Journal of Cardiology 63 (2014) 379–384

Contents lists available at ScienceDirect

Journal of Cardiology

journal homepage: www.elsevier.com/locate/jjcc

Original article

Platelet activation and thrombus formation relates to the presence of myocardial inflammation in patients with cardiomyopathy

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ARTICLE INFO

Article history:
Received 21 March 2013
Received in revised form 6 August 2013
Accepted 18 September 2013
Available online 11 November 2013

Keywords:
Cardiomyopathy
Platelet activation
Myocardial inflammation

ABSTRACT

Background: Patients with cardiomyopathy show a significantly increased risk for thromboembolic events due to a hypercoagulable state and platelet dysfunction. The pathophysiologic mechanism underlying the increasing platelet activity in patients with cardiomyopathy remains unclear. We performed a clinical study to elucidate the link between myocardial tissue alterations and platelet activation in patients with cardiomyopathy.

Methods: A total of 30 patients with suspected cardiomyopathy and 10 healthy control patients were included in our study. Hemodynamic parameters were measured by catheterization and echocardiography. Endomyocardial biopsies were taken to determine myocardial inflammation. Flow cytometry was performed to examine the platelet activation by quantification of p-selectin and thrombospondin expression on platelets.

Results: The p-selectin (8.46 ± 3.67 AU) and thrombospondin (26.56 ± 23.21 AU) expression was significantly correlated with the amount of CD3+ T cells (p-selectin: $r = 0.573$, $p < 0.05$; thrombospondin: $r = 0.488$, $p < 0.05$) and the endothelial/interstitial activation (p-selectin: $r = 0.521$, $p < 0.05$; thrombospondin: $r = 0.39$, $p < 0.05$). This was found to be independent of hemodynamic parameters, age, and gender. The platelet activation of patients ($n = 3$) with echocardiographically documented ventricular thrombi was significantly increased (p-selectin: 12.57 ± 5.5 AU vs. 8.1 ± 3.2 AU, $p < 0.05$) and this was associated with elevated myocardial inflammation scores.

Conclusion: Myocardial inflammation is associated with a significant increase in platelet activation and ventricular thrombus formation independently of the hemodynamic conditions.

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Introduction

Non-ischemic cardiomyopathy (CM) is a serious disorder leading to high morbidity and mortality because of arrhythmic events and sudden death [1]. The myocardial disorder is characterized by an impaired systolic or diastolic heart function combined with changes in ventricular diameters and pressures. These alterations predispose to ventricular thrombus formation with increased risk for stroke, peripheral arterial occlusion, and pulmonary embolism [2].

The etiology of CM includes various factors such as myocardial viral infections, genetic variations, autoimmune disorders, or toxic agents. After exclusion of a possible ischemic genesis for impaired cardiac function, CMs are classified into 5 groups according to their ventricular diameters, intra-ventricular pressures, and myocardial tissue alterations: dilated cardiomyopathy (DCM), hypertrophic cardiomyopathy, restrictive cardiomyopathy, arrhythmogenic right ventricular cardiomyopathy, and unspecified cardiomyopathies (UCM) [3,4].

DCM is characterized by impaired contractile heart function and ventricular chamber enlargement. One important causal factor for the development of DCM is the presence of a myocardial viral infection, triggering inflammatory processes such as increased inflammatory cytokine and chemokine expression as well as infiltration of proinflammatory T cells and macrophages. A chronic state of these inflammatory processes might result in myocardial alterations as defined by the existence of DCM.

Higher rates of ischemic strokes in patients with symptoms of heart failure have already been demonstrated [5]. As described by the "Virchow' Triad" abnormal blood flow, vessel wall abnormalities, and pathologic changes in blood constituents may lead to an increased risk of thrombogenesis [6]. Thus far, clinical studies have failed to show a direct relation between only depressed hemodynamic conditions and thromboembolic events, pointing

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http://dx.doi.org/10.1016/j.jjcc.2013.09.014

to additional pathophysiologic alterations that mediate blood hypercoagulability in patients with CM. Therefore, clear guidelines about the necessity of anticoagulant therapies in patients with non-ischemic CM are still missing [7].

A pro-thrombotic state is at least in part characterized by increased platelet activation [8–10]. Patients with CM have already been reported to have significant abnormalities in platelet function, that contribute to a hypercoagulable state [11]. Chronic cardiac tissue alterations including increased myocardial inflammation and vascular constriction have been suggested to contribute to endothelial and platelet dysfunction in cases of CM [12,13].

Thus our clinical study was performed to elucidate the link between myocardial alterations and platelet function. Therefore, platelet activation was assessed by quantification of activation-dependent protein expression on the platelet surface in patients with suspected CM and correlated with markers of endothelial activation and myocardial inflammation.

Methods

Study design

A total of 30 patients with suspected non-ischemic CM and 10 healthy control patients were included in our study, considering medical history, symptoms, physical examination, and non-invasive test. The patients were enrolled into the study if they had both (1) clinically suspected CM by history and symptoms (dyspnea or exercise intolerance, angina, and palpitations) or by history and electrocardiographic changes (rhythm disturbances, ST-segment, or T-wave deviations) and (2) echocardiographic findings of left ventricular dysfunction (regional wall motion abnormalities or global left ventricular dysfunction). The presence of coronary artery disease was excluded in all patients with coronary angiography. Left ventricular ejection fraction (LVEF) was determined by left ventricular catheterization and ventriculography. Right ventricular catheterization was performed in all patients to take endomyocardial biopsies from the right ventricular septum with a standard bioptome. Patients with severe respiratory infections, tumor disease, renal insufficiency, hematological disorders or other severe disease were excluded from the study. Furthermore, echocardiographic analysis was performed at the time of enrollment to assess left ventricular end-diastolic diameters (LVEDD), left atrium diameter, and to detect possible ventricular thrombus formation. Patients were classified into 4 groups of non-ischemic CM according to their hemodynamic and cardiac inflammatory characteristics: (1) DCM without inflammation (LVEF < 50%, LVEDD > 55 mm, negative cardiac inflammation score); (2) DCM with inflammation (DCMi: LVEF < 50%, LVEDD > 55 mm, positive cardiac inflammation score); (3) CM with inflammation (CMi: LVEF ≥ 50%, LVEDD < 55 mm, positive cardiac inflammation score); (4) UCM (LVEF ≥ 50%, LVEDD < 55 mm, negative cardiac inflammation score, symptoms of heart failure or electrocardiographic changes). A positive myocardial inflammation score was defined according to the Dallas criteria and criteria of the World Heart Federation (WHF) [14].

The study protocol was approved by the local ethics committee. It was performed in accordance with the ethics principles in the Declaration of Helsinki. Written informed consent was provided by each patient before participation in the study.

Immunohistochemical assessment of myocardial biopsies

Myocardial biopsies were prepared and evaluated as previously described. HLA-1, HLA-DR, and ICAM-1 were evaluated as markers for activation of endothelial and interstitial cells within the myocardial tissue. Expression of HLA-1, HLA-DR, and ICAM-1 on endothelial and interstitial cells was semiquantitatively scaled 0 (normal), 1 (intense), 2 (abundant), 3 (very abundant) according to the intensity of immunoperoxidase staining of the cells [15]. The activation of the endothelial and interstitial cells was graded according to the sum of HLA-1, HLA-DR, and ICAM-1 expression on each cell type (minimum: 0; maximum: 18). Further cellular infiltrates (CD3+ cells, CD45+ cells, and activated macrophages) within the myocardial tissue were also assessed as a measure of myocardial inflammation as previously described [16,17].

Measurement of in vivo platelet degranulation

Venous blood was drawn from all patients into a platelet-stabilizing agent containing 134 mmol ethylenediaminetetraacetic acid and 20 units heparin (ratio 9 volumes blood plus 1 volume reagent). The blood was immediately fixed in 0.5% paraformaldehyde (pH 7.4). Platelet-rich plasma was prepared for the measurement of in vivo platelet degranulation as described before [18]. The platelets were incubated with mouse IgG (unspecific binding) or a monoclonal antibody against p-selectin, thrombospondin, or fibrinogen (all antibodies from Coulter Immunotech, Marseille, France), followed by a secondary fluorescein isothiocyanate-labeled antibody (SIGMA, St Louis, MO, USA). Flow cytometry was thereafter performed (FACScan, Becton Dickinson, San José, CA, USA) as previously described [19–21].

Statistical analysis

SPSS statistical software version 16.0 (Chicago, IL, USA) was used for all statistical analyses. Graphical illustrations were done by SPSS statistical software version 16.0 and GraphPad Prism Version 4.03 (San Diego, CA, USA). All data were expressed as the mean ± standard deviation (SD), except for data in the figures that are presented as the mean ± standard error of the mean (SEM). A p-value < 0.05 was regarded as statistically significant. Quantitative variables with normal distribution were analyzed with Student's t-test, and variables without normal distribution with a two-tailed Mann–Whitney U-test. The correlation analyses were performed with Pearson's and Spearman's test. Bonferroni's correction was not performed. Multivariate linear regression was calculated to analyze the factors independently affecting specific variants. An α error <5% was considered statistically significant. Our multivariate linear regression was performed as a stepwise backward elimination process, deleting statistically non-significant parameters subsequently. Possible multicollinearity was tested by determination of the variance inflation factor (VIF) and the tolerance of each included parameter. A VIF >5 was regarded as a critical sign of existing multicollinearity.

Results

Thirty patients with non-ischemic CM were included during the study period. Patient characteristics are shown in Table 1, demonstrating hemodynamic and myocardial inflammation patterns of the study patients. A total of 7 patients were classified into the DCM group, 7 patients into the DCMi group, 8 patients into the CMi group, and 8 patients into the UCM group. The platelet activation was evaluated by flow cytometry and the expression of p-selectin (8.46 ± 3.67 AU), thrombospondin (26.56 ± 23.21 AU), and fibrinogen (25.47 ± 8.62) on the platelet surface was quantified (Fig. 1). Compared to a healthy control group (n = 10), p-selectin (5.8 ± 1.99 AU, p < 0.05), thrombospondin (12.8 ± 9.21 AU, p < 0.05), and fibrinogen (14.1 ± 3.55 AU, p < 0.05) expression were significantly increased in patients with non-ischemic CM.

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