

MONITORING OF REPERFUSION INJURY DURING LOWER LIMB REVASCULARIZATION SURGERY

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INTRODUCTION

During peripheral vascular surgeries, done to improve circulation, while procedure is going on, involved vessels are clamped. After revascularization procedure, a blood circulation of higher volume and pressure will affect the system; this might cause reperfusion injury which is in direct relationship with the level of occlusion, duration of the procedure, and general condition of the tissue. In clinical experiences these parameters can hardly be monitored, but it is important to notice that they can highly affect the post operative results, so attempt to stop or reduce these parameters requires great attention in surgical studies.

Today's clinical experience can easily solve the question of recanalization of roughly occluded arteries, but sometimes it requires high attempt. During ischemia-reperfusion (I/R) a cascade of pathophysiological process starts, which usually ends up by cell death, while applying a general distress to the body.

In a critical period, of post acute ischemic revascularization (e.g. embolectomy), subfascial edema happens which is originated by muscle swelling. This might highly affect the lymphatic circulation as well. If infection is present the long lasting edema can be followed. Surprisingly after reperfusion the injury to unaffected tissues might still continue, at the same time a so called "oxygen paradoxon" may come into existence in which oxygen derivative free radicals play an important role. In acute arterial cases either sudden occlusions or acute bleedings are dominant, in both cases; late diagnosis can cause greater and irrevocable results. Followings are the main causes for acute occlusion of arterial system: embolus (thrombus, tumor, fat, foreign body), arterial thrombosis (arteriopathy of stenosis, or trauma origin), arterial spasm, and external compression (anatomic anomaly, or traumatic)

AIMS OF OUR STUDIES

Post operative vascular ischemia-reperfusion injuries attitude, is mainly up to ischemia duration, amount of involved tissue, and tissue's general condition, as mentioned these highly affect the post operative side effects and risk factors. Although during last decades, techniques in vascular surgery have performed a great development, but at the same time, mainly in developed countries, civilization dependent diseases (Diabetes, Hypertonia, and atherosclerosis) have shown a dramatic progression. This poses a great challenge for vascular surgery field. Since patients' prevention compliance is usually low, in advanced cases, even beside modern technique applications, complications are frequent. Our main goal has been to monitor reperfusion injuries exact character, degree, and characterization during revascularization surgery, and to decrease it by antioxidant therapy. Our next goal is to introduce a protocol for post reperfusion compartment syndrome conservative and surgical treatment.

1. In the first part of our study we monitored the **reperfusion injuries in those patients undergone revascularization surgery for acute ischemia (embolus, thrombosis)**. Reperfusion injuries have been characterized by prooxidant and antioxidant systems assay as

well as leukocyte activation. A week of monitoring as early post operative period as well as late post operative complication has been considered. We tried to figure out reasonable method for treatment and observation of patients with high ischemic risk.

2. Study's second part is focused on the **E-vitamin's antioxidant effect on reperfusion procedure, after elective lower limb revascularization in a selected group**. With these measurements we tried to find out how effective is the E-vitamin to reestablish the intracellular oxidative balance in an ischemic-reperfusion model, at the same time we monitored its efficacy on reducing or stopping leukocyte activation resulting inflammation regression.

3. In our study's final part we monitored a group of **postoperatively observed patients, with high risk of compartment syndrome**. We applied continues monitorisation of fascia pressure and tissue oxygen saturation to figure out a reasonable treatment, and diagnostic protocol for such a patient group. Up on the measured parameters (evidence based medicine) and parallel to generally known criteria's we aimed to find a new line of clinical indications for surgical treatment in this group. We applied respectively, measure of intracompartmental pressures and tissue saturations with NIRS techniques, in circumstances under which surgical treatment (fasciotomy) is certainly needed and conservative therapy is no more successful to save the limb.

1. MONITORIZATION OF STRESS PARAMETERS DURING ACUTE LOWER LIMB REVASCULARIZATION SURGERY

PATIENTS AND METHODS

All the patients taking part in this study has been operated in Department of General and Vascular Surgery, Baranya County Hospital. We made laboratory measurements in the Department of Surgical Research and Techniques of Pécs University.

10 adult patients suffering from critical lower limb ischemia and undergone revascularization operation have been involved in a prospective randomized study. The average age was $58,1 \pm 7,3$. In 8 cases the ischemia was embolus originating (one art.iliaca, one aorto-byiliacal, and six at the superficial and deep femoral art. bifurcation), in one of the cases rupture of infra renal aorta, and in the last case acute arterial thrombosis at the level of femoral art were the causatives of ischemia.

Peripheral blood samples have been taken before operation and at 2 and 24 hour as well as a week after operation. Leukocyte free radical activation has been evaluated by detection of phorbol miristate acetate (PMA); this has been monitored by luminometric method. We measured the time interval between induction and production of free radicals, free radicals production acceleration and its peak production respected to the measure of leukocytes. Among the antioxidant enzymes, we determined the superoxide dismutase (SOD) activation, and glutathion (GSH) concentrations. Lipidperoxidation measure has been demonstrated by malondialdehyd (MDA) level. The expression of leukocyte adhesion molecules has been determined by flowcytometry technique.

RESULTS

The level of leukocytes in the healthy group was $6,5 \pm 0,34 \times 10^3$, the same parameter in study group, preoperatively proved to be $11,5 \pm 1,9 \times 10^3$ ($p < 0,05$), this parameter did not show any remarkable change within post operative first week. Superoxide production respectively to leukocyte level, showed a significant variation (control group: $10,85 \pm 2,64 \text{ U}/10^3$ preoperative, study group: $93,44 \pm 20,15 \text{ U}/10^3$, $p < 0,01$). In the study group this parameter within the first post operative week continuously elevated. By the influence of PMA free radical production in the study group has started significantly faster ($135 \pm 40,09 \text{ sec}$), comparing to the control group ($383,14 \pm 78,14 \text{ sec}$, $p < 0,01$), this value during early reperfusion kept decreasing constantly. This change, by post operative 24 hr, has reached the preoperative value, but still measured much lower comparing to control group.

The GSH and thiol group measurements have not shown remarkable differences between the two groups. During early reperfusion period GSH level dramatically diminished ($p < 0,01$) at the same time $-SH$ groups levels also decreased. Regarding SOD activation a notable difference has been noticed between the two groups (control: $894,34 \pm 86,85 \text{ U/ml}$, study group: $415,43 \pm 75,22 \text{ U/ml}$), and after 24 hrs a significant reduction has followed ($p < 0,05$). According to our measurements these changes had reached the preoperative level after a week, but this did not exist in the case of SOD.

Malondialdehyd plasma level has shown significant elevation after the operation and during reperfusion, it remained almost constant during first post operative week, this determines lipidperoxidation and membrane impairment. The plasma MPO level also showed a significant elevation which decreased after 24 hrs, but elevated again during late reperfusion. Comparing to the base level both parameters diminished by 2nd hrs of post operation period, but during late reperfusion elevated again, and after one week were slightly above the base level (before operation). In control group the granulocytes CD11a and CD18 expressions were respectively: $172,5 \pm 29,3 \text{ AU}$ and $306,7 \pm 90,2 \text{ AU}$. Further evaluation of adhesion molecules quantitative changes (CD11, CD11b, CD18, CD49d, and CD97) has not shown any difference.

2. THE EFFECT OF VITAMIN-E ON THE ISCHEMIC-REPERFUSION INJURY FOLLOWING PERIPHERIAL REVASCULARISATION SURGERY

PATIENTS AND METHODS

Observed group has taken part in a prospective randomized procedure, during which chronic obliterative arteriosclerosis has proved by angiography and Doppler techniques, in this group respected to peripheral limb ischemia, lower limb revascularization surgery happened. All patients have proved stenosis at the level of femoral superficial art. So arterial clamping at the level of common femoral artery was done in all cases. Affected limbs blood supply was intact only through deep femoral art. The operating technique was similar in all cases (femoro-popliteal supragenaal graft bypass and AFS thrombendarterectomy), in such a way duration of ischemia ($42,8 \pm 16,3 \text{ min}$) and amount of involved tissue was almost the same in all cases. Trophic disorder was not present in any of the selected cases; there was no evidence of

infection, or inflammation going on. Group 1 patients (treated with vitamin-E) received 1x200 mg vitamin-E per day since the day before the operation till the 7th post operative day. Patients in the second group did not received vitamin-E; the operation technique was the same.

Sample taking method and parameters are the same as previously mentioned techniques. We monitored the production of free radicals by WBCs; we measured the time interval between induction phase and free radicals production period, the acceleration of free radicals production and its peak value respectively to the measure of leukocytes. Among antioxidant enzymes, we determined the superoxide dismutase (SOD) enzyme activation and glutathione (GSH) concentration. Lipidperoxidation measures have been defined by the level of malondialdehyd (MDA). WBC adhesion molecules expressions has been detected by electro cytometry technique

RESULTS

During acute operations, at any time, the level of measured WBCs, were significantly higher in the operated group comparing to the control group, but there was no significant difference to the WBC level in the electively operated group (base level). So probably the differences observed between above mentioned groups, are not up on WBC measured amount.

Free radical production, inducted by the effect of PMA in the electively operated group was almost the same as the measured level of the same parameter in the control group. In the case of acute operations (embolectomy) the measured level of free radical production, inducted by PMA, even before operation was five times higher than the normal level, this parameter increased continuously during the monitoring period. In the first group (treated by vitamin-E) radical production after operation reduced, comparing to the second group, but statistically it could not be proved significant. In the electively operated group, the free radical production was not proved pathologic, not even in a single case.

The time interval between (PMA) inductor use free radicals production initiation which is called "lag time", in the case of acutely operated patients was significantly shorter comparing to other groups. These measures became significantly shorter immediately after operation and a week after that in this group, comparing to the base level. In the other groups this phenomenon was not observed.

In the acutely operated group's free radical production diagram, the elevating curve proves more gradient and more elevated comparing to the other two groups. In the same group during post operative period this value shows a statistically significant elevation comparing to the base level, while in the first group (treated with vitamin-E) its decrease was notable. Although this difference statistically was not proved significant.

In both operative groups superoxide dismutase (SOD) enzyme activation was significantly lower comparing to the control group, even before the operation. In elective group the measured value at the base remained almost the same, during the observation period notable change was not registered.

In the acute group SOD activation even before operation was significantly higher than the group treated with vitamin-E, this difference was registered during reperfusion and 24 hrs later as well. In the acute group this low level shows further decreasing tendency within first 24hrs post operative period, comparing to measured preoperative value.

The level of glutathion in the hemolized blood is the same preoperatively in all groups. In the acute and vitamin-E non treated groups, level of glutathione , comparing to base level has slightly but significantly reduced after operation.

Thiol groups concentration in the serum post operatively (early period) reduced significantly just in the case of acute and non vitamin-E treated groups respectively to preoperative value. During the post operative reperfusion period, thiol group plasma concentration was significantly lower in non vitamin-E treated group than vitamin-E treated group. Plasma myeloperoxidase activation was basically higher in all groups comparing to control group.

During our study expression of adhesion molecules CD11a, CD11b, CD18 and CD49d of granulocytes, lymphocytes and monocytes have been monitored. During early post operative reperfusion period, CD11b and CD18 decreased dramatically, and then elevated continuously for 7 days. Their characterization is almost the same as the acute cases. In the vitamin-E treated group, during early post operative reperfusion period there was a minimal expression noticed, by the 7th day it was higher than the non treated group.

During the whole period CD49d showed constantly higher expression, in vitamin-E treated group than non vitamin-E treated group. Among the population of monitored leukocytes there was no significant change in the granulocyte group, while on the post operative day a significant lymphocyte reduction and monocyte elevation was noticed.

3. THE CLINICAL MANIFESTATION OF REPERFUSION INJURY IN THE COMPARTMENT SYNDROME

PATIENTS AND METHODS

16 adult patients all suffering from acute critical ischemia of lower limb, for more than 4 hrs have taken part in our study (12 M and 4 F, average: $62,7 \pm 9,5$ yrs), all undergone revascularization surgery. Ischemia causative origin in five cases was embolus at iliac artery level, in rest of the cases occlusion was at the level of femoral artery (embolus or thrombosis). In all embolization cases, embolectomy with Fogarty catheter was the solution of choice. In rest of the cases (4 thromboses), intraoperative angiography and femoro-popliteal suprageneal graft bypass was performed. Post operatively in all cases, peripheral pulsation was detectable. During operation all patients had received 5-10 000 IU Heparin respectively to body weight, postoperatively LMWH (Low Molecular Weight Heparin) prophylaxis therapy (2x5000 IU Fragmin sc) was applied.

For the aim of further microcirculation improvement, patients also received daily infusion of 500 ml ISOHAES. On the first day after operation routine lab test was performed in all cases (CBC, ions, blood gas, and CN, Creatinin, urine test, liver function, and CK, serum and urine myoglobin). On the post operative day different scales of edema and muscle swelling was noticed at the level of foreleg, in some of the critical cases. Secondary injuries were obvious in lab results as well. Notable pain, paresis, parestesia, pulse absence, and biochemical changes were all considered indications for fasciotomy. At this stage, we monitored the m. tibialis anterior intracompartmental pressure, and oxygen saturation of foreleg.

MEASUREMENT OF FASCIA COMPARTMENT PRESSURE IN FORELEG

Regardless to objective signs, and symptoms, fascia compartment pressure measurement proved to be a capable method for diagnosis and indication of compartment syndrome. For such procedure we had to insure a standard and enforceable technique. Regarding fasciotomy indications, fascia pressure measures, have a great role in making correct statements.

In a healthy human, capillary's critical end pressure is around 40 Hgmm. If ICP reaches or precede this level, then capillary perfusion stops, it poses a certain indication for fasciotomy. In our department we used a so called KODIAG digital manometer (BRAUN, AESCULAP. produced: MIPM Mammendorfer Institute für Physik und Medizin GmbH Oskar-von-Miller-Strasse 6, Mammendorf, Germany.)

For measuring the pressure, after foreleg disinfection, we insert a canule into body of tibialis ant. muscle. After removing the guiding gauge, we connect the canule to KODIAG device, at this time LCD monitor is fixed on „0“. Then we pull the canule back from the catheter which is fixed into the muscle, in this way tissue pressure would be determined in Hgmm.

DETERMINATION OF TISSUE OXYGEN SATURATION. (NIRS, INSPECTRA)

Determination of the tissue oxygenation is done by the use of Inspectra Tissue Spectrometer (Model 325, Huthcinson Technology Inc., and Arnhem, Netherlands) Inspectra spectrometer can detect the value of hemoglobin light absorption characteristic, within near infrared borders. (Near infrared spectroscopy; wavelength 680-800 nm). The light deflected from tissue and absorbed by the spectroscopy, can confirm the exact oxyhemoglobin and deoxyhemoglobin concentrations.

We placed the detector on the body of the gastrocnemius muscle and during calibration, continuous value registration applied. NIRS has been proved as a noninvasive method for evaluation of tissue perfusion. Since, light deflection happens in different layers, so its absorption by INSPECTRA and the final evaluation can figure out the Hgb content of the arterioles, capillaries, venules and tissue beds. This is the advantage of this method comparing to pulseoxymeter, by which only pulsating blood oxygen content can be detected. We found this method useful for the tissue perfusion evaluation of single, or permanent use.

It's capable for detection of all perfusion changes happening in the area under detector segment, so in clinical practice it's useful in diagnosis of obliterative atherosclerosis (measurement of perfusion changes under stress), determination of operative perfusion and reperfusion, confirm indications for fasciotomy, and detection of its effectiveness.

EXPERIMENTS ON SKELETAL MUSCLE STRUCTURAL AND ULTRASTRUCTURAL CHANGES

A muscle specimen from 3 patients was taken under this procedure. These specimens, as wide as 1x1 cm have been removed from tibialis ant. muscle during fasciotomy procedure. A side of specimen has been placed in isopentane, got frozen by liquid nitrogen, and then cross sections of 10 μ m wide has been made by cryostat. To view under light microscope Hematoxylin-eosin (HE), PAS, Oil-red, modified Gömöri (mGm), enzyme histochemical dyes (ATP-ase, acid phosphatase, NADH, SDH, and COX) has been used. The other side of the specimens, for evaluation by electron microscopy, has taken under fixation by wax.

OUR RESULTS

DETERMINATION OF MUSCLE COMPARTMENT PRESSURE AND TISSUE OXYGEN SATURATION IN LOWER LIMB

For evaluation of our data, we considered accepted literature's normal values as the base. We considered intracompartment pressure (ICP) of above 40 Hgmm as absolute pathologic; at this stage fasciotomy is certainly indicated. According to Cohn et al (They examined normal tissue perfusion in the thenar region in 707 patients) we considered 87% as normal oxygen saturation level. In 12 patients ICP exceeded the critical pressure of 40 Hgmm. Nevertheless, the complete recanalization in this group, the StO₂ was 50-53 %. At this stage urgent fasciotomy was performed. After fasciotomy continuous monitorization was the next step. Within few hours StO₂ level got close to normal level, but ICP in contempt of significant drop, was not close to the normal parameters even by next day. Experiments did not show urgency for fasciotomy in 4 patients, in these patients clinical manifestation of compartment syndrome was proved.

In this group ICP level was 25-35 Hgmm, at the same time StO₂ was almost within normal borders. Intensive clinical, biochemical and experimental observation applied to all members of this group. Fasciotomy was not performed in this group, within few days' lab results, Compartment pressure and perfusion parameters became normal.

SKELETAL MUSCLE CHANGES DURING ISCHEMIA

Light microscopy has shown myopathic variations in all cases. Mild fiber size changes, sporadically atrophic fibers, predominance of type I. fibers, diminishing of type II/B fibers, and mild patchy proliferation of connective tissue; all has been diagnosed by this technique. In almost all cases presence of fragmented red fibers (RRF) was notable, this shows the pathologic mitochondrial accession. Their number altered between 1-5%. Succin dehydrogenase (SDH) level and Cytochrom oxydase activation in fragmented red fibers were either constant or slightly elevated. COX activation was noticed in all of the cases.

An examination by electron microscope has shown a variation of nonspecific changes. In two milder instances, myofibrils moderate impairment (separated filaments, fragmentation), as well as mild swelling of sarcoplasmic reticulum, subsarcolemmal mitochondria and moderate glycogen proliferation, and intracellular edema was observed. In one of the cases other than extreme fiber impairments, huge amount of injured mitochondria (swelling, fragmented crista system) was noticed, expressly high intracellular edema, lipid and glycogen accession were also present.

NOVEL FINDINGS

1. Our studies have primarily monitored ischemic-reperfusion injuries development and procession in those patients undergone acute peripheral revascularization surgery, acute ischemic cause was either emboly or thrombosis. Our measurements during preoperative period, has proved characteristic changes in prooxidant and antioxidant systems.

We measured the oxidative balance changes for 7 days, postoperatively, in a group of patients undergone revascularization surgery, (ischemic origin of sudden onset), belong to the first 12-24hrs post operative period. We also specifically monitored free radical productions, and decreasing antioxidant defense mechanism during early reperfusion period. Then during late post reperfusion period, free radicals remarkable drop, and continuous elevation in plasma antioxidant enzymes concentration was noticed.

In clinically acute cases, we primarily monitored expression changes, in leukocyte adhesion molecules. We noticed that adhesion molecules expression which is a marker for leukocytes activation, significantly diminished during early reperfusion, and then during next 7 days gradually elevated.

Our results and measures can be useful in building up strategies for treatment, as well as determination of prognosis in high risk patients (long ischemic period, occlusion at higher level, main circulation occlusion, and delicate collateral circulation). In such cases, especially within first 24-to-48 hours, intensive monitorization and observation are required.

2. Second part of our study has focused on vitamin-E antioxidant effect, during reperfusion procedure after lower limb elective revascularization surgery.

For the first time, we could prove that preoperative daily administration of 200mg E-vitamin (from the preoperative day till post operative 7th day), could significantly decrease the "ischemic-reperfusion" injury, oxidative extent, as well as prooxydant-antioxydant balance discord.

According to our experiments, we also could prove as the first, that pre and postoperative administration of vitamin-E in the case of elective revascularization surgeries, could remarkably diminish the leukocytes activation during post operative period, as a result inflammation incidences decreased as well.

3. By the final part of our study, **muscle compartment pressure, and tissue oxygen saturation changes has been monitored, in a group of patients undergone recanalization surgery of lower limb, with high risk of compartment syndrome.**

For the first time we applied two monitoring methods of, noninvasive tissue oxygen saturation and minimally invasive muscle compartment pressure measurements, for surgical diagnosis and treatment of compartment syndrome.

Our experiments show that by elevation of compartment pressure to 40 Hgmm and above that, oxygen saturation of microcirculation will drop below the critical level of 50%, at such stage fasciotomy is certainly indicated to prevent tissue necrosis and following systemic impairments.

In our experiments beside routine therapeutic protocols, **measured parameters (evidence based medicine) play an important role, in diagnosis, surgical indication and therapeutic strategy** of compartment syndrome. Our measurements of pressure and saturation, help in making correct decision regarding conservative or surgical treatment.