SURGICAL METHODS FOR REDUCING REPERFUSION INJURY AFTER REVASCULARISATION INTERVENTIONS

PhD Thesis

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1. INTRODUCTION

In vascular surgery during the manipulation on the vessels the periferial tissues always suffer from a more or less severe ischaemia. In acute ischaemia the time of ischaemia could be also serious, thus after reconstruction we always have to face with reperfusion injury. The aim to reduce these extent of these reperfusion injury associated pathways has real clinical importance in vascular surgery.

Reperfusion injury is an inherent response to the restoration of blood flow after ischaemia, 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 depends on the ischaemic-time, the ischaemic tissue volume, and the general state of the endothelium-leukocyte-tissue functional complex (diabetes, chronic ischaemia, drugs). The pathogenesis of reperfusion injury is a complex process involving numerous mechanisms exerted in the intracellular and extracellular environment.

2. AIMS

In the first series of our investigations we aimed to examine the protective effects of ischaemic postconditioning on peripheral tissues in a rat model.

After infrarenal abdominal aortic occlusion we applied ischaemic postconditioning and measured the evoked oxidative stress, and inflammatory responses. We aimed to measure the extents of lipid-peroxidation (serum peroxide level) for characterize the reperfusion induced oxidative stress. To feature the reperfusion-induced inflammatory response, we measured cytokine expression (TNF-alpha), the leukocyte activation, serum myeloperoxidase (MPO) levels, and the free radical production of leukocytes.

Furthermore to confirm the protective effect of the applied ischaemic PostC we monitored the activation of intracellular anti- and proapoptotic common signaling pathways (pGSK, pAKT, pERK1/2, pp38MAPK) during the early phase of reperfusion.

In the second series of our investigations we focused on the effect of ischaemic PostC in human revascularization operations. After aorto-bifemoral bypass surgery we applied ischaemic PostC and observed the protective effect.

To describe the oxidative stress we measured the serum malondialdehyde level – to quantify the rate of lipidperoxidation, and the antioxidant enzymes (SOD, GSH, SH). To see the

inflammatory changes we measured serum MPO levels, free radical production of leukocytes, and the expression of leukocyte CD11a and 18 adhesion molecules.

In the third series of the investigations we aimed to examine the protective effect against reperfusion injury with controlled reperfusion in animal model. After a long infrarenal aortic occlusion we started the reperfusion for 30 min. with a crystalloid diluted blood perfusion with low pressure to the periphery. We hypothetised, that this low saturated, diluted blood perfusion with a low pressure could reduce the evoked oxidative stress, and thus consecutively the reperfusion injury. We aimed to determine the antioxidant- prooxidant state, the rheological changes in peripheral and diluted blood, and the inflammatory responses.
We aimed to follow the animals up to one week, and to make pathological examinations from tissue samples (skeletal muscle, lung, kidney, heart, liver, small bowels) to see the late effects of controlled reperfusion.

3. THE EFFECT OF ISCHAEMIC POSTCONDITIONING ON THE PEROXIDE FORMATION, CYTOKINE EXPRESSION AND LEUKOCYTE ACTIVATION IN REPERFUSION INJURY AFTER ABDOMINAL AORTIC SURGERY IN RAT MODEL

The concept of 'Ischaemic PostC' was first described by Vinten-Johansen's group. This study was performed in a canine model of 1 hr coronary occlusion and 3 hrs reperfusion. In this study the PostC algorithm was 30 sec. of reperfusion followed by 30 sec. of coronary occlusion, which were repeated for three cycles at the onset of reperfusion. Although this seminal study used the term 'Ischaemic PostC', subsequent studies of these and other authors omit the term 'Ischaemic' because it is not clear whether the brief periods of ischaemia, the preceding and/or the subsequent periods of reperfusion, or their combination, provide the key stimulus for cardioprotection. In general, PostC can be defined as intermittent interruption of coronary flow in the very early phase of a reperfusion, which leads to protection against reperfusion injury. The duration and number of these stuttering periods of reperfusion and ischaemia has been one of the aims of early studies on this topic.

3.2 MATERIALS AND METHODS

Animal model

24 Wistar rats in both sexes, weighed between 200-250 g were used in the study. The animals were anaesthetized with an intraperitoneal injection of 50 mg/kg ketamine hydrochloride (Vetalar, Fort Dodge) and were placed on a heating pad. ECG was placed and the carotid artery was catheterized (22 gauge) for blood pressure measurement (Siemens Sirecust 1260, Düsseldorf, Germany). The skin was aseptically prepared and a midline laparotomy was performed. The inferior caval vein was gently catheterized for collecting blood sample, fluid equilibration and supplemental anesthetic. The abdominal aorta was exposed by gently deflecting the intestine loops to left. After fine isolation of the infrarenal segment, an atraumatic microvascular clamp was placed on the aorta for 60 minutes. The abdomen was then closed and the wound was covered with warm wet compress to minimize heat and fluid losses. The microvascular clamp on the infrarenal abdominal aorta (IAA) was then removed and IAA was reperfused for 120 minutes. Aortic occlusion and reperfusion was confirmed by the loss and reappearance of satisfactory pulsation in the distal aorta.

Experimental groups

Rats were divided into three groups (8 animals in each group). In the first (ischaemia-reperfusion, IR) group the aorta was closed for 60 min and then a 120 min of reperfusion followed without interruption.

In the second (ischaemic postconditioning, PostC) group the infrarenal aorta was clamped for 60 min, and the early reperfusion was interrupted with 4x15 sec total reclamping of the aorta with an intermittent 15 sec perfusion. The animals then underwent 120 min of reperfusion.

In the third group (control group) animals underwent a sham operation without aortic clamping.

Peripheral blood samples were collected before the operation, and in the early (5; 10; 15; 30; 60 and 120 min) reperfusion periods. The serum samples were harvested and stored at minus 78°C until biochemical assays.

Serum peroxide determination

Low density lipoproteins (LDL) are the most sensitive compounds in the blood for oxidative stress. Oxidation of LDL by oxidative stress in biological systems is principally a free radical process, where polyunsaturated fatty acids (PUFAS) in LDL are converted by lipid peroxidation to lipid hydroperoxides. For quantitative determination of serum peroxides OyxStat (Biomedica Medizinprodukte GmbH, Wien, Austria) colorimetric assay was used, following the manufacturer's instructions.

The results show a direct correlation between free radicals and circulating biological peroxides and thus allow the characterization of the oxidative status in reperfusion.

Serum TNF-alpha quantification

For measurement the TNF-alpha concentration in serum we used Rat TNF-alpha/TNFSF1A ELISA kit (R&D Systems, Inc. Minneapolis, USA), following the manufacturers protocol. This method determines the free i.e. biological active TNF-alpha concentration.

Serum myeloperoxidase assay

Anticoagulated blood was centrifuged with 2000g, and 200 µl plasma was mixed with 1 ml working solution (0,1 M sodium-citrate 10,9 ml, 0,05% Triton-X 100 5 µl, 1mM H_2O_2 1 ml, 0,1% o-dianisidine 100 µl). The mixture was incubated at 37 ºC for 5 minutes, then 1 ml 35% perchloric acid was added. Photometry were done at 560 nm. Plasma myeloperoxidase was expressed as nM/l. Hematologic measurement: Red blood cell count, white blood cell count, platelet numbers, haemoglobin concentration, haematocrit level were measured by Minitron automatic analysator (Diatron Ltd, Budapest, Hungary).

PMA-induced leukocyte ROS production

The induced ROS production of leukocytes was measured in whole blood. The superoxide anion production was induced with 0.2 µg/ml phorbol 12-myristate 13-acetate (PMA) (Sigma-Aldrich Ltd, Budapest, Hungary), and was detected with luminol 3.33 µg/ml (Boehringer Gmbh Mannheim, Germany;) on a Chrono-log 560-VS lumino-aggregometer (Chrono-log Corp., USA). We have registered the maximum rate of ROS production.

Pro- and antiapoptotic signaling pathways measurements

Western Blot Analysis

Tissue samples were collected from the skeletal muscle, heart, liver, kidney and lung. We examine the effects of ischaemia-reperfusion and PostC on the signaling pathways in these organs as well.

3.3. RESULTS

Serum peroxide results

We measured in our animal model the serum total peroxide concentration during infrarenal aortic cross clamping ischaemia and reperfusion. In both groups we have detected a typical curve of serum peroxide changes with a rapid elevation ($p< 0.05$ vs. before surgery) in the immediate phase of reperfusion and that was followed with a slow elimination period. Our data showed significant ($p< 0.05$) differences in the extent of early elevation between the two groups. The peroxide concentration was higher in IR group in the 5^{th} (16.91±3.67 µM/l vs

10.04±1.9 μM/l) and in the 15th minutes (20.42±3.17 μM/l vs 13.77±2.84 μM/l) comparing to postconditioned group (PostC). In the depletion phase there was no difference between the groups.

Serum TNF-alpha results

We measured the serum TNF-alpha levels before the ischaemia and after the ischaemia-reperfusion in the experimental groups. We have found that on the end of the reperfusion protocol the serum TNF-alpha concentration was elevated both in the postconditioned (PS) (116.55±12.04 pg/ml vs. 36.31±7.91 pg/ml) and in the non-conditioned (IR) (167.41±21.26 pg/ml vs 38.27±4.31 pg/ml) groups in comparison to the values before the ischaemia.

While both in the postconditioned and in the non-conditioned groups the serum TNF-alpha concentration after the reperfusion was higher than in the control group, in the postconditioned group the concentration was significantly lower than in the non-conditioned group (116.55±12.04 pg/ml vs. 167.41±31.26 pg/ml).

Serum myeloperoxidase results

To characterize the neutrophyl activation we measured the plasma myeloperoxidase (MPO) level and the induced ROS production of leukocytes. MPO level increased significantly after ischaemia-reperfusion in non-conditioned group (group IR: 1.759 ± 0.239 μM/ml vs 1.108 ± 0.143 μM/ml). In the postconditioned group there was no elevation before and after the ischaemia-reperfusion (1.22 ± 0.126 μM/ml vs 1.179 ± 0.182 μM/ml). To compare the values of postconditioned and non-conditioned groups we found a significant difference (1.759 ± 0.239 μM/ml vs 1.22 ± 0.126 μM/ml $p<0.05$).

PMA-induced leukocyte ROS production

The phorbol-myristate-acetate (PMA) induced maximal ROS production of the leukocytes shows their activation level. The results are demonstrated on figure 7. We have found a significant elevation in ROS production in the non-postconditioned group (IR) before and after the ischaemia-reperfusion period (2.59 ± 0.95 AU/10^3 cells vs 5.7 ± 0.96 AU/10^3cells; $p<0.05$). In the postconditioned group there was no significant change detected (3.89 ± 0.94 AU/10^3cells vs 4.63 ± 0.69 AU/10^3cells).

There was no significant change in the white blood cell (WBC) count during the protocol in both groups (IR: 9.25 ± 5.22 x10^3cells/μl vs 13.45 ± 7.23 x10^3cells/μl; PostC: 5.08 ± 0.54 x10^3cells/μl vs 9.38 ± 2.35 x10^3cells/μl).

Pro- and antiapoptotic signaling pathways

Western-blot analysis shows that in the 2nd hour of reperfusion in the ischaemia-reperfusion groups in skeletal muscle, in myocardium and in the lung pAKT was activated, and ischaemic PostC could further increase this activation in these tissues. In the liver we could not detect real pAKT activation, while in the kidney the activation was detectable, but neither ischaemia-reperfusion, nor PostC increased this.

PGSK in skeletal muscle, and in the heart was increased in I/R group vs controll, and as seen in figure PostC caused further elevation. In the liver, kidney and lung a similar tendency could be seen.

The activation of pERK1 and 2 could be detected in all examined tissues, and this antiapoptotic marker was increased with ischaemic PostC.

As seen in picture the proapoptotic pp38MAPK was increased in ischaemic-reperfusion group in all tissues, and this activation was less in the PostC group.

4. EFFECTS OF ISCHAEMIC POSTCONDITIONING IN HUMAN VASCULAR SURGERY

4.1. INTRODUCTION

Ischaemic postconditioning was found effective to reduce reperfusion injury not only in experimental animal models, but in humans as well in cardiac interventions. Our first series of examinations has confirmed that ischaemic PostC could also be effective in peripheral tissues, thus has real clinical potential in vascular surgery. To examine the effectiveness of PostC in humans we applied ischaemic PostC during aorto-bifemoral bypass surgery and measured the extent of reperfusion injury.

4.2. PATIENTS AND METHODS

All patients completing the study suffered from general atherosclerosis with distal aortic or aorto-biiliac occlusion., and underwent an aorto-bifemoral bypass surgery.

Human ischaemic postconditioning protocol

In the postconditioned group (10 patients) after the completion of the distal anastomosis, before starting the reperfusion we made two cycles of 30 sec reperfusion-reocclusion on the graft. After this two cycles of reperfusion-reocclusion we let the continuous reperfusion to the distal artery.

In the ischaemia-reperfusion group (10 patients) after the distal anastomosis we started the continuous perfusion.

The measurement of oxidative stress parameters:

Measurement of malondialdehyde (MDA): Malondialdehyde was determined in anticoagulated whole blood, by photometric method.

Measurement of reduced glutathione (GSH) and plasma thiol (SH) groups: GSH and plasma SH levels were determined from anticoagulated whole blood (ethylene diamine tetraacetic acid (EDTA)) by Ellman's reagent according to the method of Sedlak and Lindsay.

Measurement of Superoxide dismutase (SOD) activity in washed red blood cell (RBC):

The main principle of this measurement was that adrenaline is able to spontaneously transform to adrenochrome (a detectable colorful complex). This transformation can be blocked by SOD, and SOD containing cells or tissues. The difference in the rate of rise of control and sample curves obtained at 415 nm, are proportional to SOD activity.

Measurement of inflammatory response, leukocyte activation

Determination of free radical production from whole blood: Free radical production was induced by 30 µl phorbol-12 myristate 13-acetate (PMA; 0,2µg/ml) (Sigma Aldrich Budapest); in the mixture of whole blood (20 µl), phosphate buffered saline (1400 µ) and 50 µl luminol (3.33 µg/ml; Boehringer Mannheim Gmbh Germany), and was detected by Chrono-Log Lumino-aggregometer.

Leukocyte adhesion molecule measurement: The leukocytes were marked with fluorescein isotiocianide (FITC) labeled antibodies for adhesion molecules (CD11, CD11b, CD18, CD49d, és CD97) (Becton Dickinson Biosciences, Pharmingen, USA), and measurements were performed on BD FacsCalibur (Becton Dickinson Biosciences, Pharmingen, USA) flowcytometer.

RESULTS

Plasma malondialdehyde concentration before surgery was similar to the control group. A significant increase was detected in both group right after the reconstruction, but this elevation was significantly higher in the non-conditioned group. Same results were measured 24 hours later and the MDA plasma concentration decreased to the initial values after 7 days.

Measuring the antioxidant enzyme plasma levels we observed that the thiol group concentration in non-conditioned group significantly decreased in the early reperfusion period. The 24 hours values did not show significant changes compared to control and initial values, but after a week in the non-conditioned group a slight decrease was detectable (the

second waves of reperfusion injury: mediated by not the ischaemia-reperfusion, but the inflammatory response activated leukocytes.

In the plasma level of reduced glutathion, a significant decrease was detectable in the early reperfusion in both groups. From the first day a continuous elevation was observed until the 7th day and the plasma level in both groups returned to the values before surgery.

The activity of superoxide dismutase before surgery was lower in both groups compared to the control group, and did not show any changes right after the operation. 24 hours later in the non-conditioned group we detected a significant decrease, which disappeared at the end of the week.

Leukocyte activation increased significantly immediately after revascularisation surgery in the non-conditioned group, and this elevation could not be observed in the postconditioned group. In the late reperfusion period the maximum of leukocyte-derived free radical production were elevated in both group withouth significant difference between the two groups.

The plasma myeloperoxidase (MPO) concentration was higher in both investigated groups than in healthy control group. We did not observed any significant changes until the 7th day. On the last day of the protocol the plasma MPO concentration elevated significantly in the non-conditioned group, and this elevation was not detectable in the postconditioned group.

Granulocyte surface adhesion molecules were detected by flowcytometer. The detectable expression of CD11a adhesion molecules were significantly lower in the postoperative first samples than before surgery. There was no significant difference at this time between the two groups. After 24 hours in the non-conditioned group a significant expression was observed, which was not detected in the postconditioned group. At the end of the one week period the values reached the starting values.

CD18 showed a significant decrease in the immediate reperfusion period in both groups, and after these changes were the same as the control values.

In the results of the red blood cell count, white blood cell count, platelet numbers, haemoglobin concentration and haematocrit level we did not detected any difference between the two groups of patient.

4.4. Conclusion

Postconditioning has the advantage of being a way to influence and modify reperfusion injury after it has occurred. This may open a therapeutic alternative in situations of unexpected and

uncontrolled ischaemic-reperfusion injury, for instance in the situation where technical complications occur during surgery, making a simple procedure into a complicated one, and making aortic cross-clamping longer than anticipated.

We think, that many more examinations are needed to describe and understand in details the mechanism of ischaemic PostC. We are sure, that this manoeuver is easy to perform, quick, and does not any expensive instruments, so it may have a place in the therapeutic arsenal of vascular surgeons.

5. THE EFFECT OF CONTROLLED REPERFUSION ON REPERFUSION INJURY USING A SIMPLIFIED PERFUSION SYSTEM

5.1. INTRODUCTION

Persistent and acute ischaemia of the extremity, including neurologic dysfunction of the compromised leg, is associated with high morbidity and mortality. The most common reasons for acute limb ischaemia are embolism of cardiac or arterial origin and in situ thrombosis of arteriosclerotic vessels. Since the late 1960s, surgical revascularization with the use of Fogarty catheter primarily has been the therapeutic gold standard. It must be emphasized that the results of surgical therapy have not improved over the decades. Even the introduction of new interventional treatment options such as intra-arterial thrombolysis did not reduce the high rates of mortality and amputation. Crucial in treating acute lower-limb ischaemia is that restoration of arterial blood flow, essential for limb salvage, can further damage ischemic tissue in a phenomenon known as reperfusion injury. Reperfusion injury and its systemic effects on remote organs can cause severe local and systemic complications such as renal and pulmonary failure. There is experimental evidence that modifying the initial perfusion modalities, especially perfusion pressure and composition of the initial perfusate, can reduce reperfusion injury. The therapeutic principle named “controlled reperfusion” was first used to treat myocardial ischaemia. Reduction of the initial reperfusion pressure is aimed at reducing edema development, the modification of the initial reperfusate is aimed at counteracting the known biochemical changes that occur with ischaemia-reperfusion, such as the breakdown of aerobic metabolism, metabolic acidosis, an increase in intracellular calcium, and the development of oxygen-derived free radicals with the onset of reperfusion. In an animal model of acute lower-limb ischaemia, local and systemic complications were reduced by the

use of controlled reperfusion. The concept of controlled reperfusion has been successfully used in clinical practice for treating patients with severe, prolonged lower-limb ischaemia.
All techniques described so far have required the use of a heart-lung machine or roller pumps. Beyersdorf et al. have developed a new blood bag reperfusion system that allows the application of controlled reperfusion on acute ischemic limbs with minimal technical effort.

5.2. MATERIALS AND METHODS

Study protocol.

We used ten Yorkshire pigs for the animal model. Five of these animals undervent a 4-hour infrarenal aortic occlusion followed by continuous reperfusion without any therapy. (Control group)
Five of these animals were treated with controlled reperfusion. In these cases after a 4-hour aortic occlusion we made the controlled reperfusion for 30 minutes and after that we started the continuous reperfusion with normal blood flow.

Surgical Preparation

Yorkshire pigs of either sex, weighing between 18 and 22 kg and free of clinically evident disease, were entered to this study.
A median laparatomy was made, and with gentle retraction of the bowels we isolated the infrarenal aorta. We occluded the aorta with DeBakey clamps. After a 4-hour ischaemic period we removed the clamps and checked the restoration of continuous blood flow with the pulsation of the iliac arteries.

Management of controlled reperfusion

Controlled limb reperfusion was performed as follows: After the aortic occlusion and before restoration of blood flow, a 10-Charrier (CH) (1 CH is equivalent to .33 mm) cannula was inserted proximally into the aorta, another 10-CH cannula was inserted distally. The proximal cannula was connected to the blood line, and oxygenated blood was drawn into the first blood bag where it was mixed with the crystalloid reperfusion solution (blood:reperfusion solution ratio, 1:1). According to the hemodynamic status of the animals, either 200 mL or 300 ml of blood was taken every cycle. A 12-gauge cannula was inserted into the aorta distal to the reperfusion cannula for continuous pressure control. Perfusion pressure was kept strictly 60 Hgmm. In most cases, the blood reperfusion solution was returned to the leg by gravity alone. The procedure was repeated for 30 minutes. After removal of the cannulas, the arteriotomy was closed with direct suture, and normal blood flow was re-established.

Oxidative stress parameters and the leukocyte activation measurements

The methods are described in the previous capture.

Histological examinations

The animals both from treated and control groups were anesthetized one week after terminating ligation and biopsy was taken from quadriceps muscle and large parenchymal organs (liver, kidney, lungs, heart) as well as large and small intestines.

5.4. RESULTS

Hemodynamic Data

Baseline heart rate, systolic and diastolic pressure were not significantly different among the various groups, and controlled reperfusion did not differ from control at any measurement period.

Oxidative stress parameters

The plasma MDA concentration elevated in the full reperfusion group right after the beginning of reperfusion and decreased for the 24th hour of reperfusion. This elevation was much milder in controlled reperfusion group, we measured significant difference between the two groups. On the end of the investigated period we detected a recurrent elevation in the reperfusion group, but lower than in the early reperfusion period.

We observed changes in the –SH group in the first hour of reperfusion. There was a significant decrease in the regulary reperfused group, but this change can not be seen in the controlled reperfusion group. The measured values in the 24th hour were similar to the values before reperfusion. A second decrease was detected on the 7th day in the normal reperfusion group.

GSH plasmalevels decreased significantly in the early reperfusion period until the 24th hour. We did not detected differences between the two groups. On the 7th day of reperfusion the measured values in both group reached the start values.

We did not measure changes in SOD activity during the ischaemia and in the early reperfusion. A significant decrease in SOD activity was detected in the 24th hour of reperfusion in the normal reperfusion group. This decrease could not be detected in the controlled reperfusion group. On the last measure time both groups showed the same values as before the ischaemia, and there was no any difference between them.

We detected significant increase in the speed of leukocyte radical production in the early period – in the 15th and in the 60th minutes – in the totally reperfused group. These

changes could not be observed in the controlled reperfusion group. In the 24th hour we measured physiological values in both group.

The maximum of free radical production first elevated in the reperfusion group in the 15th min of reperfusion and this elevation could be detected until the examined period. Significant difference between the groups could be measured in the first hour of reperfusion. On the seventh day we measured increased values in both groups.

We detected a significant increase in the reperfusion group in the 24th hour. At this time there was no elevation in the controlled reperfusion group, and a significant difference could be seen between them. We measured still elevated values at the end of the experimental period.

The results of histological investigations

In the first group of animals the basic tissue structure is mainly kept in the striated muscle tissue, there is no fibrosis and necrosis cannot be defined with absolute certainty and neither significant inflammation cannot be observed. At the same time the muscle tissues show well-observed size and shape differences. Regular-morphology was seen, normal staining muscle fibers, where the desmin immunohistochemical reaction is strong, smooth and fully covers the area of fibers. At the same time the other fibers are swelled, irregular-shaped and the interstitial space between the fibers is pressed, decreased. The fibers staining are paler, less even and slightly basophile- shade, in places the sacroplasma seems tattered and the nucleuses are fewer. In smaller areas the replacement of nucleus can be observed from the edge of fibers to their central area, and same round-celled inflammative infiltration and regenerative signs also appear, the last can indicate to necrosis occasionally.

The rate of affected muscle fibers by degeneration was 61.7% in the first-untreated-animal group (617:383), and it exceeds 50% in every areas. The rate of damaged fibers is 42.4% (424:576) in the second treated group, and it stays under 50% in every area

5.5. CONCLUSION

Our results strongly support the hypothesis that controlled reperfusion can improve outcome after acute severe lower-limb ischaemia even though this study was limited by the small number of animals. We have seen that controlled reperfusion significantly reduced the postischaemic oxidative stress and inflammatory responses in the early reperfusion period.

Our pathohistological results confirm, that controlled reperfusion has real beneficial effect not only on the ischaemic skeletal muscle, but also protects against reperfusion syndrome in the lung, kidney and liver.

Our results confirm, that controlled reperfusion might be also a potential therapeutic approach in vascular surgery against reperfusion injury in acute limb ischaemia.

6. NOVEL FINDINGS

In the first series of our investigations we observed the protective effects of **ischaemic postconditioning** after aortic occlusion-induced reperfusion injury in an experimental animal (rat) model.

We have three important observations in the study.

1. Our results demonstrated that ischaemic PostC significantly reduced the reperfusion induced early oxidative stress and the inflammatory responses (leukocyte activation, cytokine expression, myeloperoxidase elevation) after a sustained skeletal muscle ischaemia-reperfusion.

2. In our molecular biology investigations we observed that skeletal muscle ischaemia-reperfusion could activate both pro-, and anti-apoptotic pathways in myocardial, lung, kidney and liver. Thus peripheral ischaemia-reperfusion simultaneously induces survival and death signalisation in central organs that were not suffered from ischaemia-reperfusion.

3. Ischaemic postconditioning after peripheral muscle ischaemia-reperfusion showed significant reduction of proapoptotic signal activation and significant increase in antiapoptotic (protective) signal activation in central organs. Thus these results suggest that ischaemic PostC could reduce not only the local reperfusion injury but also can protect the other parenchymal organs in reperfusion injury.

The second series of our investigations we described the beneficial protective effect of **ischaemic PostC in human** vascular surgery interventions. Our results showed that ischaemic PostC after aortic occlusion in aorto-bifemoral bypass surgery could significantly reduce the oxidative stress in the early phase of reperfusion, preserves the endogenous antioxidant capacity, and depress the local and systemic inflammatory pathways (leukocyte activation, surface adhesion molecule expression).

PostC seems to be a beneficial and simple surgical method in cases of relatively long ischaemia affecting a mass of tissue to lower the surgical complications of revascularization.

In the third series we described the effect of **controlled reperfusion** on aortic occlusion-induced lower limb skeletal muscle reperfusion injuy in a pig model. We confirmed that with chrystalloid solution-diluted low pressure blood reperfusion significantly reduced the reperfusion induced oxidative stress and leukocyte activation and preserved the plasma antioxidant capacity.

The histological investigations showed reduced cellular necrosis and intracellular edema in the skeletal muscle treated with controlled reperfusion.

Controlled reperfusion also reduced lung, kidney, liver and myocardial injury after a long aortic occlusion.

Controlled reperfusion seems to be an effective method in long-lasting ischaemia – critical lower limb ischaemia in vascular surgery to reduce local and systemic reperfusion injury.

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