INFLAMMATORY RESPONSE AND OXIDATIVE STRESS ASSOCIATED WITH CARDIOPULMONARY BYPASS

PhD Thesis

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INTRODUCTION

Majority of operations in cardiac surgery necessitate the applying of cardiopulmonary bypass (CPB). Despite the advances of CPB in safety and over 50 years of practice it is known to provoke complex cascades of particularly unknown physiological processes. Activation of acute inflammatory response is the most important and versatile effect of CPB resulting from operative trauma, blood exposure to artificial surface, damage of barrier of intestinal mucosa, abnormal blood gas interfaces and reperfusion injury after global ischemia of the heart.

Although more and more contributors of CPB- associated inflammatory response are elucidated, several events of inflammatory processes and its dynamic are unclear yet. Majority of therapeutic measures failed to improve clinical and even experimental outcomes. Better therapeutic strategies are based only on solid understanding of mechanisms involved in CPB-mediated inflammatory cascade.

As an initial step exposure of blood to non-endothelial surfaces activates the complement system. Both alternative and classic pathways are involved in complement activation as a consequence of contact of blood with extracorporeal circuit, heparine and protamin administration and endotoxin release.

Upon complement activation WBC convert to activated state leading to changes in gene expression and function which is pivotal part of inflammatory response to CPB with further important influences.

Responding to CPB polymorhonuclear leucocytes (PMN) release oxidants and proteases and further inflammation- amplifying products resulting in tissue damage. Mononuclear cells, monocytes (MC) and lymphocytes (PBL) also play a pivotal role in regulation of inflammatory processes and the exact change in phenotype of mononuclear cells during CPB is not well determined and appears to be ambiguous.

As a general consequence of CPB, adhesion molecules are presented on WBC leading to interaction between the endothelial cells (EC). Upon expressing adhesion molecules WBC can attach to endothelial cells and transmigrate through vessel wall thereby extending tissue damage

Similarly to leukocytes, platelets exhibit change in shape, presentation of adhesion molecules and activation responding to CPB. Upon platelet activation WBC-platelet conjugates can be formulated leading to intravascular obstruction. Even though, release of intracellular contents can lead to tissue destruction and disturbances in coagulation.

Essential consequence of inflammatory-cell activation is the release of different cytokines. Cytokines might be the most crucial and central contributor of inflammatory processes thus prolonging and enhancing or even blunting the inflammatory reactions. The sensitive balance between different cytokines can be blunted implicating in development of impaired healing processes and complications. Some cytokines in extremely elevated concentration can modulate the function of organs locally and even at distant location from involved organ.

The balance between pro- and anti-inflammatory cytokines is essential to appraise the genuine effect of different cytokines and the characteristics of cytokine network. Temporal change of the balance between pro- and anti-inflammatory cytokines is less investigated.

Evidence suggests that the myocardium is capable of synthesizing biologically active cytokines upon several conditions. The effect of coronary surgery with or without CPB on myocardial cytokine production has not been investigated in detail yet.

One of the most important consequence of the CPB-mediated inflammation is excessive generation of reactive oxygen species (ROS) resulting from activation of inflammatory cells or endogen release from mitochondrion. Oxidative stress, damaging effect of ROS is manifested if generation of free radicals is excessive in relation to antioxidant defenses. Cells evolve a defense mechanism to prevent harmful effect of free radicals as enzymatic or non-enzymatic antioxidants.

The function of poly (ADP-ribose) polymerase (PARP) contributes to oxidative stress -induced DNA repair and maintenance of genomic stability by forming ADP-ribose polymers (PAR). Formation of PAR (ribosilation) also regulates the function of transcription factors and expression of various proteins,

nonetheless, excessive generation of ROS causes overactivation of PARP which consumes NAD+ and ATP leading to cellular death and/or enhancing inflammatory processes.

ROS takes important place in regulation of cellular signal transduction.. Drugs of antioxidant effect act as influencing of signaling pathways of oxidative stress. However signal transduction mechanisms of CPB-associated event has been less studied already.

CPB is often associated with pathophysiological changes involving systemic activation of inflammation together with clinically manifested symptoms. This condition is similar to circumstance which occur in sepsis or shock and is known as systemic inflammatory response syndrome (SIRS). Activation of cellular and humoral cascades of inflammation can be exaggregated in certain cases and its effect at organ level is believed to play a major role in the pathophysiological events leading to organ dysfunction or multiple organ failure (MOF).

A number of different strategies, including new pharmacologic agents, CPB circuits and components, and surgical techniques, have been employed in attempts to minimize inflammatory response and the impact of SIRS and MOF on patients. However, non of these has not allowed the use of a single strategy.

AIMS

The aim of the thesis was to investigate clinical, pathophysiological and biochemical aspects of inflammatory response associated with CPB and accordingly try to find modalities, agents which might be able to diminish the deleterious effects of CPB.

In the first section of this thesis it was aimed to compare the complications, adverse outcomes occur following CPB and in course of OP surgery and what is the incidence of these complications among our study population.

Although many details of inflammatory processes during and after CPB are elucidated, several factors of inflammation have not been investigated yet or contradictions are present. According to our hypothesis patients undergoing open-

heart operation develop an extensive and prolonged pro-inflammatory response, which is not counterbalanced by anti-inflammatory mediators at later period after surgery. Dominancy of pro-inflammatory forces involves several contributors of process and results in systematic oxidative injury, which together may blame for postoperative unwanted events, especially during the first weeks after surgery. Regarding our hypothesis, we tried to clarify the following aspects of inflammatory process in patients undergone operation with or without CPB:

-The balance between pro and anti-inflammatory batteries and temporally changes of this balance during a longer postoperative period.

- The contribution of myocardial tissue in pro-inflammatory processes.

- Release of IL-12 as a marker of cell mediated immunity

- Influence of CPB on the expression of adhesion molecules on leukocytes

- Activation of different subsets of leukocytes as a result of application of CPB or OP surgery.

- Expression of CD97 on different subsets of leukocytes

- The extent and temporal change of oxidative injury during and following CPB or OP technique

- Change in activity of antioxidant enzymes during CABG with or without CPB.

- Activation of PARP following CABG surgery with or without CPB

PATIENTS AND METHODS

For statistical comparison of patient's outcomes receiving operation with CPB or OP technique pre, peri, and postoperative outcomes of 50 patients (25 operated wit CPB and 25 with OP) were analyzed retrospectively, at random. Furthermore 30 subjects were randomized in the study receiving elective CABG. 20 of the patients were operated with conventional method using CPB (CPB group), while 10 patients underwent operation with OP technique (OP group).

Pre- and postoperative data

Age, gender, severity of coronary artery, classification of angina, prior surgery or percutan coronary intervention, existence and type of diabetes, preoperative risk score, preoperative ejection fraction (EF) (%).

Data of operation number of grafts, arterial grafts, time of operation were compared. Postoperative blood loss, transfusion requirements, the time of ventilatory support, length of intensive care unit stay and hospitalization, were documented furthermore ejection fraction on the sixth postoperative day was calculated in both groups.

Blood sampling

Blood samples from peripheral vein were taken just after the induction of anaesthesia and 5, 30 minutes after the beginning of reperfusion, and on the 1st, 2nd, 3rd, and 7th postoperative days. Further blood samples were taken from coronary sinus (CS) using a catheter in both groups 5 minutes after reperfusion.

Measurement of cytokines

The plasma concentrations of cytokines were determined using cytometric bead array (CBA Human Inflammatory Kit) (TNF, IL-1, IL-6, IL-8, IL-10, IL-12) Our outcomes were corrected to cell count thus expressing the final values in pg ml^{-1} per 10^6 cells.

. The cytokine levels of CS samples were compared to their corresponding peripheral vein levels (100%) thus expressing the values in percentage.

Assessment of CD 97 and adhesion molecule expression

The samples were incubated with FITC- labeled monoclonal antibodies against CD 97, CD11a and CD18 molecules for 15 minutes. The samples were measured by BD FacsCalibur flowcytometer.

Measurement of reactive oxygen species producing capacity

Respiratory burst of leukocytes was assessed by measuring the amount of reactive oxygen species in whole blood via modified chemiluminescense (CL) method based upon the reaction of luminol with free radicals. Following blood sample collection, leukocytes were stained with cerium-chloride and propidium iodide for nuclear staining and validation was performed by confocal laser microscopy.

Determination of lipid peroxidation, activation of antioxidant enzymes and reduced glutatione

Malondialdehyde (MDA) concentration and the activity of superoxide dismutase (SOD) and catalase (CAT) were analyzed from haemolysates by colorimetric, original assays. Moreover the level of reduced glutatione (GSH) level was quantified from haemolyzates by commercially available assay kit

Determination of PAR polymers

The mononuclear cells were separated using Ficoll-Paque. Intracellular staining was carried out using commercially available primer and secunder antibodies and staining . was quantified by flow cytometric measurement.

Statistical analysis

The data are presented on tables and figures as mean ± standard error of mean (SEM).

The data between the two groups were compared with unpaired Student's t test. In a given group comparisons between control data were made using paired Student's t test.

RESULTS

Intubation-time was significantly higher in CPB group related to OP group. In addition the postoperative blood loss of CPB group through the chest drain exceeded the level of OP group markedly however this difference was not proved statistically significant. 18 patients in CPB group and 11 in OP group required blood transfusion, and the quantities of transfusion was significantly higher in CPB group. Postoperative EF of patients operated with CPB decreased significantly related to preoperative values. Troponin I and CK-MB levels of CPB group were many fold higher compared with OP group in all time points

Citokine levels

The balance between inflammatory and anti-inflammatory forces was determined by calculating pro-inflammatory cytokine/IL-10 ratio. All ratios were similar in the CPB and OP group. In CPB group, an early drop was observed during surgery and

afterwards the ratio increased extremely throughout the observation period. In OP group, the ratio of given pro-inflammatory cytokine and IL-10 tended to decrease reaching it's minimum value on the 1st or 2nd postoperative day thereafter it normalised gradually.

In CPB group, all of observed cytokines from CS exceeded the concentrations of peripheral vein samples. The difference between sinus and peripheral vein samples was proved to be significant for the IL-1, IL-6, IL-8 and TNF levels. During OP surgery the cytokine concentrations of the CS and peripheral vein were roughly equivalent

Appearance of CD97 on granulocytes and monocytes

Biphasic modification in surface expression of CD97 was noted on myeloid cells (PMN and MC) in CPB group. First, an intensive drop was present in CD97 activity of PMN in course of CPB. On the first day, the CD97 level of PMN was close to the control value and subsequently it started to rise considerably, reaching its maximum 3 days after operation. In OP group the PMN CD97 consistency showed marked decrease in the ischaemic period. It finally remained around the control value.

In CPB group, the rate of active CD97 positive PBL tended to rise gradually and markedly from the beginning of reperfusion to the 3rd postoperative days when 30,12±5,86% was determined to CD97 positive (preoperative value: 8,3±1.56%).

In OP group, the percentage of active CD97 positive PBL showed a peak during operation at 30 minutes of reperfusion and it was also slightly elevated on the 1st postoperative day (10,44±1,97% versus control value of 7.9±0,65%).

The integrin (CD11a, CD18) levels on the surface of PMN tended to decrease in the early phase of reperfusion and afterwards they increased. The expression of both integrins was markedly higher on granulocytes of CPB group than of OP group, especially on the 2nd postoperative day. Expression of CD11a and CD18 on monocytes were similar. Lymphocytes increased their expression of integrins gradually with maximum on the 2nd postoperative day in CPB group and on the 3rd day in OP group

Production of reactive oxygen species

CPB induced extreme increase in ROS generating capacity of leukocytes. It elevated gradually during the intervention, 30 minutes after cessation of aorta cross-clamping peaking on the 2nd postoperative days. OP operation was also associated with considerable elevation in ROS generation but it was definitely lower than in CPB group. Qualitative imaging of Ce-deposits, showed similar results.

Level of malondialdehyde

MDA level of CPB group peaked 30 minutes after beginning of reperfusion and it remained highly elevated on the 1st, 2nd and 3rd POD. However, only a slight elevation can be documented in OP group peaking on the 2nd POD.

Change in activity of antioxidant enzymes and level of reduced glutation

Contrasting alteration in SOD activity can be noted in two groups. In CPB group it decreased continuously reaching its nadir on the 2nd POD and it normalized to the 7th POD. With regard the OP group SOD activity rose gradually during the operation while on the 1st POD it was closed to control thereafter it increased again.

Less expressed change can be observed in CAT activity in both groups.

GSH level decreased markedly soon after beginning of ischaemia in both groups. Although in CPB group GSH levels remained decreased until the end of the first postoperative week the values of OP group tend to recover to the 1st POD.

Activation of PARP enzyme

The binding of PAR specific antibodies, which is indicative of PARP activation, was markedly increased in patient operated with open-heart surgery from the early reperfusion to the 2nd POD. OP technique was able to abolish the increased activation of PARP. Considering OP patients, appearance of PAR did not differ from control level during the whole observation period.

SUMMARY

- *Among our study population*, the intensification of blood loss, increased need of blood transfusion, lengthened hospitalization and ventilation time are associated with CPB.

- Despite the early elevation of pro-inflammatory cytokines are counterbalanced by anti-inflammatory forces, prolonged and considerable pro-inflammatory processes are present during days following application of CPB.
- Moreover these findings can refer to timing of anti-inflammatory therapy after open-heart surgery.
- It was demonstrated that myocardial outflow of pro-inflammatory cytokines occur during CPB and it is less expressed during off-pump technique. Thus jeopardized myocardial tissue can contribute to inflammatory processes and amplify local inflammatory insults.
- It was proved for the first time that off-pump surgery can decrease IL-12, thereby decreasing the contribution of cellular immune response.
- Novel to this work was the demonstration of CD97 activation on leukocytes after CPB representing activation state of white blood cells. Adhesion molecules are also presented markedly in course of CPB.
- We were able to demonstrate that activated leukocytes can exert condition of oxidative stress as a result of CPB. Oxidative injury remains significant over the postoperative days following CPB. Off-pump surgery is associated with more moderate oxidative processes.
- Exhaustible oxidative effects after CPB provoke decreased activity of antioxidant enzymes.
- Our biochemical measurements provided evidence for the first time that marked oxidative injury led to systemic PARP activation in patients receiving open-heart surgery. Off-pump surgery was able to reduce manifest activation of PARP enzyme.