Endothelin-1 is involved in plasma mediated stimulation of neutrophil adherence during coronary artery bypass grafting

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Abstract

Objective: Myocardial ischaemia followed by reperfusion during coronary artery bypass grafting (CABG) is known to result in the activation of polymorphonuclear neutrophils (PMN). The activation of PMN during ischaemia/reperfusion may be a result of their direct contact with activated endothelial cells and/or an effect of stimuli released from ischaemic myocardium. Increased expression of adhesion molecules on the PMN surface, after activation, leads to coronary capillary plugging with a subsequent decrease in blood flow. The purpose of the study was to evaluate plasma-mediated stimulation of PMN adhesion during CABG and to verify if endothelin-1 (ET-1), known to be a potent stimulus for PMN, is involved in stimulation of neutrophils adhesion mediated by integrins.

Methods: Coronary sinus, peripheral artery and peripheral venous plasma samples were taken from 11 patients undergoing coronary surgery before aortal cross-clamping, at the beginning of reperfusion and 30 min thereafter. PMN isolated from five healthy volunteers were incubated with the plasma (20 samples per patient) in the presence of saline or a specific ET-1 receptor blocker, and PMN adherence to a microtiter plate covered with a monoclonal antibody against CD 18 antigen (β-subunit of the integrin family of adhesion molecules) was evaluated.

Results: We have observed a significant increase in adhesion of PMN incubated with the plasma taken from coronary sinus at the beginning of reperfusion (7.79 ± 1.64% of adhering cells) as compared with plasma obtained before aortal cross-clamping from the same place (6.78 ± 1.3%, P = 0.04) and from peripheral artery at the beginning of reperfusion (6.64 ± 1.1%, P = 0.04, means ± SEM). ET-1 receptor blocker, significantly decreased stimulation of PMN adhesion by coronary sinus plasma obtained at the beginning of reperfusion (6.7 ± 1.51%, P = 0.02). Plasma levels of ET-1 (ELISA) in the samples taken from coronary sinus at the beginning of reperfusion, were higher than in samples obtained before myocardial ischaemia or 30 min after reperfusion.

Conclusions: We conclude, that soluble stimuli capable of stimulation of PMN adhesion are released following myocardial ischaemia during CABG and ET-1 may be involved in PMN stimulation. © 1999 Elsevier Science B.V. All rights reserved.

Keywords: Coronary artery bypass; Endothelin; Neutrophil

1. Introduction

The mortality in patients undergoing coronary artery bypass surgery (CABG) is known to be related to ischaemic myocardial injury. It is well documented that the major role in this process is played by polymorphonuclear neutrophils (PMN). Several investigators have shown that myocardial ischaemia/reperfusion injury may be reduced by neutrophil depletion [1]. Neutrophil-induced damage of myocardium involves several mechanisms: (a), aggregation and adhesion to endothelial cells with subsequent coronary capillary plugging [2] (b), the release of cytotoxic compounds [2,3] and (c), vasoconstriction resulted from B4 leukotriene release [4].

Activation of neutrophils results in the expression of surface adhesion molecules on PMN. These molecules belong to one of two distinct families: selectins and β-integrins [5,6]. β-integrins consists of three heterodimers that share a common β-subunit (CD 18) and differ with respect to their
α-subunit CD11a, CD11b or CD11c. They are stored in secretory granules in neutrophils and can be rapidly mobilized to the cell surface as a result of cell activation. β2-integrins recognize their receptors on the surface of endothelial cells including ICAM-1 (intercellular adhesion molecule). Clinical and experimental studies have suggested that β2-integrins are upregulated during cardiopulmonary bypass (CPB) [7].

The increased adhesion molecule expression may be involved in the development of neutrophil mediated myocardial tissue damage during CPB.

Ischaemia followed by reperfusion during the cardiac surgery initiates a cascade of events leading to neutrophil activation. Intravascular neutrophil activation during myocardial ischaemia/reperfusion may occur as a result of a direct neutrophil contact with endothelial adhesion molecules expressed within ischaemic heart. This process may also be a result of an influence of certain stimuli released from ischaemic myocardium to the peripheral blood. It was previously shown that peripheral venous plasma obtained from patients with myocardial infarction can induce chemotaxis, adherence and superoxide anion production by neutrophils obtained from healthy donors [8,9]. Previous studies have found that several potent neutrophil-oriented stimuli, including the complement cascade, interleukines, platelet activating factor (PAF) and tumour necrosis factor α (TNFα) are released from ischaemic myocardium during ischaemia/reperfusion [1].

It has been shown that endothelin-1 (ET-1), a potent vasoconstricting peptide, may act as a stimulus for PMN in an inflammatory focus [10]. Since ET-1 is released during cardiac surgery it may be involved in PMN activation following CABG.

The aim of this study was to evaluate the plasma mediated activation of neutrophil adhesion during CABG grafting, as well as to verify the possible involvement of ET-1, a potent stimulus for PMN, in this process.

2. Materials and methods

The study group consisted of 11 patients undergoing CABG. The group included two women and nine men aged 37–65 years (mean age 51). Patients with diabetes mellitus, renal failure, neoplastic conditions and fresh myocardial infarction were excluded. Six patients from the study group had suffered from previous myocardial infarction. Ejection fraction estimated with echocardiography ranged from 32 to 68% (mean 50%). The operative procedure was performed using CPB at moderate hypothermia (30–32°C). During bypass hematocrit was maintained between 20 and 25%, pump flow was maintained between 2 and 2.2 l/min per m². Cardiac arrest was induced by administration of cold high-potassium cardioplegic solution and maintained by the delivery of additional doses in 15–20 min intervals. After graft implantation, the patients were rewarmed to 36°C and separated from extra-corporeal circulation (ECC) by the gradual reduction of venous return to the bypass circuit. Venous drainage was by a two-stage cannula and an internal mammary artery was implanted as the last anastomoses. In all procedures Stockert roller pump was used for CPB in each patient. The bypass circuit period ranged from 83 to 168 min (mean 115 min), complete ischaemia (aortal clamping) 34 to 84 min. (mean 51 min). Reperfusion-period from aortal clamp release to bypass circuit stopping was 41 to 87 min (mean 57 min).

Blood samples were collected before extra-corporeal circulation installation from peripheral vein (V₀). Other plasma samples were taken in each patient from the peripheral vein (V), peripheral artery (A) and coronary sinus (CS) before myocardial ischaemia (aortal clamping) (V₁, A₁, CS₁), at the beginning of reperfusion (aortal clamp release) (V₂, A₂, CS₂) and 30 min after reperfusion (V₃, A₃, CS₃). The samples were centrifuged immediately and plasma was frozen (~7°C) until analysis. Patients’ plasma samples (20 per patient) were incubated with isolated PMN obtained from five healthy volunteers in the presence of saline or a specific ET-1 receptor (ET₄) antagonists cyclo-Asp-Pro-Val-Leu-Trp (Calbiochem) in a final concentration of 10 pg/ml (V, A and CS₁–₃b, respectively).

Peripheral blood neutrophils were isolated from blood samples obtained from five healthy volunteers. PMN were isolated by a single step centrifugation procedure on Gradi-sol G (Polfa) gradient which is a modification of the method described by Bøyum [11]. Neutrophils obtained after centrifugation were washed twice with 0.9% NaCl and then suspended in 10 ml of saline. Red blood cells were lyzed for 5 min with haemolytic buffer (0.828 g NH₄Cl and 0.1 g KHCO₃ dissolved in 100 ml distilled water with 20 μl 1N NaOH). The residual PMN were resuspended in Hank’s solution (Sigma).

The PMN adhesion measurements were performed using a microtiter-plate-based spectrophotometer (Multiscan MS, Labsystems). The plastic plates were coated with a monoclonal antibody (MAB) against CD18 antigen (Becton Dickinson). Anti-CD18 MAB (50 μl) per well in a concentration of 0.2 pg/ml was placed on the plate and incubated for 24 h at 4°C. Fifty microlitres of investigated plasma and 50 μl of cell suspension (1 × 10⁶ PMN/ml) was added into each well. The plates were incubated for 15 min in 37°C and washed three times with 0.9 NaCl. Residual granulocytes were lysed in 50 μl 0.5% hexadecyltrimethylammonium bromide (HATB) and incubated for 10 min in 37°C. After that 40 μl of 0.32% o-dianizin and 200 μl of 30% H₂O₂ were added to each well. The number of granulocytes adhering to the plate covered with anti-CD18 MAB was evaluated by the measurement of myeloperoxidase concentration produced by PMN. Light emission was measured at 450 nm wave length.

The ET-1 concentration was measured with R and D Systems’ ET-1 assay utilizing a sandwich immunoassay technique for the quantitation of ET-1 in extracted samples.
This involves two antibodies against different epitopes of the ET-1 molecules. Any present ET-1 molecules form a bridge between the two antibodies. The amount of conjugate bound to the well (and thus ET-1) is detected by reaction with substrate specific for the enzyme which yields a coloured product that is quantified photometrically.

Non-parametric statistics were used because our data were not normally distributed, as assessed by the Kolmogorov–Smirnov test. Evaluation was performed using the Friedman test and Dunn’s Post-test for comparisons within study group (patients’ plasma taken in different time points) and Wilcoxon signed rank test for comparison of saline and ET_A blocker treated PMN. Results are expressed as the percentage of adhering PMN ± SEM. The study protocol was accepted by local Ethics Committee.

3. Results

We have found a significant ($P = 0.04$) increase of PMN adhesion in samples incubated with plasma taken from coronary sinus at the beginning of reperfusion (CS_1 = 7.79 ± 1.6%) compared with samples incubated with plasma obtained before aortal clamping (CS_1 = 6.78 ± 1.3%), and in 30 min of reperfusion (CS_1 = 5.83 ± 0.85%) from the same place (Fig. 1). There was also a significant ($P = 0.04$) increase in CS_2 samples compared with samples incubated with plasma taken from the peripheral artery (A_2 = 6.64 ± 1.1%) and vein (V_2 = 6.65 ± 1.17%) at the beginning of reperfusion. PMN adhesion in the samples incubated in the presence of ET_A blocker with plasma taken from coronary sinus at the beginning of reperfusion (CS_2b = 6.7 ± 1.51%) was significantly lower ($P = 0.02$) than in samples incubated with saline in the same plasma (CS_2) (Fig. 1).

We have also found that neutrophil adhesion, stimulated by plasma obtained from peripheral vein taken before aortal cross-clamping (V_1 = 7.29 ± 2.8%), was significantly higher ($P = 0.04$) than adhesion stimulated by plasma samples obtained 30 min after the start of reperfusion (V_3 = 6.06 ± 0.91%) from the same vein. The PMN adhesion induced by plasma samples obtained from peripheral arteries before aortal clamping (A_1 = 7.05 ± 1.25%) was significantly higher ($P = 0.04$) than those induced by samples obtained after 30 min of reperfusion from that same place (A_3 = 5.76 ± 0.81%).

In the current study we have measured ET-1 levels in plasma samples used for neutrophil stimulation. Coronary sinus ET-1 concentration was 1.57 pg/ml, at the beginning of reperfusion and 0.93 pg/ml after 30 min of reperfusion. In arterial blood samples the concentrations were 0.73 and 0.47 pg/ml, respectively.

4. Discussion

It was previously confirmed that the β_2-integrin family is involved in increased neutrophil adherence to endothelium during myocardial ischaemia. Increased expression of CD 11b/CD 18 complex was shown in neutrophils taken from the coronary sinus of patients with unstable angina compared with neutrophils taken from the aorta, indicating a transcardiac increase of neutrophil adherence molecules as a result of myocardial ischaemia [14]. An increased level of CD 18 was found in patients with a brief period of ischaemia during coronary angioplasty [17]. In experimental infarctions followed by reperfusion administration of monoclonal antibodies against the CD18 subunit, inhibited neutrophils accumulation in myocardium [9] and significantly increased coronary blood flow [19].

The current study indicates increased plasma mediated stimulation of neutrophil adhesion is dependent on β_2-integrins as a result of myocardial reperfusion during CABG, i.e. just after aortal clamp release. We have compared the influence of plasma obtained from blood taken from peripheral vein, artery and coronary sinus before aortal clamping, at the beginning of reperfusion (aortal clamp release) and after 30 min of reperfusion. The largest neutrophil stimulation was observed under the influence of plasma samples taken from the coronary sinus immediately after aortal clamp release, and decreased in the presence of plasma samples taken 30 min after aortal clamp release. The result of our study suggests that myocardial ischaemia during coronary artery surgery results in the release of soluble factors capable of the induction of neutrophil expression of CD 18 antigen. Furthermore, the decreased adhesion in samples taken in 30 min after aortal clamp release indicates elimination of neutrophil activating factors from myocardium throughout the reperfusion. Our results correspond to previous observations indicating the increase of expression of CD 11b/CD 18 in isolated cardiopulmonary circuits [12,15], in animal models [13,18], and in patients [7,16]; however they contrast to the work of Galinanes et al. [19]. The reason for the discrepancy is unknown, but Galinanes et al., suggested that the use of the centrifugal pump may have played some role.
The release of soluble forms of endothelial adhesion molecules is well documented. Kalawski et al. [28] have shown that plasma levels of soluble ICAM-1 (sICAM-1) in blood samples taken from coronary sinus, after 30 minutes of reperfusion during CABG, have been significantly higher than in samples taken at the beginning of reperfusion. Moreover the level of sICAM-1 in blood samples taken from coronary sinus after 30 minutes of reperfusion have been significantly higher than in samples obtained from arterial blood at the same time, indicating a transcardiac release of soluble adhesion molecules. This suggest that soluble form of ICAM-1, being a receptor for PMN integrins may modify plasma indicating stimulation of neutrophils during CABG.

Several neutrophil-oriented stimuli are known to be released from the myocardium during ischaemia and reperfusion. Previous studies have indicated that compounds of complement cascade, being strong chemotactic factors (C5a, C5a des Arg) for the PMN that additionally initiate neutrophil activation with subsequent adhesion to the endothelial cells, are released during experimental ischaemia [21,22] and CABG [23]. A previous study reported that C5a intra-coronary administration decreases coronary blood flow and impairs coronary circulation [24–26].

Some reports indicate, that IL-8 connected to the endothelium, increases the neutrophils adherence through augmenting of β2-integrin expression on PMN [25]. Platelet activating factor (PAF) is a potent stimulus for PMN activation and has been reported to be released during ischaemia and subsequent reperfusion. PAF plays an important role in increasing CD 11b/CD 18 expression on the neutrophil surface [27]. Neutrophils themselves are a source of PAF [1] that is released after exposure to certain stimuli (e.g. endothelin).

Endothelins have been shown to act as stimuli for granulocytes [20] and thus, play some role in neutrophil dependent myocardium injury during ischaemia. Current study indicates that the use of ET₄, endothelin receptor blocker decreases the integrin-mediated neutrophil adherence in samples incubated with plasma taken from coronary sinus at the beginning of reperfusion. Moreover plasma ET-1 concentration was highest in these samples.

These findings suggest that ET-1 may play some role in neutrophil activation during CABG and, therefore, be involved in the development of neutrophil-mediated systolic dysfunction as a result of myocardial ischaemia during the procedure. The results of our current study correspond to the very recent observations of Gonon et al. [29], performed on an animal model. Isolated rat hearts were perfused during ischaemia and reperfusion with PMN suspension in the presence of ET₄ receptor antagonist LU 135252. ET₄ blockade significantly decreased PMN-related myocardial dysfunction [29].

Thus, future pharmacological intervention based on ET-1 receptor blockade may, therefore, affect the clinical outcome in patients undergoing coronary artery bypass grafting.

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References


