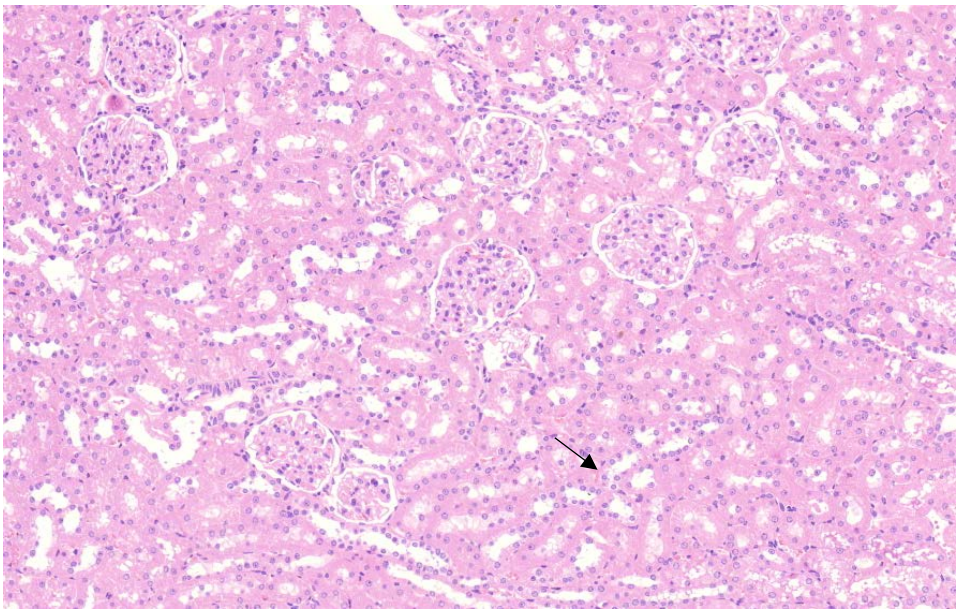


Fig. No. 10. Renal parenchyma in 5 mmHg group.

In group 5 mmHg IPC we saw disintegration of glomerular and tubular cells, minimal bruising in parenchyma, and damage of urethral epithelial cells; Fig. No 11.

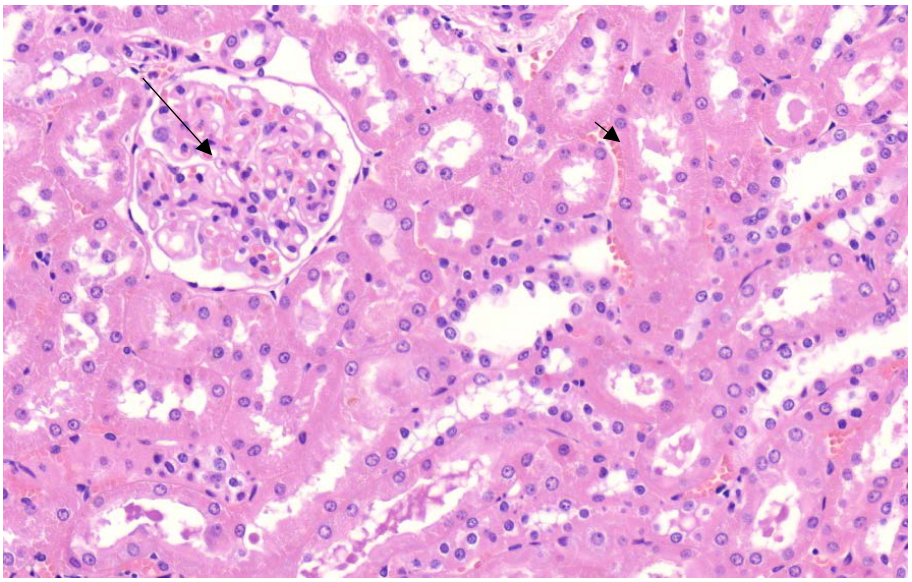


Fig. No. 11. Renal parenchyma in group 5 mmHg IPC.

In group 5 mmHg IPoC 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. Fig No 12.

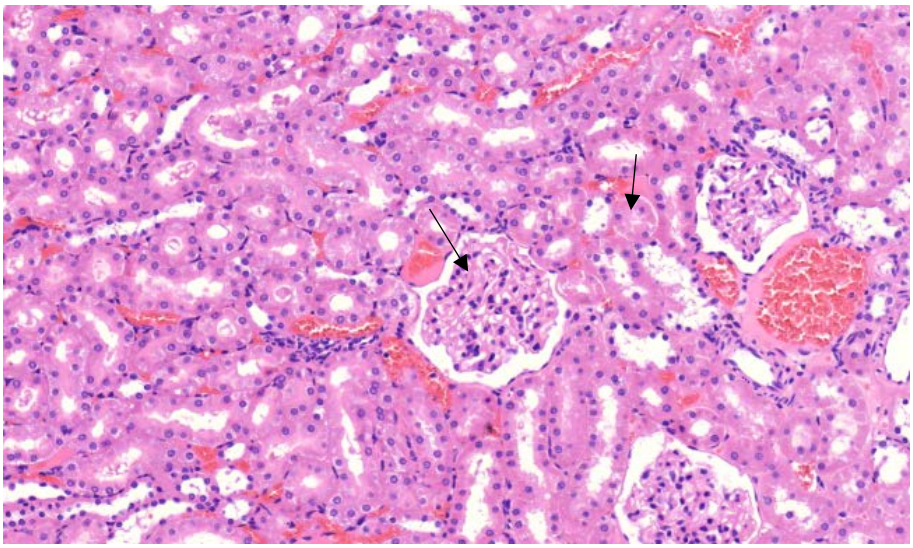


Fig. No. 12. Renal parenchyma in group 5 mmHg IPoC.

In group 10 mmHg we saw bruising in glomeruli and tubuli as well. Fig No. 13.

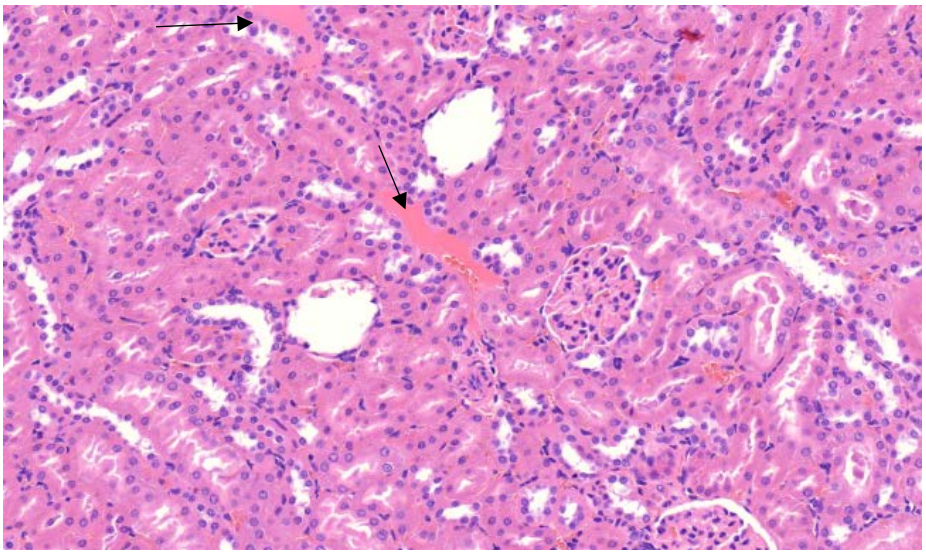


Fig. No. 13. Renal parenchyma in 10 mmHg group.

In group 10 mmHg IPC we noticed swollen nuclei, bruising in glomeruli, renal parenchyma, and calyx. Glomerular tubuli remained intact. Fig No.14.

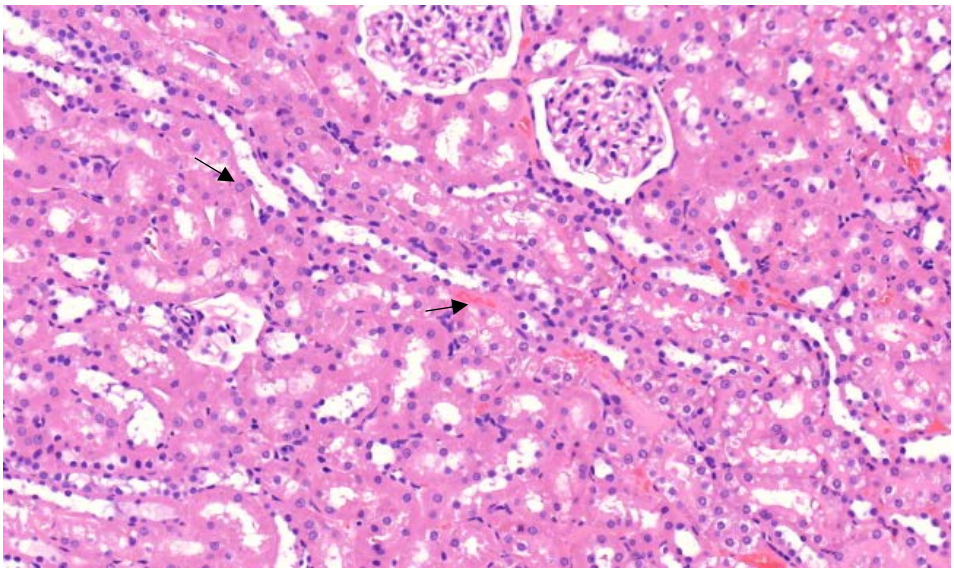


Fig. No.14. Renal parenchyma in 10 mmHg IPC group.

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. Fig No. 15.

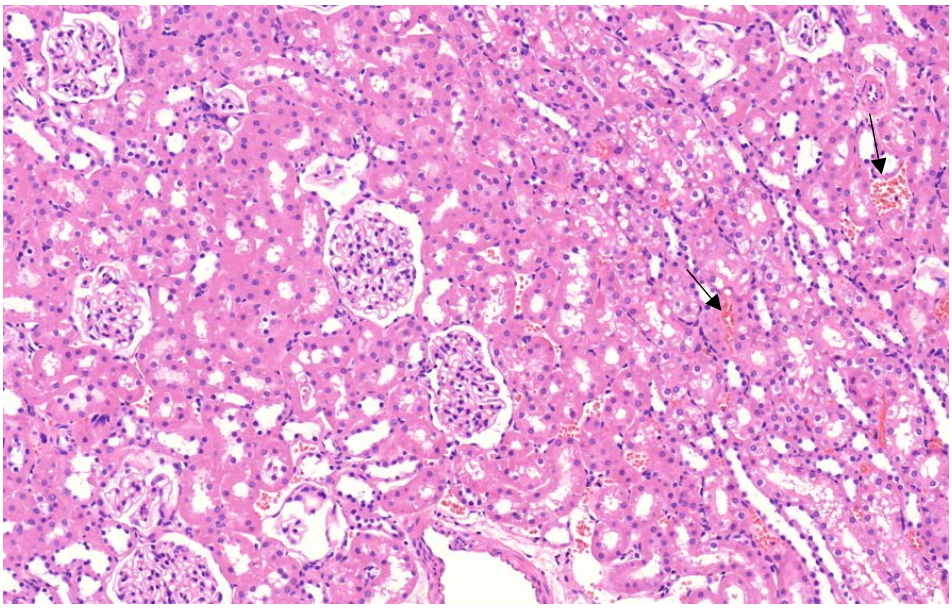
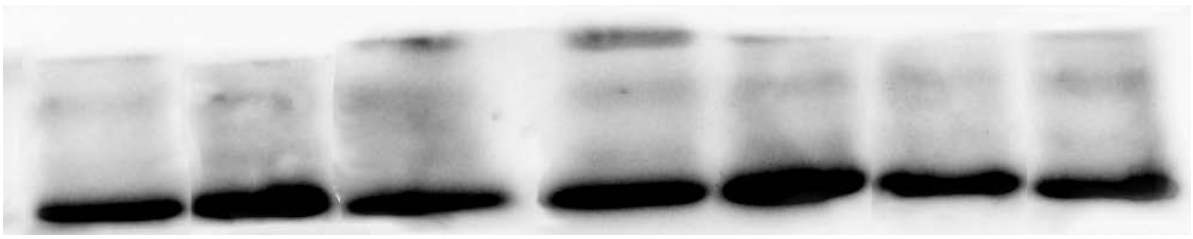


Fig. No 15. Renal parenchyma in 10 mmHg IPoC group.

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. Fig. No.16.



Sham **5mmHg** **5mmHg IPC** **5mmHg IPoC** **10 mmHg** **10 mmHg IPC** **10 mmHg IPoC**

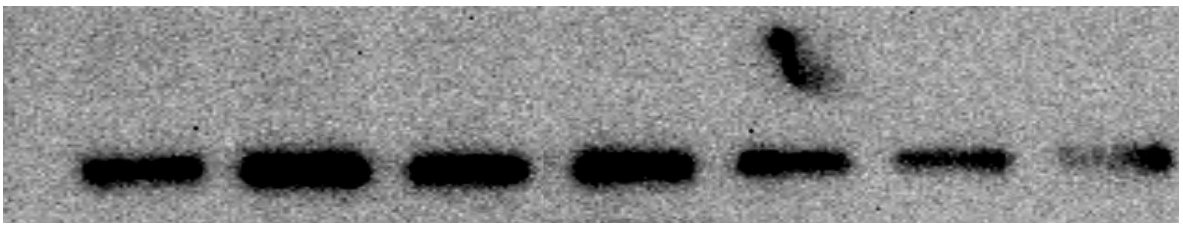
Fig. No. 16. The expression of proapoptotic Bax signal protein

The expression of antiapoptotic (bcl-2) 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. Fig. No. 17.

Sham **5mmHg** **5mmHg IPC** **5mmHg IPoC** **10 mmHg** **10 mmHg IPC** **10 mmHg IPoC**

Fig No.17. The expression of antiapoptotic bcl-2 signal protein.

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. Fig. No. 18.



Sham **5mmHg** **5mmHg IPC** **5mmHg IPoC** **10 mmHg** **10 mmHg IPC** **10 mmHgIPoC**

Fig. No. 18. The expression of p53 signal protein.

4.5. Discussion

Any form of trauma, including surgery, is known to result in oxidative stress. The majority of studies demonstrate grate immediate oxidative stress after open surgery compared to laparoscopic. Increased intra-abdominal pressure during pneumoperitoneum and inflation-deflation may cause ischemia reperfusion and, hence, oxidative stress. Oxidative stress happens when an imbalance occurs between the production of free radicals and antioxidant levels. It is stated, that it can cause distant organ damage.[1] The formation of free radicals is increased during abdominal surgery as a result of ischemia-reperfusion, such as leukocyte activation, and mitochondrial dysfunction [1,35] Additionally there is a depletion of antioxidants in the postoperative period due to their redistribution and increased consumption.[36]. Laparoscopic surgery may be less invasive and can be associated with less systemic inflammation and preserved immune function [37,38]. Splanchnic microcirculatory changes during high-pressure CO2 pneumoperitoneum include a decrease in mesenteric

arterial blood flow, and decreased gastric perfusion with a drop in gastric pH in experimental studies. Microcirculatory changes in abdominal organs under clinical conditions with a low pressure CO2 pneumoperitoneum are unknown. As Schilling MK et al. in their above mentioned study concluded that laparoscopic procedures with a CO2 pneumoperitoneum should be performed at a pressure of 10 mm Hg or lower to avoid splanchnic microcirculatory disturbances, so we set the pressures at 5 and 10 mmHg.[33] Gutt and Schmandra examined abdominal blood flow at different intra-abdominal pressures (0–12 mmHg) caused by CO2 pneumoperitoneum in rats. They observed that by increasing the intra-abdominal pressure, blood flow will decrease and at 12 mmHg it becomes minimal. Hypoxia will evolve and abdominal organs will be damaged by reactive oxygen species. [39]

Polat et al. performed laparoscopic cholecystectomy on 24 patients (12 male and 12 female). MDA and sulfhydryl-group concentration was investigated and they observed that concentration of these markers was increased if they applied higher intra-abdominal pressure (10 vs. 15 mmHg)[40]. We also measured increased MDA levels in all groups compared to Sham, and we noticed lower, but not significant, concentration in 10mmHg IPC group. In plasma MDA concentration there was also significantly higher MDA concentration in each group compared to Sham group, and there was a significant decrease in group 10 mmHg IPC and 10 mmHg IPoC compared to 10 mmHg group.

Based on the experiment of Yilmaz et al. we also used preconditioning as a protection. They could reduce oxidative stress caused by pneumoperitoneum with using preconditioning in rats. They investigated oxidative stress marker and inflammatory cytokine concentrations in Sham operated animals, after creating pneumoperitoneum and after preconditioning for 10 minutes. Increased intra-abdominal pressure could cause oxidative stress and preconditioning could reduce this. They used preconditioning before creation of pneumoperitoneum at a pressure of 15 mmHg and observed that using preconditioning has a better effect than lower intra-abdominal pressure (10 mmHg).[41] While beneficial effects of IPoC have also been observed in humans with acute myocardial infarction and after cardiac surgery, in the literature there are only few articles with the keywords laparoscopy, postconditioning.

Hafize Oksuz et al. in their study investigated the effects of pre- and post-laparoscopic conditioning, zinc, pentoxifylline (PTX), and N-acetylcysteine (NAC) on markers of I/R injury in an animal model, using 56 male rats.15 mmHg pressure pneumoperitoneum caused I/R injury on kidney samples, and they used zinc, pentoxifylline, N-acetylcysteine, pre and post-laparotomy conditioning to reduce oxidative stress. They found, that zinc, pentoxifylline, N-acetylcysteine, and post-laparotomy conditioning significantly reduced markers of

oxidative stress caused by laparoscopy.[42] Bulbuloglu E et al. in their experiment used the same model as Hafize Oksuz, the only difference was that they examined small intestine samples tested for malondialdehyde (MDA), catalase (KAT), glutathione peroxidase (GPX), and superoxide dismutase (SOD). In their case laparoscopy caused small intestinal ischemia proven by elevated markers of tissue I/R injury, which effect was significantly attenuated by zinc, pentoxifylline, and N-acetylcysteine, but not by prelaparoscopic conditioning and postlaparoscopic conditioning.[43]. Compared to this we found, that both IPC and IPoC attenuates the oxidative stress caused by pneumoperitoneum, but this attenuation could be seen mostly on 10 mmHg groups.

Sahin et al. with stepwise elevation of IA pressure tried to reduce the negative effect of pneumoperitoneum (5 Hgmm for 10 minutes then 10 Hgmm for 10 minutes and pneumoperitoneum for 60 minutes). They investigated the concentration of oxidative stress markers and inflammatory cytokines. There was a more serious injury in the stepwise elevation group compared to the Sham-operated group. If they used a 15 mmHg intraabdominal pressure then oxidative stress and inflammatory response was also greater compared to the stepwise elevation group. [44].

4.6. Conclusion

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/

5.1. Introduction

The peroxisome proliferator-activated receptor gamma (PPAR–γ) is a fito hormone that can be synthetized by mammals as well, has anti-inflammatory effect and can regulate the glucose uptake. In many countries it is used as a nutritional supplement. Laparoscopic surgery is performed widely because it causes less tissue trauma associated with shorter healing time compared with open surgery. Nevertheless, concerns regarding systemic complications and pathophysiology are still being investigated.[45] Clinical and experimental studies have established that the increase in intra-abdominal pressure that develops depending on the degree of pneumoperitoneum during laparoscopic surgery can cause hypoperfusion of intraabdominal organs.[46] Increases in ischemia and the oxidative stress response were observed with pneumoperitoneum dependent impairment of splenic perfusion. [47,48] After desufflation, reperfusion injury occurred with the fall in intra-abdominal pressure. Free radicals formed as a result of pneumoperitoneum cause plasma antioxidants to decrease. [49] Thus one of the main results of I/R due to pneumoperitoneum is the disturbance of the balance between the oxidative and antioxidative systems. The imbalance is defined as oxidative stress.[50] The severity of oxidative stress is determined by the measurement of total oxidant status (TOS) and consumed antioxidant status.

Various pharmacological agents have been tested to combat oxidative stress, and some antioxidants and vasodilators have been shown to be successful in animal models [51,52]. Administration of dopamine and endothelin 1 antagonists greatly improved the portal circulation in rats subjected to carbon dioxide and helium insufflation, but oxidative stress markers were not measured [53]. Pretreatment with the calcium channel antagonist verapamil significantly reduced oxidant levels and increased antioxidant levels in a rabbit model of retroperitoneoscopy [54]. This was based on previous studies of verapamil showing a reduction in renal and hepatic ischemia–reperfusion injury by acting on calcium influx, although a specific molecular interaction between calcium channel antagonists and ROS was not evident [55]. Erythropoietin is a hypoxia-inducible growth factor expressed mainly in the

kidney. It has multiple protective effects against oxidants and apoptosis106. Administration of erythropoietin before laparoscopy in a rat model significantly decreased plasma MDA levels compared with those in controls [56]. Similarly, melatonin administered 5 min before insufflation and immediately before desufflation significantly reduced mean MDA levels in liver, small intestine and kidney, and improved small bowel histological parameters in a rat model [57]. Other endogenous antioxidants, such as tocopherol, glutathione and superoxide dismutase, and various synthetic antioxidant drugs, such as xanthine oxidase inhibitors, provide possible avenues to minimize tissue injury in laparoscopic surgery, and also in open surgery. [58]

In this experimental study we aimed to investigate the effect of PPAR –γ on oxidative stress in the ischemia-reperfusion injury due to pneumoperitoneum.

5.1.1. PPAR –γ Agonist

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.[59] They play a role in controlling lipid cell differentiation, insulin sensitivity and inflammatory processes, [60,61] 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. Human granulocytes exposed to physical or chemical stimuli release ABA and the hormone stimulates migration, phagocytosis, reactive oxygen species and nitric oxide production in an autocrine manner. The signaling pathway activated by ABA in granulocytes sequentially involves binding to its G protein coupled receptor, activation of adenylate cyclase (AC), cAMP-dependent activation of protein kinase A (PKA), phosphorylation of the cADPR CD38 and consequent cADPR overproduction, leading to an increase of the intracellular Ca2+ concentration [62].

5.2. Aims

We administered PPAR-γ agonist (abscisinic acid, ABA) 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.

5.3. Materials and methods

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). Blood samples were taken 2 hours after the procedure, and at the end animals were terminated. Oxidative stress parameters: malondialdehyde (MDA), reduced glutathione (GSH), sulfhydryl group (-SH) concentrations, superoxide-dismutase enzyme (SOD) activity and inflammatory cytokines, TNF-α and IL-6 levels were measured with the same procedure as mentioned in first part.

5.3.1. Animal model and operation

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. The present study conforms to the Guide for the Care and Use of Laboratory Animals published by the US National Institutes of Health (NIH Publication No.85-23, revised 1996) and was approved by the local institutional Committee on Animal Research of Pécs University (BA02/2000-29/2001). Rats were fasted 24 hours prior to operation, water was given ad libitum. Animals were anaesthetized with a combination of 37,5 mg/kg ketamine and 3,75 mg/kg diazepam intraperitoneally, and were placed in a supine position on an operating table, then a Veres needle was inserted. For the creation of pneumoperitoneum an automatic insufflator (Karl Storz GmbH&Co.KG, Tuttlingen, Germany) was utilized, applying CO_2 gas at a constant 10 mmHg pressure. 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.

5.3.2. Analysis

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 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. According to each group the kidneys were homogenized in ice TRIS puffer (50 mM, pH 8,0). The protein content of the surfactant from the homogenate was elutriated by bicinchonicic acid to get an 8 Laemmli solution with 1mg/ml protein content. The samples were stored in a double concentrated SDS polyacrylamide gel electrophoretic puffer. The proteins were run in an SDS polyacrylamide gel and separated then blotted on nitrocellulose membrane. After blocking the membrane was incubated with the primary antibody on 4 ºC for a night. Next, the membranes were washed six times in TBS-Tween for five minutes then we added the secondary antibody marked by horseradish peroxidase (1:3000 dilution; Bio- Rad, Budapest, Hungary). Then the membrane was washed six times in TBS-Tween for five minutes again, we made the blot visible by chemiluminescent solution, finally quantified the results with Scion Beta 4.02 software.

5.3.3. Statistical analysis

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.

5.4. Results

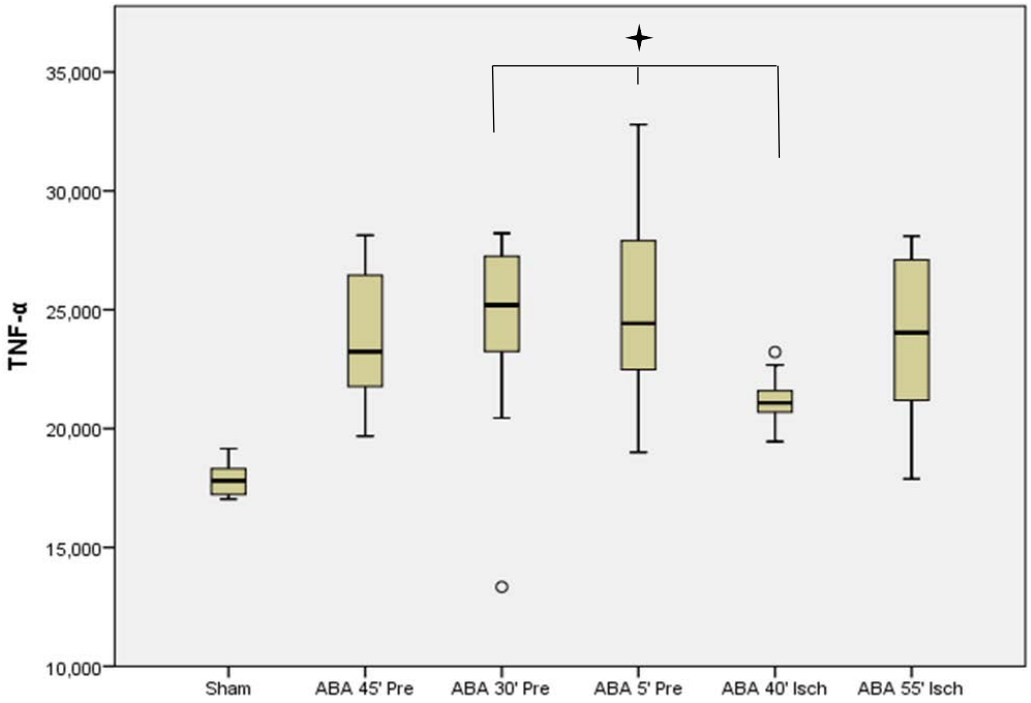


Fig. No. 19. TNF- γ levels in PPAR- γ administered groups. TNF-α levels in each group were higher compared to Sham. In group 40 Isch we found significantly lower levels compared to 5 Pre and 30 Pre groups.

We detected higher TNF-α levels in each group compared to Sham. In group 40 Isch we found significantly lower levels compared to 5 Pre and 30 Pre groups. (Sham 17,89 ± 0,79; 45Pre 23,74 ± 2,72; 30 Pre 24,16 ± 4,54; 5 Pre 25,24 ± 4,03; 40 Isch 21,19 ± 1,12; 55 Isch 23,80 ± 3,59) Fig. No. 19.

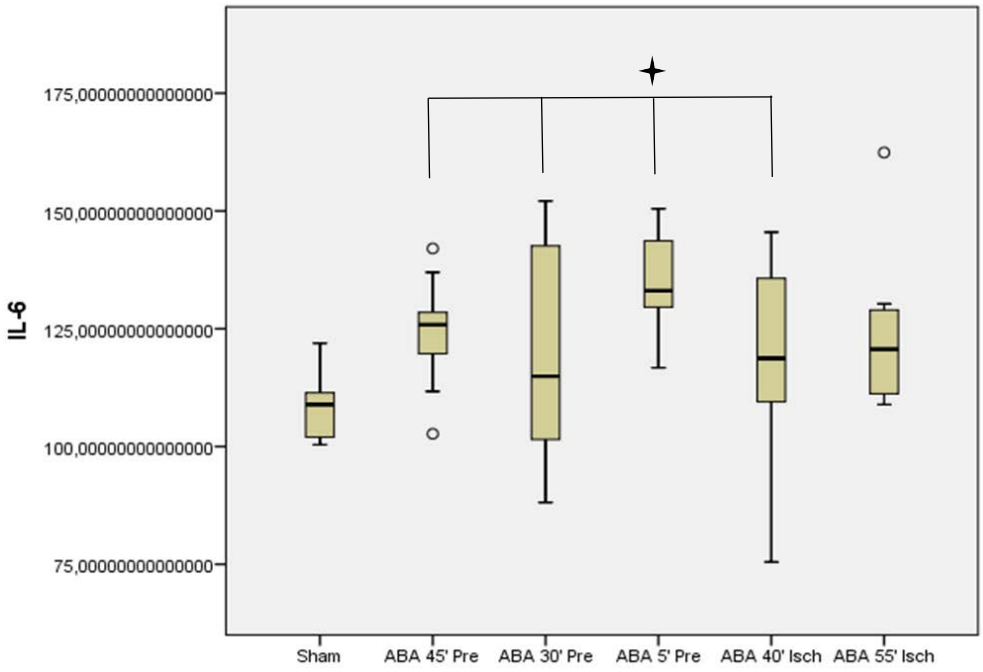


Fig. No. 20. IL-6 levels in PPAR- γ administered groups. IL-6 levels in all groups were increased compared to Sham group. Comparing groups to each other we found the above mentioned data: in 30 Pre, 40 Isch, 55 Isch groups we found significantly lower IL-6 levels compared to 5 Pre group.

Examining IL-6, we found elevated IL-6 levels in all groups compared to Sham group. Comparing groups to each other we found the above mentioned data: in 30 Pre, 40 Isch, 55 Isch groups we found significantly lower IL-6 levels compared to 5 Pre group. (Sham 108 ± 6,34; 45Pre 124,13 ± 10,81; 30 Pre 118,88 ± 22,26; 5 Pre 134,71 ± 9,32; 40 Isch120,02 ± 19,46; 55 Isch 123,56 ±15,06). Fig. No. 20.

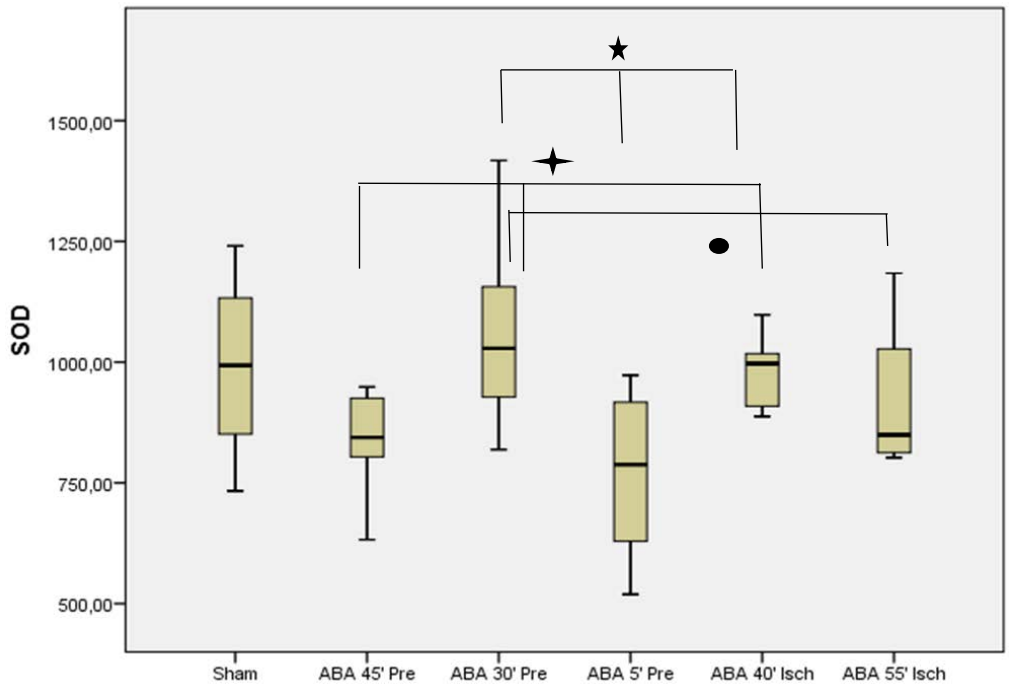


Fig. No. 21. SOD activity in PPAR- γ administered groups. SOD activity in 5Pre group was significantly lower compared to Sham, 40 Isch and 30 Pre groups. In group 45 Pre we found a significant decrease compared to 40 Isch and 30 Pre groups. We also found a significant decrease in group 55 Isch compared to 30 Pre group.

In SOD activity we found that 5Pre group data was significantly lower compared to Sham, 40 Isch and 30 Pre groups. In group 45 Pre there was a significant decrease compared to 40 Isch and 30 Pre groups. We also found a 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. (Sham 977,91 ± 163,06; 45Pre 827,339 ± 158,206; 30 Pre 1047,79 ± 177,63; 5 Pre 767,331 ± 206,38; 40 Isch 980,977 ± 43,78; 55 Isch 919,57 ± 179,59). Fig. No. 21.

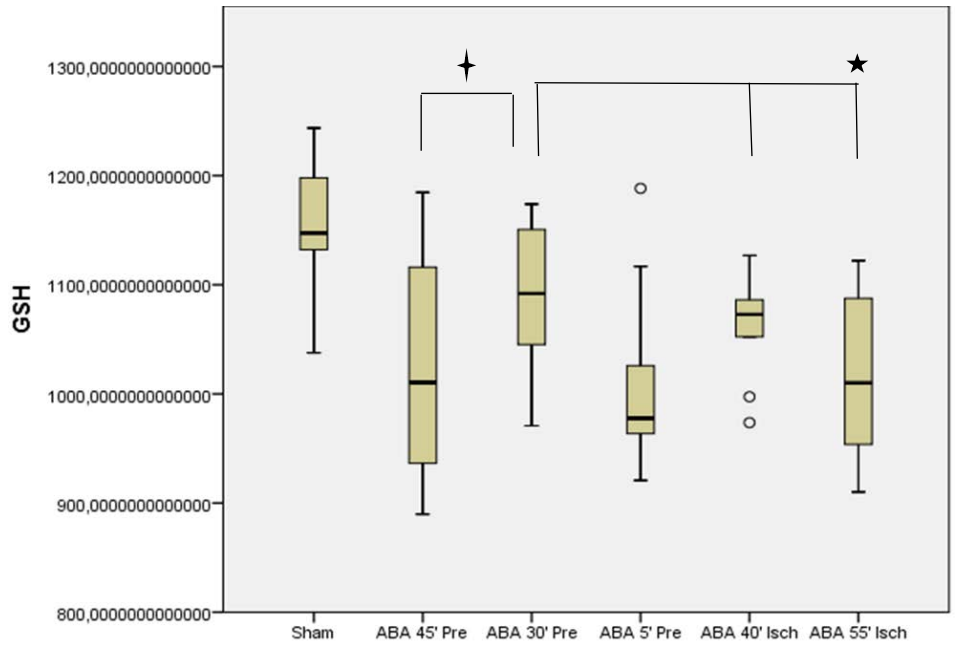


Fig. No. 22. GSH Concentrations in PPAR- γ administered groups. There was a significantly lower GSH concentration in all groups compared to Sham. We noticed a significantly lower GSH concentration in 55 Isch goup compared to 30 Pre and 40 Isch groups.

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. (Sham1153,96 ± 55,35; 45Pre 1029,39 ± 103,39; 30 Pre 1087,50 ± 67,02; 5 Pre 1005,40 ± 84; 40 Isch 1061,35 ± 45,06; 55 Isch 1009,50 ± 74,07) Fig. No. 22.

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. (Sham 69,67 ± 2,38; 45Pre 74,9 ± 7,14; 30 Pre 73,86 ± 10,53; 5 Pre 77,11 ± 7,17; 40 Isch 76,67 ± 5,77; 55 Isch 78,24 ± 11,06) Fig. No. 23.

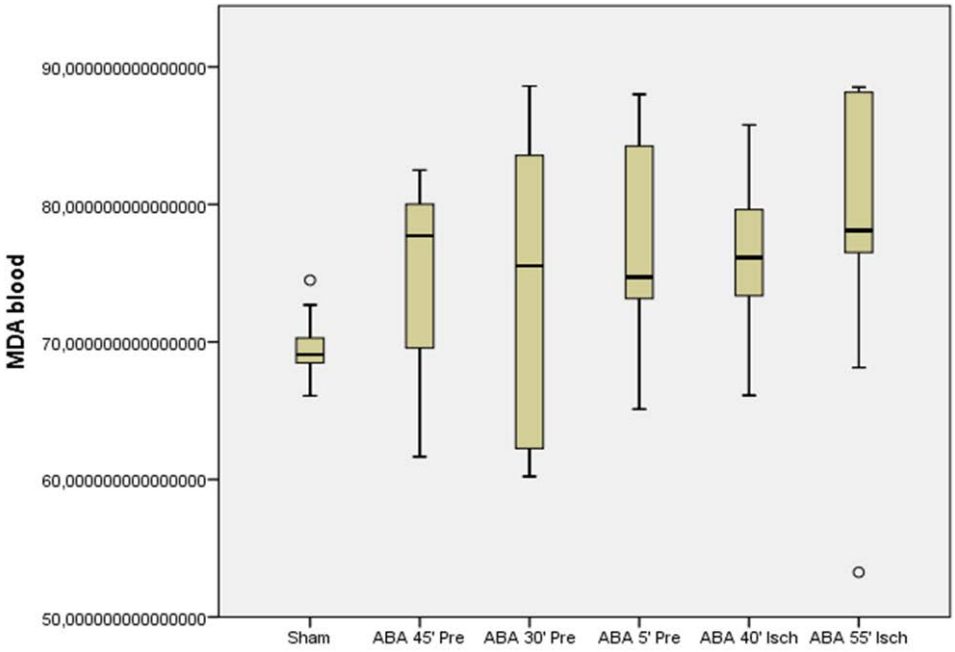


Fig. No. 23. MDA blood levels in PPAR- γ administered groups.

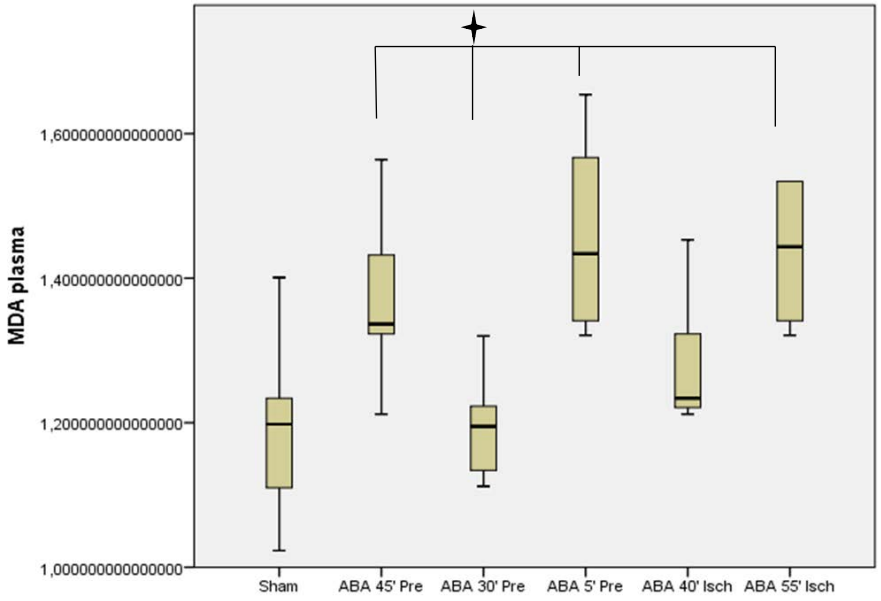


Fig. No. 24. MDA plasma levels in PPAR- γ administered groups. MDA concentration in 30 Pre group was significantly lower compared to groups 45 Pre, 5 Pre and 55 Isch.

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. (Sham 1,17 ± 0,1;

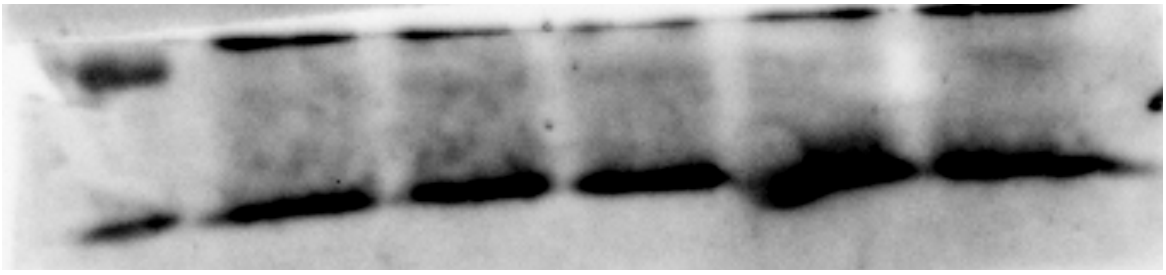
45Pre 1,38 ± 0,11; 30 Pre 1,19 ± 0,06; 5 Pre 1,46 ± 0,124; 40 Isch 1,27 ± 0,07; 55 Isch 1,42 ± 0,089) Fig. No. 24.

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.Fig. No. 25.

Sham **45'Pre** **30'Pre** **5'Pre** **40'Isch** **55' Isch**

Fig. No.25. The expression of Bax signal protein in PPARGA administered groups. The expression 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 anti apoptotic bcl-2 level was measured in 40 -, and 55 Isch groups. Fig. No. 26.



Sham 45'Pre 30'Pre 5'Pre 40' Isch 55' Isch

Fig. No. 26. The expression of antiapoptotic bcl-2 signal proteins in PPAR- γ administered groups. Higher expression of anti apoptotic 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. Fig. No. 27.

Sham 45’Pre 30’Pre 5’Pre 40’ Isch 55’ Isch

Fig. No. 27. The expression of p53 protein in PPAR- γ administered groups. Phosphorilated p53 expression showed diminution in 30’ Pre group. We detected no significant difference between groups in p53 expression.

5.5. Discussion

In this study we aimed to investigate the effects of a PPARGA on ischemia reperfusion injury in a laparoscopic rat model. We performed this experiment to evaluate the best administration time of the PPARGA. The 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.[63] Ali Çay et al. in their study administered Melatonin as prophylaxis to prevent potential adverse outcomes of laparoscopy related to increased oxidative stress in splanchnic organs. Group I: gasless (control); group II: 15 mmHg intraabdominal pressure (IAP) with CO_2 pneumoperitoneum for 60 min; group III: 15 mmHg IAP with CO_2 pneumoperitoneum for 60 min, and melatonin (10 mg/kg) was administered at two occasions, 5 min before insufflation and immediately before the desufflation. Comparisons among the groups revealed that highest mean MDA levels in liver, small intestine and kidney were in the group II, followed by the group III and control group. There was a significant difference between mean MDA levels in small intestine, liver and kidney of groups II and III ($p< 0.0005$). However, no significant difference was found between mean MDA levels in small intestine, liver, and kidney of the group III and control group. Mucosa and submucosa were affected significantly in the 15 mmHg IAP group (no prophylaxis) when compared with the control and melatonin prophylaxis groups ($P = 0.002$). However, there was no significant difference in the mean damage score of mucosa, submucosa, and muscular layers in the control group, compared to the melatonin prophylaxis

group. This experimental study indicated that melatonin prophylaxis, with anti-oxidant and anti-inflammatory actions, may have an important role in the prevention of potential complications related to oxidative stress injury on splanchnic organs induced by laparoscopy. [64] Atsushi Nakajima et al in their study sought to determine whether PPARγ could function as an endogenous anti-inflammatory pathway in a murine model of intestinal ischemia-reperfusion (I/R) injury. In their experiment PPARγ-deficient and wild-type mice were examined for their response to I/R procedure. Treatment with a PPARγ-specific ligand was also performed. In a murine model of intestinal I/R injury, they observed more severe injury in PPARγ-deficient mice and protection against local and remote tissue injury in mice treated with a PPARγ-activating ligand, BRL-49653. Activation of PPARγ resulted in down-regulation of intercellular adhesion molecule 1 expression by intestinal endothelium and tissue tumor necrosis factor α messenger RNA levels most likely by the inhibition of the NF-κB pathway. Their data strongly suggested that an endogenous PPARγ pathway exists in tissues that may be amenable to therapeutic manipulation in I/R-related injuries. [65]

5.6. Conclusion

Elevated intraabdominal pressure triggers oxidative stress due to pneumoperitoneum. 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 /Clinical pilot study/

6.1. Introduction

Laparoscopy is considered as a substantial diagnostic and therapeutic method in current surgical practice. Laparoscopic technique is much more beneficial than conventional open technique, regarding many aspects like less postoperative pain and shorter hospitalization, etc. However, there are some concerns about its adverse effects. Pneumoperitoneum created for better visualization and for working place causes an increased intraabdominal pressure (12-15 mmHg) which decreases perfusion of the splanchnic area. Due to hypoperfusion and I/R injury, reactive oxygen radicals and inflammatory cytokines accumulate. [29,66]

6.1.1. Laparoscopic surgery

Laparoscopy, the technique of examining the abdominal cavity was first described in 1901 by Kelling W.[67,68]. By introducing a cystoscope through the abdominal wall, he was able to visualize the effect of intraabdominal air insufflation on the abdominal content. That same year, Ott [69] examined the abdominal cavity of a pregnant women using a head mirror and a speculum introduced into a culdoscopic opening. In 1911, Jacobeus [70] reported from Sweden, laparoscopy in humans and Bernheim [71] in the USA, reported his experience with laparoscopy using a proctoscope and ordinary light. In 1960 Kurt Semm, a German gynecologist, developed an automatic insufflator to establish pneumoperitoneum, and in 1985 Erich Muhe reported the first successful laparoscopic cholecystectomy.[72]
The range of laparoscopic surgical techniques are increasing in complexity and now include i.a. cholecystectomy, adrenalectomy, nephrectomy, fundoplication, hernia repair, bowel resection and gynecological procedures.

6.1.2. The systemic effect of pneumoperitoneum

Laparoscopy requires the establishment of pneumoperitoneum in order to provide adequate surgical exposure and maintain operative freedom. The insufflation of carbon dioxide into the

peritoneal cavity can affect several homeostatic systems, leading to alterations in blood gases, acid-base balance, cardiovascular and pulmonary physiology. These alterations may be well tolerated by healthy individuals, but they may increase physiologic stress in patients with pre-existing morbidity, placing them at increased risk for perioperative complications. An understanding of the physiologic changes caused by capnoperitoneum is therefore essential for the identification of high-risk patients and formulation of appropriate treatment plans, which may include preoperative cardiorespiratory optimization and perioperative monitoring. Under optimal conditions, debilitated patients should be able to tolerate pneumoperitoneum safely and thereafter, reap the benefits associated with minimally invasive surgery.[73] Morbidly obese patients have 2 to 3 times higher intraabdominal pressure compared to nonobese patients. The adverse consequences of pneumoperitoneum in morbidly obese patients are similar to those observed in non-obese patients. Laparoscopy in the obese can lead to systemic absorption of CO_2 and increased requirements for CO_2 elimination. The increased intraabdominal pressure enhances venous stasis, reduces intraoperative portal venous blood flow, decreases intraoperative urinary output, lowers respiratory compliance, increases airway pressure, and impairs cardiac function. Intraoperative management to minimize the adverse changes include appropriate ventilatory adjustments to avoid hypercapnia and acidosis, the use of sequential compression devices to minimizes venous stasis, and optimize intravascular volume to minimize the effects of increased intraabdominal pressure on renal and cardiac function.[74]

6.2. Aims

Aim of our investigation was to evaluate the protective effects of ischemic preconditioning (IPC) during laparoscopic cholecystectomies (LC)

6.3. Patients and Methods

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 Hgmm 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.

6.3.1. Statistical analysis

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 $p \leq 0.05$. The graphic expression of the data was performed in box plots.

6.4.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 ± 1.047 days in the LC group, and 2.29 ± 0,72 days in the IPC group. (Mann-Whitney test $p = 0.377$)

SOD

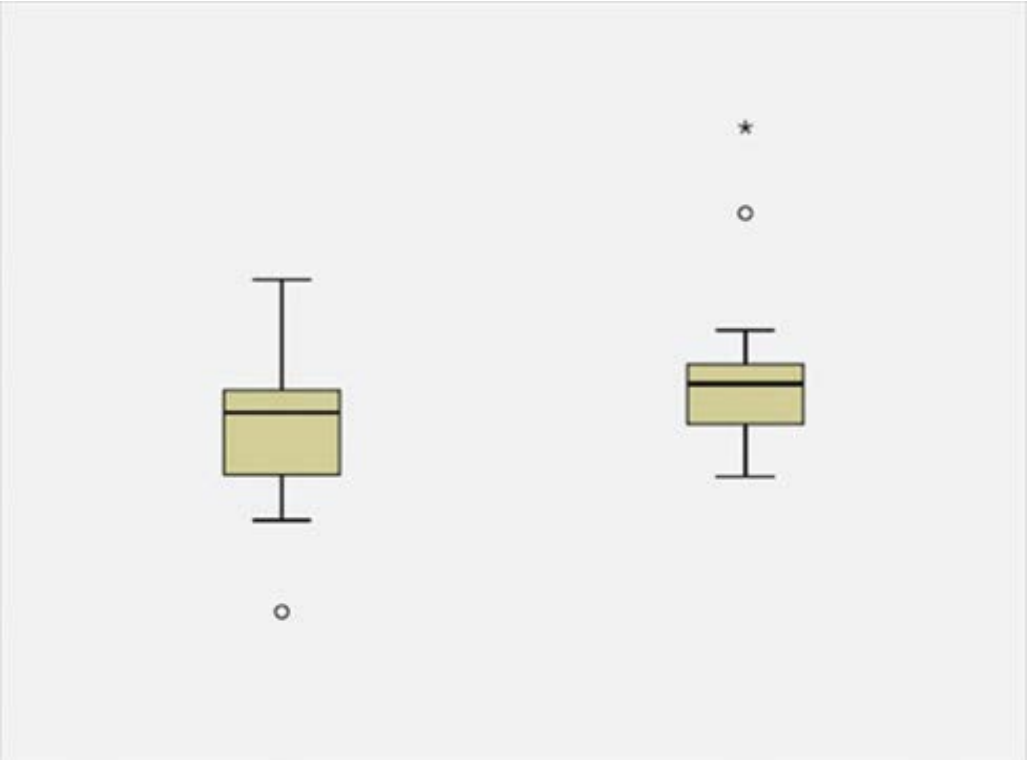
Data was very heterogeneous, therefore no statistical analysis was carried out. During control (C) we measured almost the same SOD activity in both groups. (LC 720.71 ±334.18 U/ml; IPC 720.56 ± 251.22 U/ml) According to the B.S data it can be concluded that anesthesia causes decrease in SOD activity in both groups, but this decrease was not significant (LC 655.54 ± 308.95 U/ml; IPC 645.91±210.32 U/ml). The following was found A.S.: LC 674.67± 303.60 U/ml; IPC 690.58 ± 223.37 U/ml and 24 h after operations: LC 730.76 ± 217.19U/ml; IPC 631 ± 169.34U/ml.

MDA (blood)

In case of MDA concentration heterogeneity was also detected. There was no significant difference between LC and IPC groups. *C groups*: LC 82.30 nM/ml, SD: 4.15; IPC 79.43 nM/ml;SD: 5.35; *B.S. groups*: LC 82.51 nM/ml, (SD: 3.81) IPC 82.08 nM/ml; (SD: 11.49) *A.S. groups*: LC 87.03 nM/ml, (SD: 5.2); IPC 90.03 nM/ml; (SD: 21.99) *24 h groups:* LC 85.75 nM/ml, (SD:10.95);IPC 85.26 nM/ml (SD: 11.11).

GSH

In case of GSH concentrations we also noticed heterogeneity, but in the IPC group a significantly higher concentration was measured postoperatively (908.80 nM/ml, SD: 81.65) compared to the LC group (853.93 nM/ml, SD: 67.70). (M-Wht test $p = 0.05$) Fig. No.28.



LC IPC

Fig.No. 28. GSH concentrations during operations. In the IPC group a significantly higher concentration was measured postoperatively compared to the LC group.
(M-Wht test $p = 0.05$).

SH-,Catalase

No significant changes were detected in SH- concentration and catalase levels.

MPO, MDA (plasma)

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 (LC 6.60 U, SD 1.77; IPC 4.25 U, SD 2.17) ($p = 0.014$ M-Why test), and also 24 hours later (LC 3.10 U, SD 1.5, IPC 1.17 U, SD 1.03) ($p = 0.006$ M-Why test, $p = 0.005$ Wilcoxon singed –rank test). 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. Fig. No. 29.

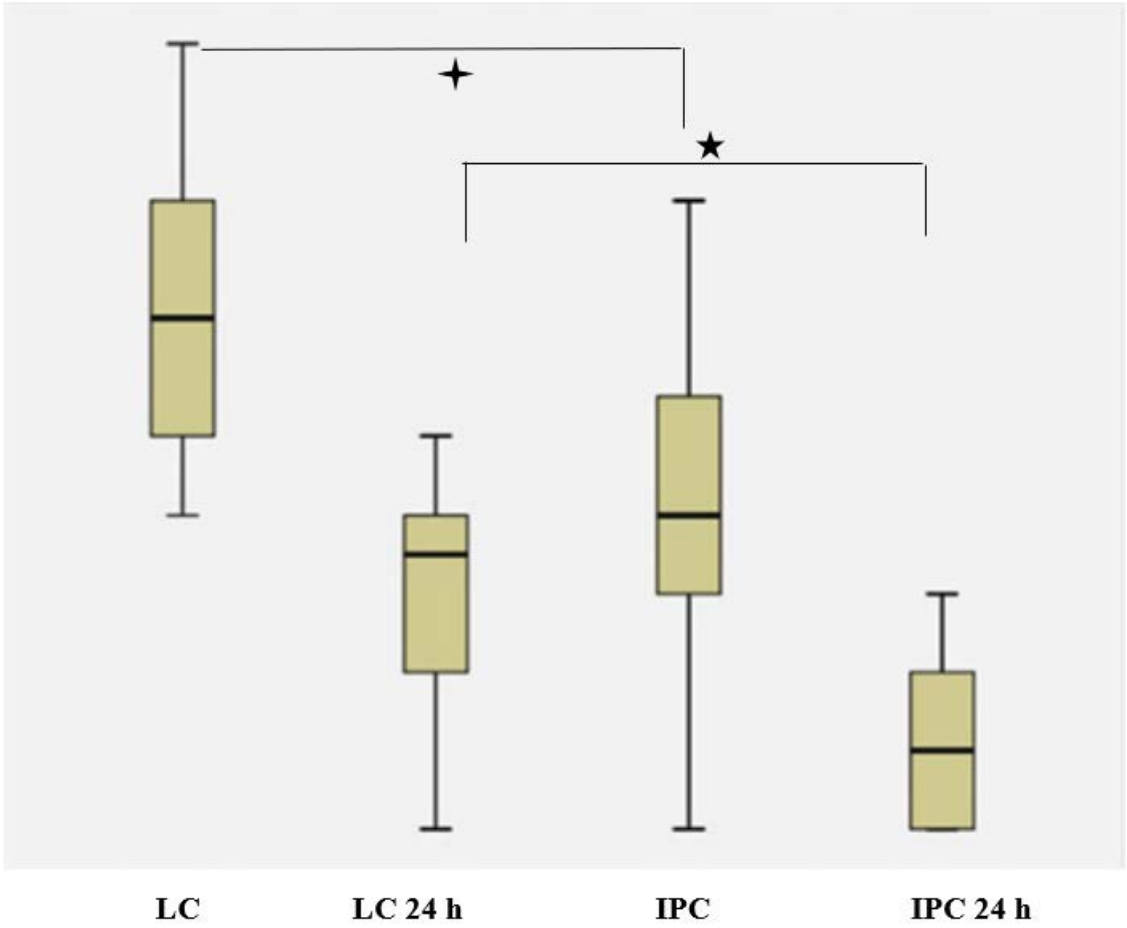


Fig. No 29. Visual Analog Scale. A significant difference can be seen in the IPC group, both after the operation (p = 0.014 M-Why test), and 24 hours later (p = 0.006 M-Why test, p = 0.005 Wilcoxon singed – rank test). Pain in IPC group was about 2–2.5 units lower at both times.

6.5.Discussion

Laparoscopic technique has many advantages over conventional laparotomy: reduced hospitalization, reduced pain-, scar, reduced postoperative hernia, etc. Operation time is notably shorter, and recovery is faster, therefore it decreases costs. Pneumoperitoneum used for adequate visualization has some side-effects too. IPC is a concept that has been employed to avoid the harmful effects of ischemia-reperfusion injury in cardiac [75], liver [76] and reconstructive surgery [77]. To our knowledge no human study on preconditioning during pneumoperitoneum has been conducted, only in animal experiments. Some concerns have also arisen that preconditioning is too lengthy for practical use.

The redox status of the body is tightly regulated and - as shown in the majority of the studies - returns to normal within 24 hours after surgery. In particular, in one study involving children, there was no change in markers of oxidative stress after either open or laparoscopic surgery.[78] Gutt and Schmandra examined abdominal blood flow at different intra-abdominal pressures (0–12 Hgmm) caused by CO2 pneumoperitoneum in rats. They observed that by increasing the intra-abdominal pressure, blood flow will decrease and at 12 Hgmm it becomes minimal. Hypoxia will evolve and abdominal organs will be damaged by reactive oxygen species.[79] Polat et al. performed laparoscopic cholecystectomy on 24 patients (12 male and 12 female). MDA and sulfhydryl-group concentrations were measured and they observed that concentration of these markers had increased if they applied a higher intra-abdominal pressure (10 vs. 15 Hgmm) [39]. Based on the above mentioned two articles, we set the IAP at 15 mmHg in our study. Yilmaz et al. could reduce oxidative stress caused by pneumoperitoneum by using preconditioning in rats. They investigated oxidative stress markers and inflammatory cytokine concentrations in sham operated animals, after pneumoperitoneum and after preconditioning for 10 minutes. They concluded that increased intra-abdominal pressure could cause oxidative stress and preconditioning could reduce that. They used preconditioning before creating pneumoperitoneum at a pressure of 15 Hgmm and observed that using preconditioning has a better protective effect than lower intra-abdominal pressure (10Hgmm). [40,80,81,82] Athanasiadis D. et al. examined the effect of remote ischemic preconditioning (RIPC) in decreasing renal ischemia-reperfusion injury (IRI) during a suprarenal aortic cross-clamping porcine model. They found, that repetitive short periods of cycles of ischemia-reperfusion (IR) ameliorated the biochemical effects of IRI on the kidneys. RIPC groups presented significantly less impaired results compared to the IR group when evaluating MDA, cystatin C, CRP and creatinine levels. Between the two RIPC groups, RIPC II presented a better response regarding CRP, NGAL, TNF-α, MDA and cystatin C values. They concluded, that remote IR and mainly repetitive short cycles of IR ameliorate the biochemical effects of IRI on the kidneys in a model of suprarenal aortic aneurysm repair. This fact also explains, that short repeated ischemia decreases ischemic-reperfusion injury. [83] In our pilot study we also found that considering GSH changes, preconditioning could reduce the IR injury. In animal studies subjects are mostly brought up under the same conditions (same food, same temperature etc.). Our data was heterogeneous, because in a human study it is impossible to assure same aged, same weighted, same fed patients. Leventi A. et al. used twenty-five female pigs in an experimental model. They used ischemic preconditioning for 15 minutes and 15 minutes desufflation before maintaining

pneumoperitoneum. They concluded, that IPC attenuated oxidative stress induced by intraabdominal hypertension, mainly by increasing antioxidative capacity and the levels of protective mediators. It was shown that IPC was effective, even in case of extremely high levels of intraabdominal pressure. [84] Nesek-Adam et al in their study demonstrated that IPC prevented hepatocyte injury and oxidative stress during CO2 pneumoperitoneum.[85] All our findings demonstrate that pneumoperitoneum has a negative effect which correlates with time and pressure. It seems that preconditioning can reduce the harm caused by pneumoperitoneum, and can reduce pain as well. There are many questions but there are a lot investigations in progress around the world to answer this assumption [86]. Thus the method of preconditioning may have important clinical implications.

6.6.Conclusion

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. Discussion

Ischemic conditioning under its different forms provides a stimulating field of research for improving organ quality, by enhancing protection against ischemia-reperfusion injury in IPC and by promoting organ repair in IPoC. Ischemic conditioning is a phenomenon through which short sequences of I/R applied to an organ confer some degree of protection towards future ischemic insults. This phenomenon was first observed in the mid-1980s in cardiac surgery, and has been since widely studied in different settings. Different sorts of ischemic conditioning exists: local vs. remote, direct or pharmacological, and with different timeframes of protection. The pathways through which ischemic conditioning works are many, offering

both preservation of cell energy, protection against oxidative stress, better blood flow to organs and protection against apoptosis. In the field of pharmacological conditioning, which tries to mimic the protective effects of traditional ischemic conditioning without the potential side-effects associated with vessel clamping, many common-used drugs including anesthetics have been shown to be effective. Significant results have been obtained in small animal models, but while ischemic conditioning is successfully used in cardiac surgery, studies in large animal models and human applications were still inconclusive.[87]

IPC was first described in the context of heart surgery in the mid-1980s.[17] IPC is a phenomenon by which sequenced short ischemic periods followed by reperfusion confer protection against further ischemic insult to the organ. Studies have shown that this phenomenon is not limited to the heart but also takes place in the kidney, liver, brain and small intestine, and covers different mechanisms and pathways. Time between IPC and ischemic insult should first be taken into consideration: one can then consider classic IPC (C-IPC), which typically confers a potent protection against further ischemia but is limited in time, usually 2 to 4 hours after initiation of the procedure,[17] and the so called ``second window of protection'' (SWOP); this SWOP happens around 24 hours after the initial IPC procedure but offers more moderate protection. Site of the IPC procedure must also be taken into account, and has led to the description of both local IPC (LIPC) in which the organ vessels are directly clamped and remote IPC (RIPC) where the organ protection is secondary to vessel clamping in a different areas.[88]

While IPC seems to be a promising path to preserve organs submitted to ischemic injury, either in emergency situations such as myocardial infarction or strokes or in a planned surgical settings, human applications of IPC have not always been as successful as preliminary animal studies would have led people to expect. First of all, IPC in animal experiments require precise timing of the conditioning itself and then of the ischemic insult. In scheduled surgical situations, it is far easier to integrate precise timing for conditioning and subsequent ischemia. While relatively easy to implement in a controlled, surgical setting such as transplantation or cardiac surgery, laparoscopy, IPC is not well-suited to emergency settings, as the onset of myocardial or brain infarction cannot be anticipated. Therefore of interest is the phenomenon of ischemic post-conditioning (IPoC), which was described subsequently to IPC. Sequential clamping and de-clamping of organ vessels after the ischemic insult can also confer some degree of protection, or higher repair potential to organs.

Pharmacoligical intervention has also been shown to mimic direct IPC procedures. This pharmacological ischemic conditioning can be considered as a subset of the RIPC.

Laparoscopic surgery was one of the greatest achievements of the last century. Many studies highlight the fact that it is a gold-standard method in many cases. After laparoscopic procedure postoperative pain decreases, hospital stay is shorter, wound healing is better and hernia formation is rarer than after conventional open surgery.

Gál et al. investigated the alteration of MDA, GSH, GSSG (oxidized glutathione) IL-6 and CRP (C-reactive protein) concentrations after laparoscopic- and open cholecystectomy. Concentration of pro-oxidant markers was increased after open operations compared to laparoscopic technique, meaning that during open operations oxidative stress is greater. Size of the oxidative harm has correlated with the size of incision.[89 90]

In our first study our observation was similar to that described in literature that during pneumoperitoneum oxidative stress develops. Reduced glutathione (GSH) concentration and superoxide-dismutase (SOD) activity were decreased and malondialdehyde (MDA) plasma concentration was increased during pneumoperitoneum. In the literature many workgroups investigated the ways how negative effects of pneumoperitoneum could be reduced. Yilmaz et al. in their randomized controlled trial evaluated the effect of preconditioning (IPC) on laparoscopy-induced I/R injury. Their subjects were 40 Sprague-Dawley male rats. Pneumoperitoneum (P) was created in all except controls, using carbon dioxide (CO_2) insufflation under a pressure of 15 mmHg. IPC consisted of 10 min of P, followed by 10 min of deflation (D). The rats were randomized to the following groups: Group P was subjected to 60 min of P. Group P/D was subjected to 60 min of P, followed by 45 min of D. Group IPC/P was subjected to IPC, followed by 60 min of P. Group IPC/P/D was subjected to IPC, followed by 60 min of P and 45 min of D. Group C (control) was subjected to a sham operation, without P. They measured plasma alanine aminotransferase (ALT) and MDA, as well as homogenized tissue MDA levels and GSH activities. Their results showed that, plasma ALT as well as plasma, liver, and kidney MDA levels and liver and kidney injury scores were increased, whereas liver and kidney GSH values were decreased in groups P and P/D, when compared to group C. Rats subjected to IPC before P had plasma ALT, kidney MDA, and kidney and liver GSH levels comparable to controls; their kidney and liver injury scores were higher than the controls' but significantly lower than nonpreconditioned animals. IPC enabled decreased plasma, kidney, and liver MDA as well as increased kidney GSH if applied before P; its efficacy on oxidative stress was limited to providing decreased kidney MDA and increased kidney GSH if applied before P/D. However, IPC significantly attenuated kidney and liver injury after P as well as P/D. They concluded: IPC consisting of 10 min of P followed by 10 min of D decreases the oxidative stress induced by sustained P in the plasma, liver, and

kidney. IPC significantly limits liver and kidney injury after prolonged P and P/D. [70] Also Yilmaz et al. used preconditioning before creation of pneumoperitoneum at a pressure of 15 mmHg and observed that using preconditioning has a better effect than lower intra-abdominal pressure (10 mmHg). IPoC appears to confer profound tissue protection across more species [91] and while the vast majority of studies have examined the myocardium as the organ of interest [92] IPoC has been shown to be protective in many different organs including the kidney.[93] Based on the above mentioned experiments by Yilmaz et al. our workgroup also used preconditioning for protection. In literature there is limited data concerning laparoscopic postconditioning. In our study we tried to compare laparoscopic IPC-, and IPoC at low pressures (5 mmHg, 10 mmHg). Within the investigated markers GSH, SOD, MPO and MDA plasma concentrations and activities were changed in the groups. Our results showed, that both IPC and IPoC attenuates the oxidative stress caused by pneumoperitoneum, but this attenuation can be seen mostly on 10 mmHg groups.

Ates in an another investigation, for protecting animals from negative effects of pneumoperitoneum, used erythropoietin, and found that, however, erythropoietin had a beneficial effect, for protection preconditioning proved to be a better method. [71]

Polat et al. performed laparoscopic cholecystectomy on 24 patients (12 male and 12 female). MDA and sulfhydryl-group concentration was investigated and they observed that concentration of these markers was increased if they applied higher intra-abdominal pressure (10 vs. 15 mmHg).[39]

Over the last quarter of a century, a huge effort has been made to develop interventions that can minimize ischemia reperfusion injury. The most potent of these are the ischemic conditioning strategies, which comprise ischemic preconditioning, remote ischemic preconditioning and ischemic postconditioning. While much of the focus for these interventions has been on protecting the myocardium, other organs including the kidney can be similarly protected. In 1993, the profound protection of IPC seen in animals was translated to humans and first investigated.[94] Since then, nearly 100 clinical trials have been published examining the effect of IPC to confer cytoprotection in the myocardium and other organs including the lung [95], liver [96], brain [97] and even in knee surgery [98]. While the published clinical studies have almost invariably demonstrated positive outcomes with IPC, the trials have generally been small, single-center trials with short-term outcomes. However, so far, no human trials investigating IPC in laparoscopy have been performed.

The typical patient presenting to the emergency department with any acute disease is most likely to be older and a smoker with a history of ischemic heart disease, obesity, hypertension,

diabetes or chronic kidney disease and has been prescribed several different medications. This is in contrast to the vast majority of animal studies that use juvenile, healthy animals with no comorbidities or medications. These differences could explain why the most potent cytoprotective strategies known to science appear to translate only into moderate benefits in humans.

8. 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.

9. Acknowledgement

First of all, my most sincere gratitude goes to my family for all their love, patience and continuous support throughout this work.

I would like to take the opportunity to express my thanks for the support I have received from my supervisors Dr. Ildikó Takács and Prof. András Vereczkei in completing this work. Their assistance, graceful guidance and leadership meant a lot over the years.

Further, I am grateful for the help and assistance of the head of Department, Dr. Gábor Jancsó and my collegues, Dr. Szaniszló Jávor, Dr. Mária Kürthy, Dr. János Lantos and Dr. Tibor Nagy. I would also thank to previous heads of the Department: Prof. Erzsébet Rőth and Prof. György Wéber, my previous Undergraduate Research Committee students Dr. Laura Petrovics, Dr. Katalin Sárvári and to the full staff at the Department of Surgical Research and Technique of Pécs University for carrying out the investigations and giving me the inward support over the years.

I would like to express my sincere thanks for giving an excellent coverage in statistical work to Dr. László Pótó and Dr. Kornélia Farkas Borbásné in the Institute of Bioanalysis. I am also very grateful for the molecular biologic methods to Mónika Vecsernyés in the Departement of Medical Biology and to Endre Kálmán for giving me the chance to carry out the pathological analysis in the laboratory of the Department of Pathology of Pécs University.

This work was supported by the Hungarian Science Research Fund OTKA-K108596 and the study was approved by TUKEB (No. ad.774/PI/2012; ad.50760/2012/EKU).

10. Publications and presentations

Publications

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 Effect of laparoscopic pre- and postconditioning on splanchnic microcirculation *Under publication*

Abstracts

1. Veres Gyöngyvér Tünde, Jávor Szaniszló, Wéber György: Az alacsony nyomáson történő prekondicionálás csökkenti a pneumoperitoneum okozta szisztémás, káros oxidatív hatásokat MAGYAR SEBÉSZET (2012)

2. T. Gy. Veres, Sz. Jávor, Gy. Wéber: Precondiotioning even using low pressure can reduce surgical stress following laparoscopic procedures EUROPEAN SURGICAL RESEARCH (ISSN: 0014-312X) (eISSN: 1421-9921) 48: (3) pp. 111-170. (2012)

3. Kürthy Mária, Miklós Zsanett, Kovács Dóra, Degrell Péter, Rantzinger Eszter, Arató Endre, Sinay László, Nagy Tibor, Hardi Péter, Kovács Viktória, Jávor Szaniszló, Veres Gyöngyvér Tünde, Rőth Erzsébet, Lantos János, Jancsó Gábor: A posztkondicionálás hatása az ischaemia/reperfúsiós károsodásokra hiperlipidémiás patkánymodellen. MAGYAR SEBÉSZET 67:(2) p. 95. (2013)

4. Kovács Viktória, Balatonyi Borbála, Borsiczky Balázs, Gasz Balázs, Jávor Szaniszló, Nagy Tibor, Hardi Péter, Veres Gyöngyvér Tünde, Dézsi László, Lantos János, Jancsó Gábor, Marczin Nándor, Rőth Erzsébet: Glutation-S-transzferáz P1 gén polimorfizmus és nyitott mellkasú szívműtét posztoperatív szövődményeinek összefüggései.
MAGYAR SEBÉSZET 67:(2) p. 95. (2013)

5. Nagy Tibor, Kovács Viktória, Hardi Péter, Veres Gyöngyvér Tünde, Kürthy Mária, Dézsi László, Sinay László, Arató Endre, Menyhei Gábor, Rőth Erzsébet, Jancsó Gábor: A glutation-S-transzferáz gátló etakrinsav hatása az ischaemia-reperfusiós károsodásokra alsó végtagi ischaemia modellen. MAGYAR SEBÉSZET 67:(2) p. 101. (2013)

6. Huszár O, Jancsó G, Ferenc S, Hardi P, Veres Gy T, Vereczkei A, Horváth Ö P, Papp A: A Harmonic ACE és Enseal vágókészülék vérzéscsillapító hatásának összehasonlító vizsgálata Copidogrel/Aszpirin Protect okozta tct aggregáció gátlásban részesült, illetve nem részesült sertéseken MAGYAR SEBÉSZET 66:(2) p. 88. (2013)

7. 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 ischaemia-reperfusió indukálta oxidatív stressz kivédésében. MAGYAR SEBÉSZET 67:(2) p. 116. (2013)

8. Hardi Péter, Nagy Tibor, Veres Gyöngyvér Tünde, Kovács Viktória, Fazekas Gábor, Pintér Örs, Nagy Ágnes, Kovács Sándor, Szelechman Ildikó, Szekeres Eszter, Takács Veronika, Bognár Laura, Jancsó Gábor. Vese iszkémiás károsodások csökkentése penthosan polysulfate natrium (PPSN) adásával állatkísérletes modellben. MAGYAR SEBÉSZET 67:(3) pp. 166-167. (2014)

9. Kovács Viktória, Nagy Tibor, Veres Gyöngyvér Tünde, Hardi Péter, Lantos János, Jancsó Gábor, Gasz Balázs. A glutathion S-transzferáz gátló etakrinsav hatása acut myocardiális iszkémia/reperfúziós folyamatokra. MAGYAR SEBÉSZET 67:(3) p. 180. (2014)

10. Nagy Tibor, Kovács Viktória, Hardi Péter, Veres Gyöngyvér Tünde, Kürthy Mária, Lantos János, Pintér Örs, Sínay László, Menyhei Gábor, Rőth Erzsébet, Jancsó Gábor, Arató Endre. Az endogén antioxidáns glutation S-transzferáz gátló etakrinsav hatása az iszkémia-reperfúziós károsodásokra alsó végtagi akut iszkémia patkánymodellen. MAGYAR SEBÉSZET 67:(3) p. 192. (2014)

11. Sárvári K, Petrovics L, Nagy T, Kovács V, Hardi P, Máthé E, Jancsó G, Wéber Gy, Vereczkei A, Veres Gy. A prekondicionálás és posztkondicionálás szerepének összehasonlítása a pneumoperitoneum okozta káros oxidatív hatások kivédésében. MAGYAR SEBÉSZET 67:(3) pp. 205-206. (2014)

12. Veres Gyöngyvér Tünde, Nagy Tibor, Sárvári Katalin, Petrovics Laura, Hardi Péter, Kovács Viktória, Takács Ildikó, Lantos János, Kondor Ariella, Baracs József, Máthé Ervin, Wéber György, Jancsó Gábor, Vereczkei András. Széndioxiddal készített pneumoperitoneum okozta káros hatások vizsgálata és csökkentésének lehetősége - Klinikai vizsgálat. MAGYAR SEBÉSZET 67:(3) pp. 221-222. (2014)

13. Veres Gyöngyvér Tünde, Katalin Sárvári, Laura Petrovics, Tibor Nagy, Péter Hardi, Ildikó Takács, Ervin Máthé, György Wéber, András Vereczkei, Gábor Jancsó. The role of pre- and postconditioning to avoid the ischaemia-reperfusion injury caused by pneumoperitoneum. EUROPEAN SURGICAL RESEARCH 52:(3-4) p. 110. (2014)

14. J Werling, T Nagy, V Kovács, L Sínay, E Arató, T Gy Veres, I Takács, I Szelechman, P Hardi, K Sárvári, G Jancsó. Controlled Reperfusion of Infrarenal Aorta Decreased Ischaemic-Reperfusion Injuries after Aortic Clamping in Vascular Surgery. EUROPEAN SURGICAL RESEARCH 52:(3-4) p. 43. (2014)

15. Sárvári Katalin, Veres Gyöngyvér Tünde, Petrovics Laura, Nagy Ágnes, Takács Ildikó, Hardi Péter, Nagy Tibor, Vereczkei András, Jancsó Gábor: PPAR-gamma agonista adásával csökkenthető a pneumoperitoneum hatására létrejövő reperfúziós károsodás mértéke patkányokban. MAGYAR SEBÉSZET 68:(3) p. 125. (2015)

16. Veres Gyöngyvér Tünde, Takács Ildikó, Nagy Tibor, Hardi Péter, Kondor Ariella, Jávor Szaniszló, Sárvári Katalin, Petrovics Laura, Baracs József, Lantos János, Jancsó Gábor, Wéber György, Vereczkei András: Prekondicionálással csökkenthető-e a laparoszkópos epeműtét során kialakult oxidatív stressz? MAGYAR SEBÉSZET 68:(3) p. 126. (2015)

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.

4. Veres Gyöngyvér Tünde, Nagy Tibor, Kovács Viktória, Hardi Péter, Takács Ildikó, Kürthy Mária, Lantos János, Sárvári Katalin, Petrovics Laura, Máthé Ervin, Vereczkei András, Rőth Erzsébet, Wéber György, Jancsó Gábor: A pre- és posztkondicionálás szerepe a

pneumoperitoneum által kiváltott oxidatív stressz kivédésében Magyar Haemorheologiai Társaság XXI., a Magyar Mikrocirkulációs és Vaszkuláris Biológiai Társaság ill. a Magyar Szabadgyökkutató Társaság IV. közös Kongresszusa, Balatonkenese, 2014. április 4-5.

5. Veres Gyöngyvér Tünde, Katalin Sárvári, Laura Petrovics, Tibor Nagy, Péter Hardi, Ildikó Takács, Ervin Máthé, György Wéber, András Vereczkei, Gábor Jancsó. The role of pre- and postconditioning to avoid the ischaemia-reperfusion injury caused by pneumoperitoneum. 49th Annual Congress ESSR 2014.05.21-24. Budapest-Hungary

6.Veres Gyöngyvér Tünde, Nagy Tibor, Sárvári Katalin, Petrovics Laura, Hardi Péter, Kovács Viktória, Takács Ildikó, Lantos János, Kondor Ariella, Baracs József, Máthé Ervin, Wéber György, Jancsó Gábor, Vereczkei András. Széndioxiddal készített pneumoperitoneum okozta káros hatások vizsgálata és csökkentésének lehetősége - Klinikai vizsgálat. Magyar Sebész Társaság 62. Kongresszusa, Győr, 2014. június 12–14.

7. Veres Gyöngyvér Tünde, Takács Ildikó, Nagy Tibor, Hardi Péter, Kondor Ariella, Jávor Szaniszló, Sárvári Katalin, Petrovics Laura, Baracs József, Lantos János, Jancsó Gábor, Wéber György, Vereczkei András: Prekondicionálással csökkenthető-e a laparoszkópos epeműtét során kialakult oxidatív stressz? A Magyar Sebész Társaság Kísérletes Sebészeti Szekciójának XXV. Kongresszusa, Pécs, 2015. május 14-16.

8. T. Gy. Veres, K. Sárvári, L. Petrovics, I. Takács, T. Nagy, P. Hardi, A. Kondor, J. Baracs, Gy. Wéber, G. Jancsó, A. Vereczkei Ischemic preconditioning may reduce oxidative stress following laparoscopic cholecystectomies – Clinical trial 50th Annual Congress ESSR 2015.06.10-06.13. Liverpool- UK

11. References

[1] Reza Arsalani-Zadeh et al. Oxidative Stress in Laparoscopic Versus Open Abdominal Surgery: A Systematic Review Journal of Surgical Research 2011;169, e59–e68

[2] Takemura G, Fujiwara H. Morphological aspects of apoptosis in heart diseases. J Cell Mol Med. 2006; 10: 56–75.

[3] Thomas S, Balasubramanian KA. Role of intestine in postsurgical complications: Involvement of free radicals. Free Radic Biol Med 2004; 36:745.

[4] Mishra V, Baines M, Wenstone R, et al. Markers of oxidative damage, antioxidant status and clinical outcome in critically ill patients. Ann Clin Biochem 2005; 42:269.

[5] Van Cruchten S, Van Den Broeck W. Morphological and biochemical aspects of apoptosis, oncosis and necrosis. Anat Histol Embryol. 2002; 31: 214–23.

[6] Tritto I, Ambrosio G. Role of oxidants in the signaling pathway of preconditioning. Antioxid Redox Signal. 2001; 3: 3–10.

[7] Zhao ZQ, Vinten-Johansen J. Myocardial apoptosis and ischemic preconditioning. Cardiovasc Res. 2002; 55: 438–55.

[8] McCord JM. Oxygen-derived free radicals in postischemic tissue injury. N Engl J Med 1985; 312:159.

[9] Jezek P., Hlavata L. Mitochondria in homeostasis of reactive oxygen species in cell, tissues, and organism. Int J Biochem Cell Biol 2005; 37:2478.

[10] Hampton MB, Kettle AJ., Winterbourn CC. Inside the neutrophil phagosome: Oxidants, myeloperoxidase, and bacterial killing. Blood 1998; 92:3007.

[11] Janssen-Heininger YM., Mossman BT., Heintz NH., et al. Redoxbased regulation of signal transduction: Principles, pitfalls, and promises. Free Radic Biol Med 2008; 45:1.

[12] Betteridge DJ. What is oxidative stress? Metabolism 2000; 49:3.

[13] Dalle-Donne I., Rossi R., Colombo R., et al. Biomarkers of oxidative damage in human disease. Clin Chem 2006; 52:601.

[14] de Zwart LL., Meerman JH., Commandeur JN., et al. Biomarkers of free radical damage applications in experimental animals and in humans. Free Radic Biol Med 1999; 26:202.

[15] Efstathios K. Iliodromitis, Antigone Lazou, Dimitrios Th Kremastinos. Ischemic preconditioning: Protection against myocardial necrosis and apoptosis Vasc Health Risk Manag. 2007 Oct; 3 (5): 629–637.

[16] Golino P., Ragni M., Cirillo P., et al. Effects of tissue factor induced by oxygen free radicals on coronary flow during reperfusion. NatMed 2: 35– 40

[17] Murry CE., Jennings RB., Reimer KA.: Preconditioning with ischemia: A delay of lethal cell injury in ischemic myocardium. Circulation 1986; 74, 1124–1136

[18] Marber M.S., Latchman D.S., Walker J.M., Yellon D.M. Cardiac stress protein elevation 24 hours after brief ischemia or heat stress is associated with resistance to myocardial infarction Circulation 1993; 88, 1264-1272

[19] Aksöyek S., Cinel I., Avlan D., et al.: Intestinal ischemic preconditioning protects the intestine and reduces bacterial translocation. Shock 2002; 18, 476–480

[20] Pajdo R., Brzozowski T., Konturek PC. et al. Ischemic preconditioning, the most effective gastroprotective intervention: involvement of prostaglandins, nitric oxide, adenosine and sensory nerves. Eur J Pharmacol 2001; 427: 263-276.

[21] Vinten-Johansen J., Zhao ZQ., Corvera J., et al. Inhibition of myocardial injury by ischaemic postconditioning during reperfusion: comparison with ischaemic preconditioning. Am J Physiol Heart Circ Physiol. 2003; 285. 579-588.

[22] Dow J., Kloner RA. Postconditioning does not reduce myocardial infarct size in an in vivo regional ischaemia rodent model. J Cardiovasc Pharmacol Ther. 2007; 12: 153–63.

[23] Tsang A., Hausenloy DJ., Mocanu MM., et al. Postconditioning: a form of “modified reperfusion” protects the myocardium by activating the phosphatidylinositol 3-kinase-Akt pathway. Circ Res. 2004 Aug 6; 95(3):230-2.

[24] Yang XM., Proctor JB., Cui L., et al. Multiple, brief coronary occlusions during early reperfusion protect rabbit hearts by targeting cell signaling pathways. J Am Coll Cardiol. 2004; Sep 1; 44(5):1103-10.

[25] Pastore A., Federici G., Bertini E., et al. Analysis of glutathione: implication in redox and detoxification. Clin Chim Acta. 2003; Jul 1; 333 (1):19-39.

[26] Placer ZA., Cushman LL., Johnson BC. Estimation of product of lipid peroxidation (malonyl dialdehyde) in biochemical systems. Anal Biochem. 1966;16:359-64.

[27] Sedlak J., Lindsay RH. Estimation of total protein-bound and nonprotein sulphydryl groups in tissue with Ellman’s reagent. Anal Biochem. 1968; 25:192-205.

[28] Misra HP., Fridovich I. The role of superoxide anion in the antioxidation of epinephrine and a simple assay for superoxide dismutase. J Biol Chem. 1972; 247:3170-5.

[29] Sinan Hatipoglu et al. Effect of laparoscopic abdominal surgery on splanchnic circulation: Historical developments World J Gastroenterol. 2014 Dec 28; 20 (48): 18165–18176.

[30] Eleftheriadis E., Kotzampassi K., Botsios D., et al., Splanchnic ischemia during laparoscopic cholecystectomy. Surg Endosc 1996; 10:324–326

[31] Jakimowicz J., Stultiëns G., Smulders F.: Laparoscopic insufflation of the abdomen reduces portal venous flow. Surg Endosc 1998; 12, 129-132

[32] R. Thuret et al. Ischemic pre- and post-conditioning: current clinical applications Progrès en urologie (2014) 24, S56-S61

[33] Javor et al. Preconditioning is a method that may reduce the negative the negative side-effect of pneumoperitoneum. Interventional Medicine and Applied Science, 2010; Volume 2, Number 3/September.

[34] Schilling MK., Redaelli C., Krähenbuhl L., et al., Splanchnic microcirculatory changes during CO2 laparoscopy. J Am Coll Surg 1997; 184: 378–382.

[35] Mishra V., Baines M., Wenstone R., et al. Markers of oxidative damage, antioxidant status and clinical outcome in critically ill patients. Ann Clin Biochem 2005; 42:269.

[36] Musil F, Zadak Z, Solichova D, et al. Dynamics of antioxidants inpatients with acute pancreatitis and in patients operated for colorectal cancer: A clinical study. Nutrition 2005;21:118.

[37] Whelan RL., Franklin M., Holubar SD., et al. Postoperative cell mediated immune response is better preserved after laparoscopic versus open colorectal resection in humans. Surg Endosc 2006; 17:972.

[38] Schwenk W., Haase O., Neudecker J., et al. Short term benefits for laparoscopic colorectal resection. Cochrane Database Syst Rev 2005; CD003145.

[39] Gutt NC., Schmandra TC.: Portal venous flow during CO2 pneumoperitoneum in the rat. Surg Endosc 1999; 13, 902–905

[40] Polat C., Yilmaz S., Serteser M.: The effect of intra-abdominal pressures on lipid peroxidation and protein oxidation status during laparoscopic cholecystectomy. Surg. Endosc. 2003; 17, 1719 –1722

[41] Yilmaz S., Ates E., Polat C., et al., Ischemic preconditioning decreases laparoscopy-induced oxidative stress in small intestine. Hepatogastroenterology 2003;50, 979–982

[42] Hafize O., Ertan B., Nimet S., et al., Re-Protective Effects of Pre- and Post-Laparoscopy Conditioning, Zinc, Pentoxifylline, and N-acetylcysteine in an Animal Model of Laparoscopy-Induced Ischemia/Reperfusion Injury of the Kidney, Renal Failure, 2009; 31:4, 297-302

[43] Bulbuloglu E. et al. Protective effects of zinc, pentoxifylline, and N-acetylcysteine in an animal model of laparoscopy-induced ischemia/reperfusion injury of the small intestine J Laparoendosc Adv Surg Tech A. 2011; Dec;21;10:947-51.

[44] Sahin DA., Haliloglu B., Sahin FK., et al., Stepwise rising CO2 insufflation as an ischemic preconditioning method. J Laparoendosc Adv Surg Tech A. 2007; 17, 723–729

[45] P. K. Garg, N. Teckchandani, N. S. Hadke, J. Chander, S. Nigam, and S. K. Puri, "Alteration in coagulation profile and incidence of DVT in laparoscopic cholecystectomy," International Journal of Surgery, 2009; vol. 7, no. 2, pp. 130–135.

[46] E. Eleftheriadis, K. Kotzampassi, K. Papanotas, N. Heliadis, and K. Sarris, Gut ischemia, oxidative stress, and bacterial translocation in elevated abdominal pressure in rats, World Journal of Surgery, 1996; vol. 20, no. 1, pp. 11–16

[47] G. K. Glantzounis, A. D. Tselepis, A. P. Tambaki et al., "Laparoscopic surgery-induced changes in oxidative stress markers in human plasma," Surgical Endoscopy, 2001, vol. 15,no. 11, pp. 1315–1319

[48] R. Seven, A. Seven, Y. Erbil, et al., Lipid peroxidation and antioxidant state after laparoscopic and open cholecystectomy, European Journal of Surgery, 1999; vol. 165, no. 9, pp. 871–874

[49] Z. Baysal, T. Togrul,N. Aksoy et al., Evaluation of total oxidative and antioxidative status in pediatric patients undergoing laparoscopic surgery, Journal of Pediatric Surgery, 2009; vol. 44, no. 7, pp. 1367–1370

[50] S. Yılmaz, C. Polat, A. Kahraman et al., The comparison of the oxidative stress effects of different gases and intraabdominal pressures in an experimental rat model, Journal of Laparoendoscopic&Advanced Surgical Techniques A, 2004; vol. 14, no. 3, pp. 165–168

[51] Cuschieri A. Adverse cardiovascular changes induced by positive pressure pneumoperitoneum. Possible solutions to a problem. Surg Endosc 1998; 12: 93–94.

[52] Gudmundsson FF, Viste A, Myking OL, Grong K, Svanes K. Effects of the aldosterone receptor antagonist potassium canrenoate on renal blood flow and urinary output during prolonged increased intraabdominal pressure (IAP) in pigs. Surg Endosc 2004; 18: 1528–1534.

[53] Kim ZG, Sanli E, Brinkmann L, Lorenz M, Gutt CN. Impact of dopamine and endothelin-1 antagonism on portal venous blood flow during laparoscopic surgery. Surg Endosc 2002; 16: 1292–1296.

[54] Guler C, Samli M, Aksoy Y, et al. Effects of carbon dioxide pneumoperitoneum on free radical formation in remote organs and use of verapamil as an antioxidant. J Endourol 2004; 18: 245–249.

[55] Karwinski W, Garcia R, Helton WS. Protective effects of the calcium channel blocker verapamil on hepatic function following warm ischemia. J Surg Res 1996; 64: 150–155.

[56] Ates E, Yilmaz S, Ihtiyar E, Yasar B, Karahuseyinoglu E. Preconditioning-like amelioration of erythropoietin against laparoscopy-induced oxidative injury. Surg Endosc 2006; 20: 815–819.

[57] Cay A, Imamodlu M, Unsal MA, Aydin S, Alver A, Akyol A et al. Does anti-oxidant prophylaxis with melatonin prevent adverse outcomes related to increased oxidative stress caused by laparoscopy in experimental rat model? J Surg Res 2006; 135: 2–8.

[58] Mittal A, Phillips AR, Loveday B, Windsor JA. The potential role for xanthine oxidase inhibition in major intraabdominal surgery. World J Surg 2008; 32: 288–295.

[59] Berger, J., Moller, D.E., The mechanisms of action of PPARs. Annu. Rev. Med. 2002; 53, 409–435.

[60] Lemberger, T. Desvergne, B., Wahli, W., Peroxisome proliferatoractivated receptors: a nuclear receptor signaling pathway in lipid physiology. Annu. Rev. Cell. Dev. Biol. 1996; 12, 335–363.

[61] Vamecq, J., Latruffe, N.,Medical significance of peroxisome proliferatoractivated receptors. Lancet 1999 354, 141–148

[62] Magnone, M.; Bruzzone, S.; Guida, L.; et al Abscisic acid released by human monocytes activates monocytes and vascular smooth muscle cell responses involved in atherogenesis. Biol. Chem. 2009, 284, 17808.

[63] Colville-Nash, P.R., Qureshi, S.S., Willis, D., et al., Inhibition of inducible nitric oxide synthase by peroxisome proliferatoractivated receptor agonists: correlation with induction of heme oxygenase 1. J. Immunol. 1998; 161, 978–984.

[64] Ali Çay, Mustafa İmamoğlu, Mesut A. et al., Does Anti-Oxidant Prophylaxis with Melatonin Prevent Adverse Outcomes Related to Increased Oxidative Stress Caused by Laparoscopy in Experimental Rat Model? Journal of Surgical Research

[65] Atsushi Nakajima et al. Endogenous PPARγ mediates anti-inflammatory activity in murine ischemia-reperfusion injury. Gastroenterology. 2001 Feb; 120 (2):460 9.

[66] Blobner M., Bogdanski R., Kochs E.,et al., Effects of intraabdominally insufflated carbon dioxide and elevated intraabdominal pressure on splanchnic circulation: an experimental study in pigs. Anesthesiology 1998; 89, 475–482

[67] William E. Kelley, Jr, MD, SLS President The Evolution of Laparoscopy and the Revolution in Surgery in the Decade of the 1990s, JSLS, 2008; Oct-Dec; 12(4): 351–357.

[68] Kelling G . Die Tamponade der Spieseroehre und des magens mit beigsamen instrumenten. Verdhandlungen der Gesellscaft Deutscher Naturtorscher und Aerzte. Vogel verlag, Leipzig 1901; 73, pp 117-119

[69] Ott DO. Ventroscopic illumination of the abdominal cavity in pregnancy. Z Akush Zhenskikl Boleznei 1901; 15: 7-8

[70] Jacobeus HC. Uber Die Moglichkeit die Zystoskopie bei Untersuchung seroser Hohlunger anzuwerder. Munch Med Wochenschr 1911; 57: 2090-2092

[71] Farr Nezhat, et al., Triumphs and Controversies in Laparoscopy: The Past, the Present, and the Future JSLS 2003; v.7(1); Jan-Mar 2003 PMC3015467

[72] Walker Reynolds, Jr, The First Laparoscopic Cholecystectomy, JSLS. 2001 Jan-Mar; 5 (1): 89–94.

[73] David B. Safran, Rocco Orlando III, Physiologic effects of pneumoperitoneum The American Journal of Surgery, Volume 167, Issue 2, Pages 281–286

[74] Ninh T. Nguyen et al. Use of laparoscopy in general surgical operations at academic centers Surgery for obesity and related diseases 2013; Volume 9, Issue 1, Pages 15–20

[75] Eisen A., Fisman EZ., Rubenfire M., et al., Ischemic preconditioning: nearly two decades of research. Acomprehensive review. Atherosclerosis; 2004; 172: 201–210.

[76] Koti RS., Seifalian AM., Davidson BR. Protection of the liverby ischemic preconditioning: a review of mechanisms and clinical applications. Dig Surg. 2003; 20: 383–396.

[77] Küntscher MV., Hartmann B., Germann G., Remote ischemic preconditioning of flaps: a review. Microsurgery 2005; 25: 346–352.

[78] V Raveenthiran Facts and factitious claims. J Indian Assoc Pediatr Surg 2010; Oct-Dec; 15(4): 122–128.

[79] Gutt NC., Schmandra TC. Portal venous flow during CO2 pneumoperitoneum in the rat. Surg Endosc 1999; 13, 902–905

[80] Cevrioglu AS., Yilmaz S., Koken T., et al., Comparison of the effects of low intra-abdominal pressure and ischaemic preconditioning on the generation of oxidative stress markers and inflammatory cytokines during laparoscopy in rats. Hum Reprod 2004; 19, 2144–2151

[81] Yilmaz S., Koken T., Tokyol C., et al., Can preconditioning reduce laparoscopy- induced tissue injury? Surg Endosc 2003;17, 819–824

[82] Ates E., Yilmaz S., Ihtiyar E., et al., Preconditioning-like amelioration of erythropoietin against laparoscopyinduced oxidative injury. Surg Endosc 2006; 20, 815–819

[83] Athanasiadis D., Kapelouzou A., Martikos G., et al., Remote Ischemic Preconditioning May Attenuate Renal Ischemia-Reperfusion Injury in a Porcine Model of Supraceliac Aortic Cross-Clamping, Journal of Vascular Research, 2015; Vol 52., No.3.

[84] Leventi A., Argyra E., Avraamidou A., et al., Attenuation of Oxidative Stress by Ischemic Preconditioning in an Experimental Model of Intraabdominal Hypertension. J Invest Surg. 2015 ;28(5):253-60.

[85] Nesek-Adam V., Rasić Z., Vnuk D., et al., Ischemic preconditioning decreases laparoscopy induced oxidative stress in the liver. Coll Antropol. 2010; Jun; 34(2):571-6.

[86] Sammour T., Mittal A., Loveday BP., et al., Systematic review of oxidative stress associated with pneumoperitoneum. Br J Surg 2009; 96, 836–850

[87] R. Thuret, T. Saint Yves, X. Tillou, et al., Ischemic pre- and post-conditioning: current clinical applications Progrès en urologie 2014; 24, S56-S61

[88] Przyklenk K., Bauer B., Ovize M., et al., Regional ischemic ‘preconditioning’protects remote virgin myocardium from subsequent sustained coronary occlusion. Circulation 1993; 87(3):893-9

[89] Gál I., Rőth E., Lantos J., et al.,: A laparoscopos cholecystectomia indukálta sebészi trauma. Orvosi Hetilap 1998; 139, 739–746

[90] Gál I., Rőth E., Lantos J., et al., Inflammatory mediators and surgical trauma regarding laparoscopic access: free radical mediated reactions. Acta Chirurgica Hung 1997; 36, 97–99

[91] Kin H., Zhao ZQ., Sun HY., et al. Postconditioning attenuates myocardial ischemia-reperfusion injury by inhibiting events in the early minutes of reperfusion, Cardiovasc Res, 2004; vol. 62 pg. 74-85

[92] Fujita M., Asanuma H., Hirata A., et al. Prolonged transient acidosis during early reperfusion contributes to the cardioprotective effects of postconditioning, Am J Physiol Heart Circ Physiol, 2007, vol. 292 pg. H2004-8

[93] Jiang B., Liu X., Chen H., et al., Ischemic postconditioning attenuates renal ischemic/reperfusion injury in mongrel dogs, Urology, 2010; vol.76 pg. 1519.e1–7

[94]Yellon DM., Alkhulaifi AM., Pugsley WB.: Preconditioning the human myocardium, Lancet, 1993; vol. 342 pg. 276-277

[95] Chen S., Li G., Long L. Clinical research of ischemic preconditioning on lung protection, Hunan Yi Ke Da Xue Xue Bao, 1999, vol. 24 pg. 357-359

[96] Amador A., Grande L., Marti J., et al. Ischemic pre-conditioning in deceased donor liver transplantation: a prospective randomized clinical trial, Am J Transplant, 2007; vol. 7 pg. 2180-2189

[97] ChanMT., Boet R., NgSC., et al. Effect of ischemic preconditioning on brain tissue gases and pH during temporary cerebral artery occlusion, Acta Neurochir Suppl, 2005; vol. 95 pg. 93-96

[98] Koca K., Yurttas Y., Cayci T., et al. The role of preconditioning and N-acetylcysteine on oxidative stress resulting from tourniquet-induced ischemia-reperfusion in arthroscopic knee surgery, J Trauma, 2011; vol. 70 pg. 717-723