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Effect of the E-selectin Gene Polymorphism (S149R) on Platelet Activation and Adverse Events After Coronary Artery Surgery

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Background and Aims. A common single nucleotide polymorphism in the E-selectin (*SELE)* gene S149R results in the loss of E-selectin ligand binding specificity. The 149R allele has been associated with severe cardiovascular diseases. We hypothesized that S149R may regulate platelet activation after stimuli associated with perioperative procedures in patients undergoing isolated coronary artery bypass grafting (CABG). Associations between the S149R polymorphism and an increased risk of perioperative acute thrombotic events with regards to a platelet count and activity were analyzed.

Methods. In elective CABG patients ($n = 152$) we analyzed associations between S149R polymorphism and an increased risk of perioperative acute thrombotic events with regard to platelet count and activity. The S149R *SELE* gene polymorphism was determined by real-time polymerase chain reaction. Platelet count and activation marker (β-thromboglobulin-βTG) were evaluated.

Results. Prevalence of the S149R genotypes was as follows: 81.8% ($n = 121$) of homozygotes (149SS), 15.5% ($n = 23$) of heterozygotes and 2.7% ($n = 4$) of homozygotes (149RR). The 149R allele carriers had significantly higher postoperative βTG levels than the homozygotes (97 [79–120] vs. 76 [66–91] IU/mL, $p = 0.03$). Sixteen patients had adverse events: myocardial infarction ($n = 14$), stroke ($n = 1$) and fatal pulmonary embolism ($n = 1$). Twelve patients were carriers of the 149R allele. Relative risk (RR) of postoperative adverse events in the 149R allele carriers was 2.03 with 95% CI (1.05–3.03).

Conclusions. Postoperative platelet activation is related to the S149R polymorphism, which enhances the risk of adverse events after CABG.

Key Words: Acute thrombotic events, E-selectin, Perioperative procedures, Platelet activity, Single nucleotide polymorphism.

Introduction

E-selectin is a membrane protein exclusively expressed in activated endothelial cells. The highest peak of E-selectin levels is observed 12 h after coronary artery bypass grafting (CABG) and remains increased up to 3 days (1). E-selectin is highly specific to some receptors presented on polymorphonuclear neutrophils (PMN) and monocytes, e.g., P-selectin glycoprotein ligand-1 (PSGL-1), CD44, and mediates their adhesiveness and rolling, leading to leukocyte migration into the subendothelial space (2). E-selectin activity blocking by a sialyl Lewis^x antagonist (TBC 1269) reduces the neutrophil infiltration during cardiopulmonary bypass (CPB) (3). Interactions between the soluble form of E-selectin, namely, E-selectin-bearing microparticles, and platelet markers-β-thromboglobulin (βTG) were also observed (4). Moreover, cell surface glycoligands are believed to contribute to the pathogenesis of acute coronary syndromes (ACS) through the cell aggregation/adhesion-related mechanisms (5).

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A variety of cardiac perioperative procedures including thoracotomy and CPB cause multi-organ injury and endothelial stimulation, which leads to the hypercoagulability state and high turnover of E-selectin and inflammation markers (3,4,6). Additionally, systemic inflammatory response is related to the increased tendency to develop endothelial dysfunction, the recruitment of leukocytes to the sites of tissue damage and platelet activation (7).

A common single nucleotide polymorphism (SNP) of the E-selectin (*SELE*) gene is a missense exchange of A>C nucleotides (rs5361) resulting in the substitution of arginine (R) for serine (S) at position 149 (S149R) within the epidermal growth factor-like domain (8). This substitution causes the loss of E-selectin requirement for alpha 1-3-linked fucose to a specific ligand binding. Clinical trials revealed that the 149R allele is associated with the early onset of severe coronary artery disease (CAD) in subjects <50 years of age and increased risk of restenosis after percutaneous transluminal angioplasty (PTA) in peripheral artery disease patients (9,10). The role of this polymorphism in the regulation of a perioperative inflammatory response (elevated CRP) was documented in our previous study (11).

We tested the hypothesis that the *SELE* candidate gene SNP (S149R) may regulate platelet activation in CABG patients. We also tried to determine any associations between this polymorphism and an increased risk of postoperative adverse events after CABG and to reveal the effect of revascularization procedures on postoperative complications with relation to genetics and other risk factors.

Materials and Methods

Patients

A clinical study was carried out at the Institute of Cardiology of the Jagiellonian University Medical College, Krakow, Poland. Patients (n = 152) with angiographically proven two- or three-vessel CAD (at least 70% stenosis of a major epicardial artery) were scheduled sequentially for elective isolated CABG. All participants were ethnologically homogeneous and of Polish descent. The local ethics committee approved the study, and the patients provided written, informed consent.

Exclusion criteria were as follows: any acute illness, hemolytic or coagulation disorders, hepatic or renal dysfunction, cancer, ACS including myocardial infarction (MI) during the previous 6 weeks, anticoagulant and antiplatelet treatment, except for aspirin, which was continued in 29 patients (19.1%).

Clinical endpoints were defined as any adverse perioperative complication including MI, death due to MI, stroke, pulmonary embolism (PE) or thrombosis. Perioperative MI was diagnosed according to the universal MI definition: MI associated with CABG-type 5.

Perioperative Management and Laboratory Tests

All procedures were performed by six experienced senior surgical staff members, coronary artery bypass grafting was performed using middle sternotomy access. In 134 patients standard CABG procedure was performed on cardiopulmonary bass (CPB), in 18 CPB was not used and beating heart coronary surgery (OPCAB) was performed. A standard anesthetic technique with the combination of etomidate or sufentanil and maintenance treatment with sufentanil or propofol was applied. Antifibrinolytic drugs were not administered. For conventional CABG, heparin (300 IU/kg) was given through the central venous line and a celite-activated clotting time ≥480 sec was maintained. Patients without CPB received 150 IU/kg of heparin. After the CPB was discontinued, protamine sulfate was administered to reverse heparin activity. Moderate hypothermia (30–32°C) was maintained during cardiac arrest. In 88.2% of the patients antegrade warm blood or cold crystalloid cardioplegia was used. Eighteen patients (11.8%) were operated on without CPB and cardioplegic arrest. Beating heart surgery was done using intracoronary shunt technique with the Octopus stabilizer to reduce epicardial movement, with pre-warmed intravenous crystalloid infusions.

Blood samples were drawn on the morning of the day of surgery and within 1 day after (18–24 h) using an appropriate blood collection system (Starstedt, Nümbrecht, Germany). Biochemical laboratory tests were carried out routinely. Fibrinogen (FBG) was measured using the Clauss method, and C-reactive protein (CRP) and cardiac troponin I (cTnI) were measured by immunoturbidimetric method (Dimension Xpand, Siemens, Marburg, Germany). Immunoenzymatic assay determined plasma βTG levels (Diagnostica Stago, Asnières sur Seine, France) and S149R polymorphism genotyping was performed by real-time quantitative polymerase chain reaction using TaqMan Universal PCR Master Mix (7900HT Fast Real-Time PCR System, Applied Biosystems Carlsbad, CA). FV Leiden G1691A and prothrombin 20210A polymorphisms were determined using commercial methods.

Statistical Analysis

Data are presented as the mean ± standard deviation (SD) or median values interquartile range. Normal distribution was confirmed (Kolmogorov-Smirnov test). Intergroup comparisons were made using the unpaired t-test or the Mann-Whitney U test. Correlations between variables were calculated by the Spearman or Pearson test. Categorical values were analyzed using the χ^2 or Fisher's exact test. One-way ANOVA was used to characterize the CPB effect on postoperative clinical and biochemical parameters. Post hoc MANOVA tested the hypothesis of no overall polymorphism effect on the set of perioperative biochemical parameters by forcing covariates into the