# The role of postconditioning in the modulation of small intestinal ischemia-reperfusion injury

Ph.D. Doctoral Thesis

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Budapest
2015

## Introduction

The acute mesenteric ischemia (AMI) is a life threatening condition, which may be present in the form of „acute abdomen”. Despite the advances in treatment modalities and intensive care, the mortality rates of acute mesenteric ischemia have not decreased in the past 20 years. According to literature data in-hospital mortality rates can be as high as 60%-80%. Although AMI accounts for only 1%-2% of all gastrointestinal diseases, its incidence is increasing with the ageing of society.

The starting point in the pathogenesis of AMI is the interruption in the oxygen supply of the highly metabolically active intestinal mucosa. The perfusion impairment may be the result of vessel occlusion (thrombi, emboli) or may be due to sistemic disorders (circulatory redistribution shock). The loss of blood supply results in ischemic injury, which paradoxically is furthermore aggravated by the reperfusion. Altough reperfusion is key to survival, it activates cell-damaging processes as well, which often exceed the harmful effects of the initial ischemic insult.

The barrier formed by the intestinal epithelial cells is damaged during mesenteric ischemia-reperfusion (I-R). The epithelial tight junction proteins expression and localization change, leading to an increment in the intestinal permeability. The injury of the intestinal unicellular epithelial layer results in the translocation of luminal bacteria into the sterile bloodstream. The progression of the local injury and the dissemination of intestinal bacteria (hematogen or lymphogen) leads to a shift in the inflammatory balance of the body. The consequent systemic inflammatory response syndrome coupled with bacteremia (sepsis) causes an excessive proinflammatory reaction, which may end in multiple organ dysfunction (MODS) and multiple organ failure (MOF).

The toll-like repectors (TLR) are important part of the innate immune system, and are capable of sensing several pathogen associated molecular patterns (PAMP). Furthermore, TLR-2 and -4 play a pivotal role in the pathogenesis of inflammation and ischemia-reperfusion, since it has been claimed that endogenous molecules released by damaged tissues (DAMP-damage-associated molecular patterns) are also triggers of the TLR-2 and -4 signal pathways. The gastrointestinal tract is in a constant "controlled" TLR expression induce by the commensal flora, which is essential for the maintenance of the intestinal homeostasis. Following mesenteric ischemia-reperfusion the balance between the luminal pathogens and the local defensive system shifts, resulting in the overexpression of mucosal TLR. Excessive TLR-expression is no longer protective against intestinal luminal bacteria, but instead induces a local inflammatory response.

Thus the intestinal IR injury has a relevant impact throughout the body, since the formation and release of large amounts of free radicals and proinflammatory cytokines can involve distant organs as well.

Considering the small intestine low ischemic tolerance, acute mesenteric ischemia, may result in serious intestinal injury, even after successful vascular reconstruction. Since surgery is frequently required for the restitution of the mesenteric circulation and the resection of necrotic bowel, postconditioning can be a suitable surgical maneuver to attenuate ischemia-reperfusion injury. Postconditioning is the application of several short cycles of reperfusion and reocclusion performed at the onset of reperfusion in the ischemic organ.

## Research objectives

**I. Experiment: Determination of the postconditioning algorithm providing the maximum protective effect against intestinal I-R.**

1. How do the different postcondiciotning algorithms affect the small intestine mucosal microcirculation?
2. What is the effect of the different postconditioning algorithms on the small intestine mucosal antioxidant status?
3. Are the different postconditioning algorithms capable of attenuating the small intestine histological alterations, and are these changes reflected in the levels of the serological markers?
4. Is there any change in the superior mesenteric vein blood pH following with the use of postconditioning?
5. Which postconditioning algorithm provides the strongest protective effect following mesenteric ischemia–reperfusion?

**II. Experiment: Analysis of changes in gut barrier function following the application of postconditioning.**

1. How does the use of postconditioning influence the expression of several tight junction proteins following mesenteric ischemia-reperfusion?
2. Can the use of postconditioning after mesenteric ischemia attenuate the rate of bacterial translocation to the mesenteric lymph nodes, spleen, liver, lung and kidneys?

**III. Experiment: Small intestinal epithelial cell TLR expression changes following the application of postconditioning.**

**1.** How does the use of postconditioning influence the local small intestinal mucosal TLR-expression?

**IV. Experiment: Analysis of remote organs following the use of postconditioning at the onset reperfusion.**

1. How does the use of postconditioning affect the liver, lung and kidney integrity after mesenteric ischemia-reperfusion?
2. Can the use of postconditioning ameloriate the sistemic inflammatory response syndrome induced by mesenteric ischemia-reperfusion?

## Materils and Methods

### Study design

The experimental design was approved by License No. 1858/000/2004 and 22.1/2408/3/2011 from the Animal Care Committee of the Semmelweis University.

### Animal model:

Male Wistar rats weighing 250–300 g were used. The total number of animals/experiment was n=50 (I.experiment), n=45 (II.experiment), n=30 (III.experiment) and n=30 (IV.experiment). The animals were anaesthetized using continuous intravenous ketamine and xylasin administration. Median laparotomy was performed and the superior mesenteric artery was isolated. Mesenteric warm ischemia was induced by clamping the superior mesenteric artery for 60 minutes. Following 1 hour of mesenteric ischemia the microclip was removed and, according to the experimental protocol, a period of 60- or 360-minutes reperfusion was permitted. Then animals were sacrificed, blood and tissue samples were collected.

### Experimental groups:

- *Sham-operated group:* the group was used to assess the surgical stress, after opening the abdomen the superior mesenteric artery was dissected, but ischemia was not induced.
- *I-R control group (I-R):* the group was used to examine the IR damage in the absence of the investigated adaptive surgical treatment.
- *Postconditioned group (PC):* the group was used to investigate the effects of postconditioning copared to the I-R group. Postconditioning was performed at the onset of the reperfusion, according to the experimental group. (Table. 1)

| | SHAM | I-R | PC |
|---|---|---|---|
| **Ischemia** | – | X | X |
| **Reperfusion** | – | X | X |
| **Postconditioning** | – | – | X |

**Table. 1.** Experimental groups

**I. Experiment:** The experiment was performed according to the general surgical model described previously. The reperfusion period lasted 60 minutes. The animals were randomly divided into 5 groups (n = 10/group): SHAM-, I-R, and three postconditioned-groups (PC-10: 6 alternating cycles of 10-10 sec reperfusion-reocclusion were performed, which lasted in total two minutes, PC-30: 3 alternating cycles of 30-30 sec

reperfusion-reocclusion were performed, which lasted in total three minutes, PC-60: 3 alternating cycles of 60-60 sec reperfusion-reocclusion were performed, which lasted in total six minutes). Sampling was performed at the end of the reperfusion: intestinal samples (duodenum, jejunum, ileum) were taken from identical anatomical parts for histological examination; intestinal mucosa scrapping was made for detailed antioxidant analysis; superior mesenteric vein blood samples were taken for pH-measurement; blood samples were taken from the right ventricule for the determination of necroenzyme and proinflammatory citokine levels.

**II. Experiment:** Twelve hours prior to operation 1mL of $1\times10^{10}$ CFU/mL GFP (green fluorescent protein)-expressing Escherichia coli suspension was administered to each animal via oroduodenal catheterization. The experiment was performed according to the general surgical model described previously. The reperfusion period lasted 360 minutes. The animals were randomly divided into 3 groups (n = 15/group): SHAM-, I-R-, and PC-group (6 alternating cycles of 10-10 sec reperfusion-reocclusion). Sampling was performed at the end of the reperfusion: intestinal samples (duodenum, jejunum, ileum) were taken from identical anatomical for histological examination; intestinal mucosa scrapping was made for detailed antioxidant analysis; mesenteric lymph node-, spleen-, liver-, lung- and kidney sample were taken for microbiological analysis; blood samples were taken from the right ventricule for the determination of intestinal fatty acid binding protein (i-FABP), d-lactate and proinflammatory citokine levels.

**III. Experiment:** The experiment was performed according to the general surgical model described previously. The reperfusion period lasted 360 minutes. The animals were randomly divided into 3 groups (n = 15/group): SHAM-, I-R-, and PC-group (6 alternating cycles of 10-10 sec reperfusion-reocclusion). Sampling was performed at the end of the reperfusion: intestinal samples (duodenum, jejunum, ileum) were taken from identical anatomical parts for histological examination; intestinal mucosa scrapping was made for detailed antioxidant analysis and determination of proinflammatory citokine and TLR-2, TLR-4 mRNA expression levels.

**IV. Experiment:** The experiment was performed according to the general surgical model described previously. The reperfusion period lasted 360 minutes. The animals were randomly divided into 3 groups (n = 15/group): SHAM-, I-R-, and PC-group (6 alternating cycles of 10-10 sec reperfusion-reocclusion). Sampling was performed at the end of the reperfusion: liver-, lung-, kidney- and intestinal (duodenum, jejunum, ileum) samples were taken from identical anatomical parts for histological examination; blood samples were taken from the right ventricule for the determination proinflammatory citokine levels.

**Investigation methods:**

-*Hemodynamic monitorization:* arterial blood pressure and heart rate were recorded invasively using a blood pressure gauge placed in the right carotid artery.

-*Intestine mucosal microcirculation:* mucosal microcirculation was measured using a laser Doppler flowmeter probe placed intraluminally in the jejunal-ileal limit. Reperfusion flow-graphs were characterized as follows:
1. Plateau maximum (PM): the average values of the last 10 min of reperfusion.
2. Reperfusion area (RA): the integral of the area under the reperfusion segment of the flow-graphs.

-*Histopathologic analysis:* samples taken from identical anatomical parts of the experimental animals were fixed in formalin and embedded in paraffin. Following hematoxylin and eosin stainning, the section were analyzed under conventional light microscope.

-*Laboratory assay:* Blood samples were centrifuged and the supernatant were collected. Serum LDH (lactate dehydrogenase), CK (creatine kinase), ASAT (aspartate aminotransferase), ALAT (alanine aminotransferase) enzyme activity levels and creatinine concentration were determined by standard spectrophotometry using an automated clinical analyser.

-*Oxidative stress analysis:* measurements were carried out in small intestine mucosal homogenates according to the following methods:
1. Total scavenger capacity was measured in a hydrogenperoxid, luminol, microperoxidase system using a luminometer. The chemiluminescence light intensity - given in relative light units (RLU) - is proportional to the concentration of free radicalsinn the sample. Results were expressed as percentage of the background activity (RLU%).
2. Free thiol groups (-SH), detected using the Sedlak method based on the Ellmanns' reaction, showed the protein-related reducing power (mmol/l).
3. The H-donating ability reflects the non-protein-bound antioxidant state of the samples. It was measured in the presence of a 1,1-diphenyl-2-picryl-hydrasyl radical at 517 nm using Blois' method. The results were expressed in inhibition-percentage (inhib%).
4. The Oyaizu method was used to determine the samples' reducing power, showing the its global antioxidant capacity. Absorption changes were measured at 700 nm and the reducing power of the samples were compared with that of ascorbic acid. Results were expressed as ascorbic acid equivalent (ASE) per gram of sample protein.

- *Blood pH analysis:* blood was taken from the superior mesenteric vein at 0, 30, 60 and 120 seconds of reperfusion, pH was measured with a clinical blood gas analyzer.

- *Enzyme-linked immunosorbent assay:* serum and small intestine mucosal homogenate TNF-α, IL-6, i-FABP, D-lactate levels were measured using commercially available ELISA kits.

- *Microbiological analysis:* tissue samples were homogenized, aliquots were cultured in Luria-Bertani agar media supplemented with L-arabinose and ampicillin. Cultures were incubated under aerob conditions at 37°C for 24-48 hours. Fluorescent colonies were counted under 312nm wavelength UV light. Number of GFP-expresser E.coli colonies were given as CFU/g tissue.

- *Immunohistochemistry assay:* formalin-fixed and paraffin-embedded duodenum, jejunum and ileum samples were immunostained for cleaved caspase-3, claudin-2, claudin-3, claudin-4, and zonula occludens-1 using polyclonal antibodies. The stainning process was performed according to the manufacturer's instructions. For negative controls, the appropriate primer antibody was omitted, for positive controls tissue samples recommended by the manufacturer were used to confirm correct immunohistochemical staining. Semiquantitative and quantitative (Leica Qwin Pro morphometry software) evaluation of the immunostained sections was performed.

- *TLR-2 and TLR-4 mRNA determination:* total RNA was isolated from the tissue samples. RNA quantity and quality was checked photometrically. RNA was transcribed into complementary DNA. TLR-2 and TLR-4 mRNA expression was quantified with RT-PCR. GAPDH was used as reference gene for relative quantification.

- *Pulmonary edema index:* the right middle lung lobe weight was measured (wet weight), then lung sample was dried at constant 80°C until reaching a constant weight (dry weight). The lung edema was calculated according to the proportion of wet and dry weights.

- *Statistical Analysis:* Results were expressed as means ± standard deviation (SD). Normality of all data was verified by the Kolmogorov-Smirnov test. Variance equality between groups was tested by Levene's statistical analysis. ANOVA was used to detect differences between groups. Post hoc comparisons were made using Scheffe's test. The difference in the histopathological grades and staining intensity scores were statistically evaluated using nonparametric Kruskal-Wallis test. Post hoc analyses for pairwise comparisons between groups were performed using Mann-Whitney *U* test. Statistical evaluation for proportional comparisons of tissue cultures was made using the Chi-square test. A value of $P<0.05$ was considered as statistically significant difference.

## Results

### Results of the I. Experiment

***Hemodynamic monitorization:*** In case of the sham-operated animals, MAP was stable throughout the study period. In the experimental groups subjected to ischemia, MAP increased after SMA occlusion and declined towards the end of the ischemia. In the IR group at the beginning of reperfusion, the blood pressure rapidly dropped and remained constantly low throughout the rest of the reperfusion period. 60 minutes after the start of reperfusion, in the three PC groups, the MAP increased approaching the preocclusion levels. In the PC-10 and PC-30 groups, MAP was significantly higher than the value found in case of the I-R group. No significant differences in MAP values were detected between the different PC groups.

***Intestine mucosal microcirculation:*** Regarding the baseline micro-circulation values (flux), no significant differences were measurable between the experimental groups. There was a drop in flux during the ischemic period in the groups subjected to ischemia-reperfusion, whereas the flow of the sham group remained constant during the entire experiment. After the start of reperfusion, there is peak in the flux of the groups exposed to mesenteric IR, which reaches the baseline then, after the first 5 to 10 minutes the curves start diverging. In the IR group the reperfusion curve gradually decreases, the PM exhibited about 70% of the baseline level at the end of reperfusion, being significantly lower ($P<0.01$) than the three postconditioned groups. In the PC-30 group, the PM showed no significant difference when compared with the PC-60 group ($P=0.317$). In the PC-10 group at the end of reperfusion, the PM was stabilized in a hyperdynamic state, which was significantly higher ($P<0.05$) than that in case of the PC-30 and PC-60 groups. Regarding the RA, the three postconditioned groups showed significantly higher ($P<0.01$) RA values compared with the I-R group. Among the postconditioned groups, the RA of the PC-10 group was significantly higher ($P<0.01$) than the values of the other two groups. There was no significant difference between the RA of the PC-30 and PC-60 groups ($P=0.657$).

***Histopathologic analysis:*** Regarding the histologic samples stained with hematoxylin and eosin in the sham group, no morphologic changes were observed in the three small intestine segments. There was no statistical difference between the duodenum sections of the four groups exposed to mesenteric I-R. Among the examined samll intestine segments the ileum presented the highest damage rates. The use of postconditioning reduced the severity of I-R injury related to the jejunum and ileum. According to the Chiu score, the grade of injury regarding the jejunum and ileum of the

PC-10 and PC-30 groups was found to be significantly reduced (PC-10 jejunum: P=0.015 vs. I-R; ileum: P<0.01 vs. I-R; PC-30 jejunum: P=0.02 vs. I-R; ileum: P=0.044 vs. I-R) compared with the I-R group. The grade of injury observed in the jejunum and ileum segments of the PC-60 group presented no statistical difference ( jejunum: P=0.165 vs. I-R; ileum: P=0.054 vs. I-R) compared with the I-R group.

***Changes in plasma LDH and CK levels:*** Plasma LDH and CK levels of the groups exposed to mesenteric ischemia-reperfusion significantly (P<0.05 vs. SHAM) raised. The marked increase in necroenzyme levels of the I-R group was significantly (P < 0.01) lower in the PC-10 and PC-30 groups. In the PC-60 group, the LDH levels did not decrease substantially (P=0.21). The elevation of plasma LDH levels was significantly higher in the PC-30 (P<0.05) and PC-60 (P<0.01) groups compared with the PC-10 group**.**

***Changes in mucosal antioxidant capacity:***

**- *Total scavenger capacity:*** Chemiluminescent intensity was significantly increased in the groups subjected to mesenteric I-R, compared to the SHAM group. The use of postconditioning was able to significantly (P<0.01) ameliorate the production of ROS in comparison with the levels detected in the intestinal mucosa of the IR group.

**- *Reducing power:*** The duodenum reducing power showed no significant differences between the experimental groups. Regarding the jejunum, the three examined postconditioning algorithms significantly (P<0.01) mitigated the impairment of antioxidant capacity detected in the I-R group. Concerning the ileum, only the PC-10 group presented significantly higher (P=0.01 vs. IR) rates of mucosal reducing power.

**- *Free thiol groups:*** The mesenteric ischemia-reperfusion injury significantly reduced the concentration of free sulfhydryl groups in examined intestinal segments. However, the PC-10 group mucosal samples presented significantly higher (P<0.05) concentration of free thiol groups, compared to the I-R group**.**

**- *H-donating activity:*** Mesenteric ischemia-reperfusion was accompanied by a significant reduction in hydrogen-donor activity of the intestinal mucosa. Regarding the duodenum and the jejunum, the three examined postconditioned groups presented significantly higher hydrogen-donor activity compared to the IR group. In case of the ileum, this difference was not statistically significant.

***Mesenteric vein pH levels:*** The venous blood pH of the groups subjected to the temporary clamping of the SMA were significantly lower compared with the sham-operated animal samples, in which case no substantial changes

were detected at any of the assessed time points. The pH levels of the groups subjected to ischemia did not differ significantly at the 0-s timepoint. At the timepoint of the second measurement (30 sec), marked differences were observed in the PC-10 group values compared to the other groups. After the first minute of reperfusion, the pH levels in the PC-10 group continued to be significantly ($P<0.01$) lower. Furthermore, the pH levels of the I-R and PC-60 groups showed significantly ($P<0.01$) faster rise in comparison with the PC-30 and PC-10 groups. At the end of the first 2 min of reperfusion, only the 30- and 10-s postconditioning algorithms delayed the pH normalization of the mesenteric venous blood compared with the I-R group. The PC-10 group showed considerably lower pH ($P<0,01$) in comparison with the PC-30 group, suggesting slower and more prolonged pH normalization at the onset of reperfusion. No statistical differences were observed between the pH levels of the I-R and PC-60 groups ($P=0.057$).

**Changes in plasma IL-6 and TNF-α levels:** The plasma IL-6 and TNF-α levels of the groups subjected to ischemiaer-perfusion were found to be substantially elevated compared to the SHAM group. All three postconditioning algorithms, significantly ($P<0.01$) reduced the levels of both inflammatory cytokines compared to the I-R group. Regarding plasma TNF-α levels, the PC-30 and PC-60 groups showed significantly ($P<0.01$) higher cytokine levels compared with the PC-10 group.

## Results of the II. Experiment

***Histopathologic analysis:*** Regarding the histologic samples stained with hematoxylin and eosin, in the sham group no remarkable morphologic changes were observed in the three small bowel segments. The duodenum was the least injured small intestine segment. This segment showed minimal subepithelial detachment in both groups exposed to mesenteric ischemia-reperfusion. There was no significant different ($P=0.71$) between the I-R and the PC group duodenum samples. However substantial tissue injury could be seen in the I-R group jejunum and ileum samples, presenting severe epithelial detachment extending towards the villi cryts and frequent denudation of the villi. The use of postconditioning mitigated the severity of I-R injury related to the jejunum and ileum segments of the bowel. Using Chiu score to quantify the lesions, the intensity of injury concerning the jejunum ($P<0.01$ vs. I-R) and ileum ($P<0.05$ vs. I-R) of the PC group was significantly lower compared with the I-R group.

***Cleaved caspase-3 immunhistochemistry:*** Cleaved caspase-3 positivity was rarely seen in the sections of the SHAM-operated group. Significantly higher ($P<0.01$) presence of cleaved caspase-3 was observed in the nucleus, as well as in the cytoplasm of apoptotic cells in the groups exposed to I-R. Postconditioning significantly decreased ($P<0.01$) cleaved caspase-3 staining

in the jejunum and ileum of the small intestine, when compared to the I-R group sections.

***Immunohistochemical Analysis of Tight Junction Proteins:*** In the samples of the sham-operated animals, claudin-2 was weakly stained in all the three small intestinal segments. A mainly granular cytoplasmic positivity was observed for claudin-2, principally in the crypts of the villi. The two groups exposed to I-R presented higher claudin-2 membrane expression. Jejunal and ileal segments of the I-R group showed significantly major ($P=0.018$; $P=0.016$) claudin-2 membrane positivity compared with the samples of the PC group. Claudin-3 gave a strong membranous reaction along the crypt-villus axis of the sham-operated group, showing no remarkable gradient in either of the intestinal segments. Mesenteric IR caused a dramatic decrease of claudin-3 expression in the epithelial cells of the jejunum and ileum. Postconditioning significantly attenuated ($P<0.01$ vs. I-R) the loss of claudin-3 membrane staining in the two above-mentioned small intestinal segments. Claudin-4 staining was poorly detected in all the examined samples of the sham-operated group and was mainly found in the cytoplasm of the epithelial cells at the tips of the villi. Ischemia-reperfusion substantially induced claudin-4 expression in all the small intestinal segments, which was restricted to the membrane of the epithelial cells located at the tips of the villi. The percentage of claudin-4 positivity did not differ significantly between the I-R- and PC-groups (duodenum $P=0.11$; jejunum $P=0.62$; ileum $P=0.62$). ZO-1 was detected as membranous staining principally on the lateral and apical surfaces of the epithelial cells. ZO-1 was uniformly observed in the duodenum, jejunum, and ileum of the sham group. Following mesenteric I-R samples taken from the jejunum and ileum displayed significantly lower ZO-1 membrane expression. However, the jejunal and ileal sections of the PC group presented significantly higher ($P<0.01$ vs. I-R) ZO-1 membrane positivity.

***Changes in mucosal antioxidant capacity:***

- ***Total scavenger capacity:*** The concentration of ROS, which is proportional to the chemiluminescent intensity, significantly increased in the groups subjected to I-R. The use of postconditioning was able to significantly ameliorate ($P<0.01$) the jejunal and ileal production of ROS in comparison to the levels detected in the jejunum and ileum mucosa of the I-R group. Regarding the duodenum there was no statistical difference between the I-R and the PC group ($P=0.09$).

- ***Reducing power:*** The reducing power of the samples exposed to mesenteric I-R significantly decreased. However, the PC group presented significantly higher ($P<0.05$) reducing power values in all the three small intestine samples compared to I-R group. The reducing power of the

duodenum and ileum samples in the PC group did not differ significantly from the results of the SHAM group.

- ***Free thiol groups:*** Compared to the I-R group, a strongly significant increase (P<0.01 vs. I-R) in the free SH-groups' concentration was measured in the PC group. There was no statistical difference between the PC and the SHAM group free thiol mucosal concentration.

- ***H-donating activity:*** The mesenteric I-R substantially decreased the small intestine mucosal samples' antioxidant capacity, mainly regarding the jejunum and the ileum mucosal samples. The hydrogen donanting activity of the samples in the PC group were significnatly higher (duodenum: P<0.01 vs. I-R; jejunum: P<0.01 vs. I-R; ileum: P=0.03 vs. IR) compared to the I-R group.

***Microbiological Analysis:*** Tissue homogenates of the sham-operated group presented no bacterial colonization. The groups subjected to IR, however, had a variable range of detectableGFP-expressing E. coli inthe cultureplates.Theuse of postconditioning not only decreased significantly the incidence of bacterial translocation to extraintestinal sites (MLN, spleen, liver, lung, and kidney) induced by IR, but also diminished markedly the concentration of translocated bacteria. (Table. 2)

| Experimental group | MLN | Spleen | Liver | Lung | Kidney |
|---|---|---|---|---|---|
| **SHAM** | – | – | – | – | – |
| **I-R** | **93%** | **86%** | **80%** | **67%** | **60%** |
| **PC** | **40%**** | **33%**** | **33%*** | **20%*** | **20%*** |

**Table. 2.** Incidence of GFP-expressing E. coli translocation.
(∗): $P$<0.05 vs. I-R group; (∗∗): $P$<0.01 vs. I-R group

***Changes in the Plasma I-FABP and D-lactate levels:*** The plasma I-FABP levels rose significantly upon mesenteric I-R, and was significantly attenuated (P<0.01) in the PC group. There was a significant elevation in the plasma D-lactate levels detected in the groups exposed to I-R, when compared with the sham-operated group. The level of plasma D-lactate measured in the PC group was significantly lower (P<0.01) than in the IR group.

## Results of the III. Experiment

**Tissue TLR-2 and TLR-4 mRNA expression:** Mesenteric I-R induced a significant elevation of TLR-2 and TLR-4 mRNA expression levels (P<0.05 vs. SHAM) in the I-R and PC groups small intestinal samples, except for the

PC group jejunum TLR-2 mRNA expression (P=0.11 vs. SHAM). The use of postconditioning significantly decreased (P<0.01) the jejunum and ileum TLR-2 and TLR-4 mRNA expression, when compared to the I-R group.

***Mucosal IL-6 and TNF-α levels:*** The examined proinflammatoric citokine levels showed similar changes in the jejunum and the ileum segments of the samll intestine. Compared with the SHAM operated group, mesenteric I-R significantly increased (P<0.01) mucosal IL-6 and TNF-α levels. However in the PC group significantly lower concentrations of jejunum and ileum IL-6 and TNF-α levels were measured compared to the I-R group.

## Results of the IV. Experiment

***Histopathologic analysis of the distant organs:*** The lung samples of the I-R group showed diffuse alveolar injury, with partial atelectasy, edema and thickened alveolar walls. In contrast, the PC group lung sections presented milder alveolar damage, with thinner alveolar walls, wider alveoli and lower amount of atelectasy, similar to the samples of the SHAM operated group.

In the liver samples of the I-R group periportal-midzonal necrosis, periportal hemorrhage, dilatated sinusoids and inflammatory infiltration was mainly observed. These histopathologic changes were substantially attenuated by the use of postconditioning.

Mesenteric I-R caused marked renal tissue injury with an increased in cellular eosinophilia, intracellular vacuolization and blurred cell borders. In the PC group kidney sections no lesions could be seen.

***Parameters of tissue injury:*** The measured necroenzyme activities correlated well with the histopathologic alterations detected in each experimental group. The LDH, CK, ALAT and ASAT levels increased significantly upon mesenteric I-R. Compared to the I-R group samples, the use of postconditioning significantly decreased the examined necroenzyme levels (LDH: P=0.03, CK: P=0.04, ASAT: P=0.04) except for the ALAT (P=0.24). Serum creatinine levels in the I-R group increased significantly (P<0.01), when compared to the SHAM operated group. In contrast, the serum creatinine levels of the PC group showed no statistical elevation (P=0.48 vs. SHAM).

***Pulmonary edema index:*** The calculated lung wet content of the I-R group was significantly higher (P<0.01) compared to the SHAM operated group. The use of postconditioning significantly decreased (P=0.013) the pulmonary edema measured in the I-R group.

***Serum IL-6 and TNF-α levels:*** The serum IL-6 and TNF-α levels, as classic markers of the systemic inflammation, were singnificantly higher (P<0.01) in the group exposed to mesenteric I-R compared to the SHAM operated

group. However, the PC group showed significantly lower ($P<0.01$) levels of both examined proinflammatory citokine compared to the I-R group.

## Conclusion

According to the results above, the following answers can be given to our questions:

1. The intestinal mucosa microcirculation was significantly improved during the reperfusion with the use the different postconditioning algorithms. The highest intestinal mucosa microcirculatory rates were reached with the 6x10 sec postconditioning algorithm.
2. The intestinal mucosa antioxidant status was enhanced in diverse rates by the different postconditioning algorithms. Among the measured antioxidant parameters the scavenging- and the hydrogen donor capacity was improved by 3x30- and 3x60 sec postconditioning cycles. However the 6x10 sec postconditioning algorithm significantly improved all the measured antioxidant parameters.
3. According to the histological sections, all the three postconditioning algorithms attenuated the morfological changes caused by mesenteric IR. The protective effects of postconditioning were properly reflected by serum levels of creatine kinase and lactate dehydrogenase.
4. During the first minutes of reperfusion only the 6x10- and the 3x30-postconditioning algorithm delayed significantly the pH normalization. The mesenteric vein pH was not influenced by the 3x60 sec postconditioning algorithm at any measured timepoint.
5. Among the examined postconditioning algorithms the 6x10 sec cycles proved to be more effective protocol in a rat modell of superior mesenteric artery occlusion.
6. The use of postconditioning maintained the expression of barrier tightening tight junction proteins, furthermore the expression of the intestinal permeability enhancer claudin-2 protein was attenuated.
7. The use of postconditioning reduced the bacterial translocation rates and the average colony forming unit count in the positive samples.
8. The use of postconditioning reduced the overexpression of mucosal TLR-2 and TLR-4 detected in the IR-group and maintained TLR-2 and TLR-4 expression rates close to pre-ischemic conditions.
9. The use of postconditioning ameliorates liver, lung and kidney injury caused by mesenteric IR.
10. The use of postconditioning following mesenteric ischemia significantly reduced the serum levels of proinflammatory citokines, thus may inhibit a systemic inflammatory response formation.

## Publications

Without conference abstracts and presentations

**Publications on the topic of the dissertation**

1. **Rosero O**, Onody P, Stangl R, Turoczi Z, Fulop A, Garbaisz D, Lotz G, Harsanyi L, Szijarto A. (2014) Postconditioning of the small intestine: which is the most effective algorithm in a rat model?. J Surg Res, 187: 427-37. **IF: 2.121**
2. **Rosero O**, Onody P, Kovacs T, Molnar D, Lotz G, Toth S, Turoczi Z, Fulop A, Garbaisz D, Harsanyi L, Szijarto A. (2014) Impaired intestinal mucosal barrier upon ischemia-reperfusion: "patching holes in the shield with a simple surgical method". Biomed Res Int, 2014: 210901. **IF: 2.706**
3. Onody P, **Rosero O**, Kovacs T, Garbaisz D, Hegedus V, Lotz G, Harsanyi L, Szijarto A. (2012) Postconditioning -- effective method against distant organ dysfunction? Magy Seb, 65: 222-9.
4. **Rosero O**, Onody P, Stangl R, Hegedus V, Lotz G, Blazovics A, Kupcsulik P, Szijarto A. (2011) Posztkondicionálás kísérletes vizsgálata vékonybél ischaemiás-reperfúziós modellben. Magy Seb, 64: 28-36.
5. Szijarto A., **Rosero O.**, Onody P., Harsanyi L. (2013) Kritikus Mesenterialis Ischaemia. Adaptív Mechanizmusok. Távoli szervi diszfunkciók. Magy Belorv Arch. 66: 13-19.
6. **Rosero O**, Kovacs T., Onody P., Harsanyi L., Szijarto A. (2014) Bakteriális transzlokáció: rés a pajzson. Orv Hetil, 155: 304–312.
7. **Rosero O**, Harsanyi L., Szijarto A. (2014) Akut mesenterialis ischaemia: biomarker egyenlő diagnózis?. Orv Hetil, 155: 1615–1623.

**Other publications:**

1. Garbaisz D, Turoczi Z, Fulop A, **Rosero O**, Aranyi P, Onody P, Lotz G, Rakonczay Z, Balla Z, Harsanyi L, Szijarto A. (2013) Alsó végtagi ischaemiás-reperfusiós károsodás hosszú távú tüdő szövődményeinek megelőzése posztkondicionálással. Magy Seb, 66: 146–154.

2. Modis K, Gero D, Stangl R, **Rosero O**, Szijarto A, Lotz G, Mohacsik P, Szoleczky P, Coletta C, Szabo C. (2013) Adenosine and inosine exert cytoprotective effects in an in vitro model of liver ischemia-reperfusion injury. Int J Mol Med, 31:437-46. **IF: 1.880**
3. Garbaisz D, Turoczi Z, Fulop A, **Rosero O**, Aranyi P, Onody P, Lotz G, Rakonczay Z, Balla Z, Harsanyi L, Szijarto A. (2013) Therapeutic option for managing lung injury induced by infrarenal aortic cross-clamping. J Surg Res, 185: 469-76. **IF: 2.121**
4. Onody P, Stangl R, Fulop A, **Rosero O**, Garbaisz D, Turoczi Z, Lotz G, Rakonczay Z Jr, Balla Z, Hegedus V, Harsanyi L, Szijarto A. (2013) Levosimendan: a cardiovascular drug to prevent liver ischemia-reperfusion injury?. PLoS One, 8:e73758. **IF: 3.534**
5. **Rosero O**, Németh K, Turoczi Z, Fulop A, Garbaisz D, Gyorffy A, Szuak A, Dorogi B, Kiss M, Nemeskeri A, Harsanyi L, Szijarto A. (2014) Collateral circulation of the rat lower limb and its significance in ischemia–reperfusion studies. Surg Today, 44: 2345-53. **IF: 1.208**
6. Garbaisz D, Turoczi Z, Aranyi P, Fulop A, **Rosero O**, Hermesz E, Ferencz A, Lotz G, Harsanyi L, Szijarto A. (2014) Attenuation of Skeletal Muscle and Renal Injury to the Lower Limb following Ischemia-Reperfusion Using mPTP Inhibitor NIM-811. PLoS One, 9:e101067. **IF: 3.534**
7. Turoczi Z, Fulop A, Czigany Z, Varga G, **Rosero O**, Tokes T, Kaszaki J, Lotz G, Harsanyi L, Szijarto A. (2015) Improvement of small intestinal microcirculation by postconditioning after lower limb ischemia. Microvasc Res, 98:119-25. **IF: 2.432**

Published or accepted cumulative impact: 19.536