Endothelial and systemic effects of percutaneous coronary intervention

Determination of circulating endothelial cells in ischaemic heart disease

Ph.D. Thesis

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Budapest
2010

Introduction

The endothelium represents a metabolically active tissue with several important functions – during physiological conditions it has antithrombotic, anticoagulant and fibrinolytic properties, moreover, producing mediators with vasoconstrictive/ or vasodilatative effect it regulates the vascular tone. Previous data confirmed, that cardiovascular risk factors may induce endothelial dysfunction/ damage and promote atherosclerotic process manifesting later as stable angina pectoris, or acute coronary syndrome.

In patients with ischaemic heart disease, the recanalisation of stenotic, or occluded coronary artery is crucial for the prevention of further ischaemic myocardial damage. Over the last two decades, invasive coronary revascularization therapy has been referred as an important therapeutic strategy in patients with ischaemic heart disease. Several trials have shown, that percutaneous coronary intervention (PCI) with implantation of coronary stents effectively restored coronary artery patency, decreased the need of coronary revascularization and improved clinical outcome. However, beside the clinical benefits of PCI, few data indicated leukocyte and platelet activation at the stented coronary area and systemic inflammatory response was recognized in patients undergoing invasive coronary intervention. Since data regarding the oxidative stress response, and the extent of PCI induced direct/or indirect endothelial damage are still lacking, further examination is required.

Early impairment of endothelial function documented in presence of several conventional risk factors, such as obesity, hypertension, hyperlipidaemia, diabetes mellitus, and smoking - might be characterized by loss of endothelial integrity and endothelial cell detachment

demonstrating as increased circulating endothelial cell count (CEC) in peripheral blood. Since the detached endothelial cells circulate in blood of healthy individuals with very low frequency and the cell number often increases in conditions associated with vascular damage, the CEC count can serve as a sensitive marker of endothelial injury.

Aims of the work

The main aim of the series of investigations was to assess the endothelial effects of PCI and to evaluate the grade of systemic oxidative stress response and the reperfusion injury following invasive coronary intervention. Our further aim was to study the endothelial effects of the known cardiovascular risk factors as well as to study the effects the cardiovascular pharmacotherapy.

Our goals were the following:

1. Endothelial injury can be characterized by elevated number of endothelial cells circulating in peripheral blood. Since, we aimed to assess the level of endothelial injury using circulating endothelial cells count as a direct marker, at first we had to standardize a specific method for the isolation of endothelial cells from the peripheral blood.

2. Several data indicated inflammatory response after invasive coronary intervention. Since, it may cause endothelial damage *via* mechanical manipulation, or by radiocontrast material, we wished to examine the level of endothelial injury after coronarography/ PCI in ischaemic heart disease patients.

3. As proved by earlier data, the cardiovascular risk factors may induce endothelial dysfunction/ and damage resulting in development of atherosclerotic plaque. In our further studies we aimed to evaluate the endothelial effects of the known cardiovascular risk factors and the complex cardiovascular pharmacotherapy in patients with stable form of ischaemic heart disease. Circulating endothelial cell count, and other endothelial and inflammatory markers were determined to investigate the correlation between the soluble factors and cellular endothelial damage.

4. The aim of the clinicians is to reperfuse the ischemic myocardium as soon as possible, in order to avoid further irreversible myocardial damage. Paradoxically, reperfusion may trigger tissue damage resulting in a spectrum of reperfusion-associated pathologies, collectively called "reperfusion injury". Since only limited human data are available concerning the effect of invasive coronary revascularization therapy, our aim was to assess the level of oxidative stress and reperfusion DNA injury in patients with acute myocardial infarction undergoing percutaneous coronary intervention.

Three separate clinical studies were conducted to answer the above questions.

Patients and Methods

1. Patient population

1.1. Endothelial effects of percutaneous coronary intervention performed in ischaemic heart disease

In the first study we recruited 74 cardiovascular patients undergoing coronarography/ or invasive coronary intervention. The enrolled patients were divided into three groups:

I. Acute ST- elevation myocardial infarction (STEMI)

Twenty-eight patients, admitted with the diagnosis of acute STEMI based on the presence of permanent chest pain (> 30 min), ST-segment elevation on ECG (≥ 2mm) and/or elevation of cardiac specific enzymes (troponin, CK-MB) were enrolled. Beside the standard therapy consisting of aspirin, clopidogrel, heparin, GPIIbIIIa receptor blocking agent, beta-blocker and statin, all STEMI patients underwent urgent coronarography and PCI with implantation of bare metal stents (BMS); in all cases, the culprit coronary artery was successfully recanalized.

II. Stable angina group with elective PCI

In this group, 23 patients with stable angina pectoris and with significant (> 70 % of diameter) coronary stenosis were enrolled. In each patient successful PCI (with implantation of bare metal stent) was performed in order to recanalize the stenotic coronary artery.

III. Stable angina group with coronarography only

To validate, whether the endothelium is also affected by the coronarography (intracoronary catheter manipulation, contrast material injection without balloon inflation and stent implantation), 23 stable angina patients undergoing electively scheduled coronary angiography without

PCI were investigated. In this patient group, coronarography revealed either three-vessel disease or non-significant coronary artery stenosis. Patients with negative coronarography were excluded.

In each patient, CEC count, vWF and sICAM-1 plasma levels were consecutively determined on admission, and 30 minutes, 24 hours and 96 hours after the coronary intervention. In patients with coronarography only, the mentioned parameters were serially measured on admission, 30 minutes and 24 hours thereafter.

1.2. Endothelial effect of cardiovascular risk factors and the effects of cardiovascular pharmacotherapy

In the second study, we recruited 80 stable angina patients with angiographically proven (at least one significant coronary artery stenosis on angiogram) coronary artery disease.

In each patient, CEC count and several endothelial (CD40L, E-selectin) and inflammatory (CRP, IL-6) plasma/ serum markers were determined. Clinical history, data regarding prior PCI, prior acute coronary syndrome, concomitant diseases and recent medication were also recorded.

As controls, 18 healthy volunteers (11 women, 7 men, mean age: 34,4 years) were enrolled.

In both studies, the exclusion criteria were history of severe renal, or hepatic disease, autoimmune disease and malignancy, in patients with ST-elevation myocardial infarction the cardiogenic shock,and the use of intraaortic balloonpump. The study protocol was approved by the institutional and regional ethics review committee and before enrolment written informed consent was obtained from all participants.

1.3. Oxidative stress and reperfusion injury following percutaneous coronary intervention

In this study, we examined 30 cardiovascular patient referred to our institution in order to perform coronary angiography or percutaneous coronary intervention. The patients were divided into three groups:

I. Acute ST- elevation myocardial infarction (STEMI)

Fifteen patients were enrolled with the diagnosis of acute STEMI based on the presence of permanent chest pain (>30min), ST-segment elevation on ECG (≥2mm) and/or elevation of cardiac specific biomarkers. In all STEMI patients urgent PCI with implantation of bare metal stents (BMS) was performed.

II. Stable angina pectoris group with elective PCI

Nine patients with stable coronary artery disease were enrolled. In these patients, PCI (with implantation of BMS) was performed to recanalize the stenotic artery.

III. Stable angina group with coronarography only

To validate the effect of coronarography only, 6 angina patients undergoing electively scheduled coronary angiography without PCI were investigated. Patients with negative coronarography were excluded.

In STEMI group, the plasma hydrogenperoxide level (as a marker of oxidative stress) and 8 – hydroxydeoxyguanosine (marker of oxidative DNA damage) was determine on admission, 15 minutes, 24 and 96 hours after the coronary intervention; in the II. a III. patient group the above markers were determined on admission, and 15 minutes after the elective PCI/ or coronarography.

2. Laboratory tests

2.1. Isolation of circulating endothelial cells from whole blood and the verification of endothelial origin by immunohistochemistry

The endothelial cells were isolated from cubital venous blood drawn in EDTA using the immunomagnetic method. Briefly, the mouse anti-human CD146 antibody (S-Endo1) (Biocytex, France) was incubated with immunomagnetic beads (4.5 μm in diameter) (Dynal, Norway) coated with anti – human IgG antibody for 1 hour at room temperature. After careful washing using magnetic device (Dynal, Norway), 100 μl of anti-human CD146 - coated immunomagnetic beads were added to venous blood and incubated for 1 hour. After repeated washing using magnetic device the isolated CD 146 + cells were resuspended in 0,1 % BSA-PSA and ready for further procedure.

The isolated CD 146 positive cells were fixed with 4 % paraformaldehyd, washed and permeabilized by 0.2 % Triton X. After careful washing (using a magnetic device) the isolated cells were stained using primary rabbit anti-human von Willebrand factor antibody (DAKO, Belgium) and the secondary anti – rabbit Alexa Fluor 466 (Invitrogen, USA) antibody. The stained cells were visualized under fluorescence microscope (Nikon, USA).

2.2. Determination of CEC count

In order to assess CEC count, 8 ml of venous blood was drawn from the cubital vein in EDTA, first 4 ml were discarded. CECs were isolated with the above described immunomagnetic method (immunomagnetic beads, anti-human CD146 antibody). The isolated CECs were resuspended in 0,1% BSA-PBS and stained with 5 μg/ml acridine orange (Sigma, USA). Cells were analyzed in Nageotte chamber under fluorescent microscope by single observer. Only matured endothelial cells with typical morphology and more than 10 immunomagnetic beads on their surface with 20-50 μm in diameter were analyzed.

2.3. Determination of plasma/ serum markers using ELISA

Plasma hydrogenperoxide, von Willebrand factor, soluble ICAM-1, CD 40 L and interleukin - 6 levels were determined from platelet- poor plasma, 8OHdG, E- selectin and hsCRP levels were determined from serum using ELISA method.

3. Statistical analysis

Baseline characteristics are listed as mean ± standard deviation (SD) for continuous variables and as counts (and percentage) for categorical variables. Results (CEC count, and plasma/serum marker levels determined by ELISA) are listed as medians (M) with interquartile ranges (IQR), or as mean and SEM (standard error of the mean). Differences between independent variables were analyzed by chi-square test, Mann-Whitney, or by Kruskal-Wallis test, as appropriate (values are considered as significant at $p < 0.05$). Friedman's repeated measures (two-way) ANOVA was used to analyze the differences between multiple dependent variables (values are considered as significant at $p < 0.05$). Correlation between the variables was analyzed using Spearmann correlation analysis (the values are significant at $p<0.05$). Regression analysis was performed to analyze the relationship between quantitative and qualitative parameters. All tests were performed using Statistics 7.0 software (Stat Soft. Inc., Tulsa, USA).

Results

1. We standardized the specific immunomagnetic method for isolation of endothelial cells from whole blood.

2. Endothelial effects of percutaneous coronary intervention performed in ischaemic heart disease

Baseline CEC count, vWF and sICAM-1 levels in stable or acute form of ischaemic heart disease

a.) We found significantly higher CEC count in patients with stable angina compared to healthy individuals indicating the presence of systemic endothelial injury. Elevated CEC count in stable angina patients might resulting from endothelial damage caused by cardiovascular risk factors; however, further examination is required.

b.) We found significantly higher baseline CEC count in acute ST-elevation myocardial infarction compared to stable angina patients. Our results indicate the presence of manifest endothelial damage during acute myocardial ischaemia. We suppose, that beside the local endothelial damage caused by coronary plaque rupture, myocardial ischaemia – related systemic endothelial injury might be responsible for the increased CEC count.

c.) Beside the significantly elevated CEC count, we found significantly higher plasma vWF levels in acute ST- elevation myocardial infarction compared to stable angina patients. These results confirm the presence of explicit endothelial injury during acute myocardial ischaemia.

d.) No significant difference in baseline sICAM-1 plasma levels was found between the stable and the acute form of ischaemic heart disease. Consequently, the sICAM-1 plasma level serves rather as a marker of chronic vascular activation and atherosclerotic process than an early marker of endothelial dysfunction during acute myocardial ischaemia.

Kinetics of endothelial markers following percutaneous coronary intervention performed in stable angina

a.) In our study, nor the coronarography, neither the elective PCI caused significant CEC count elevation. Moreover, the circulating endothelial cell count was comparable in stable angina patients undergoing PCI or coronarography alone. In contrast to previous concepts, our results indicate that elective PCI itself (similarly to coronary angiography) performed in stable form of ischaemic heart disease does not cause explicit endothelial damage.

b.) In our study - similarly to CEC count - the plasma vWF did not show significant elevation after elective PCI/ or coronarography performed in stable angina patients.

c.) Interestingly, non-significant decrease of sICAM-1 plasma levels was observed immediately after coronarography/ and elective PCI. We suppose, that this observation might be caused by the consumption of ICAM-1 molecule.

In summary, our results suggest, that percutaneous coronary intervention (similarly to coronarography) itself has no major endothelial effect under stable conditions.

Kinetics of endothelial markers following percutaneous coronary intervention performed in acute myocardial infarction

a.) Analyzing patients with acute ST- elevation myocardial infarction, we found pronounced increase in CEC count after percutaneous coronary intervention with a significant maximum at 24 hours. Decrease in CEC count was observed at 96 hours after the coronary intervention.

b.) In STEMI patients we found elevated vWF plasma levels at 24 and 96 hours after the PCI; interestingly, immediately after the percutaneous coronary intervention decrease in vWF plasma levels was detected. Platelet activation, subsequent thrombus formation at the site of the direct endothelial damage and plaque rupture might cause rapid consumption of soluble vWF. Nevertheless, this observation might be a coincidence and needs further confirmation.

c.) The kinetics of soluble ICAM-1 after percutaneous coronary intervention performed in STEMI showed similar kinetics to those in stable angina. These observation supports the assumption, that nor PCI, nor myocardial ischemia/ reperfusion have an evident acute effect on sICAM-1 plasma levels.

Based on our results, during percutaneous coronary intervention performed in conditions of manifest myocardial ischaemia more extensive endothelial damage occurs. We assume, that beside the effect of percutaneous coronary intervention, the local damage of the endothelium caused by coronary plaque rupture, and the myocardial ischemia and reperfusion – related systemic endothelial injury might be responsible for the explicit CEC count

elevation. This assumption might be supported by the facts, that the baseline CEC count in STEMI patients was significantly elevated already on admission and showed further increase with maximum at 24 hours after the recanalisation.

Correlation between the extent of endothelial injury and myocardial necrosis

We found positive correlation between baseline CKMB values and CEC count at 24h in STEMI demonstrating that pronounced myocardial necrosis at admission is accompanied by explicit endothelial damage. These observations might possess clinical significance, since – besides achieving coronary artery patency via PCI in STEMI – protection of the ischemic myocardium/ endothelium have gain importance.

3. Endothelial effects of cardiovascular risk factors and cardiovascular pharmacotherapy

In stable angina patients on clopidogrel therapy we found significantly lower CEC count, suggesting possible endothelial protective effect of the $P2Y_{12}$ ADP receptor inhibitor. Further prospective studies have to be performed to elucidate the underlying mechanism and its impact in human atherosclerosis.

4. Oxidative stress and reperfusion injury after percutaneous coronary intervention

a.) We found, that in STEMI patients undergoing PCI the total hydrogen peroxide concentrations were not affected by coronary reperfusion directly; the hydrogen peroxide values determined immediately after PCI did not differ statistically from pre - PCI values. However, a significant increase of plasma hydrogen peroxide levels was observed at 24 and at 96 hours. These results might be explained by the presence of oxidative / antioxidant imbalance caused by the exhaustion of "scavanger" capacity. Nevertheless, further studies are required to prove this assumption.

b.) In patient with STEMI we observed rapid significant 8OHdG serum level increase immediately after successful percutaneous coronary intervention; at 24 and 96 hours decrease in 8OHdG levels was detected. Our results provide the first clinical evidence of reperfusion DNA injury present in patients with myocardial infarction undergoing PCI and are consistent with the concept that local myocardial ischaemia/ and reperfusion triggers systemic responses in humans. The prognostic value of 8OHdG deserves further investigation.

Conclusions

1.) Significantly higher CEC count found in patients with stable angina compared to healthy individuals indicates the presence of systemic endothelial injury.

2.) Significantly higher baseline CEC count and plasma vWF levels observed in patients with acute ST-elevation myocardial infarction compared to stable angina patients prove the presence of explicit endothelial damage during acute myocardial ischaemia. Comparable baseline sICAM-1 levels in stable and acute form of ischaemic heart disease demonstrate, that the sICAM-1 plasma level serves rather as a marker of chronic vascular activation and atherosclerotic process than an early marker of endothelial dysfunction during acute myocardial ischaemia.

3.) Neither coronarography, nor elective PCI performed in stable angina patients cause significant CEC count, vWF, or sICAM-1 elevation. Consequently, elective PCI itself (similarly to coronary angiography) performed in stable form of ischaemic heart disease does not cause explicit endothelial damage.

4.) In patients with acute ST- elevation myocardial infarction, pronounced increase in CEC count can be observed after percutaneous coronary intervention with a significant maximum at 24 hours. Based on the results, during percutaneous coronary intervention performed in conditions of manifest myocardial ischaemia more extensive endothelial damage occurs.

5.) In STEMI patients elevated vWF plasma levels can be found at 24 and at 96 hours after the PCI; interestingly, immediately after the percutaneous coronary intervention decrease in vWF plasma levels can be detected.

6.) The kinetics of soluble ICAM-1 after percutaneous coronary intervention performed in STEMI is similar to those in stable angina

patients. Neither the PCI, nor the myocardial ischemia/ and reperfusion have an evident acute effect on sICAM-1 plasma levels.

7.) Positive correlation between baseline CKMB values and CEC count at 24 hours observed in STEMI demonstrates, that pronounced myocardial necrosis is accompanied by explicit endothelial damage.

8.) In stable angina patients on clopidogrel therapy significantly lower CEC count can be observed, suggesting possible endothelial protective effect of the $P2Y_{12}$ ADP receptor inhibitor.

9.) In STEMI patients undergoing PCI the total hydrogen peroxide concentrations are not rapidly affected by coronary reperfusion. Significant increase of plasma hydrogen - peroxide levels can be observed at 24 and at 96 hours. Conversely, rapid significant 8OHdG serum level increase can be detected immediately after successful percutaneous coronary intervention providing the first clinical evidence of reperfusion DNA injury present in patients with myocardial infarction undergoing PCI.

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