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Original article

Exercise stress testing enhances blood coagulation and impairs fibrinolysis in asymptomatic aortic valve stenosis

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ABSTRACT

Background: Increased thrombin formation and fibrin deposition on the valves were observed in patients with severe aortic valve stenosis (AS). Exercise enhances blood coagulation and fibrinolysis in healthy subjects, but its haemostatic effects in AS are unknown. We sought to investigate how stress echocardiography alters blood coagulation and fibrinolysis in AS patients free of significant atherosclerotic vascular disease.

Methods: We studied 32 consecutive asymptomatic moderate-to-severe AS patients and 32 age- and sex-matched controls. We measured peak thrombin generated using calibrated automated thrombogram, clot lysis time (CLT), and fibrinolytic markers at four time points, i.e. at rest, at peak exercise, and 1 h and 24 h after a symptom-limited exercise test.

Results: We observed that peak thrombin generated rose at peak exercise to 25% higher value in the patients than in controls ($p < 0.001$) and reached its highest level 24 h from exercise in AS patients while it decreased post-exercise in controls. Baseline CLT was 13% longer in AS patients ($p = 0.006$) and associated with thrombin activatable fibrinolysis inhibitor (TAFI) activity ($r = 0.69$, $p < 0.001$), antiplasmin ($r = 0.47$, $p = 0.007$), and plasminogen ($r = -0.55$, $p = 0.001$). In AS, CLT remained unaltered at peak exercise, while it decreased in controls, yielding the intergroup difference of 28% ($p < 0.001$). Twenty-four hours after exercise CLT became 15% longer in AS patients ($p < 0.001$). This hypofibrinolytic effect followed a similar pattern observed for TAFI activity.

Conclusions: Asymptomatic moderate-to-severe AS patients respond to exercise with more pronounced and prolonged increase in thrombin generation, together with impaired fibrinolysis as compared to controls. Repeated episodes of exercise-induced prothrombotic state in AS might contribute to the progression of this disease.

Introduction

Aortic valve stenosis (AS) is the most common valvular heart disease in adults, which could be asymptomatic for a long time [1]. Current evidence indicates that AS is an active process with lipoprotein infiltration, chronic inflammation, extracellular matrix remodeling, angiogenesis, and calcium deposition [2,3], which is to some extent similar to atherosclerosis, in which enhanced blood coagulation is involved in its progression and complications [4]. There have been reports suggesting that AS is also associated with enhanced blood clotting. Dimitrow et al. [5] have shown that there is a prothrombotic state, characterized by increased thrombin formation and platelet activation associated with maximal transvalvular gradient in patients with severe AS. Altered hemodynamic properties of blood flow with the occurrence of post-stenotic turbulence in AS can facilitate the activation of the coagulation cascade with the resultant thrombin generation [5]. Microthrombi on the aortic valve have been shown in AS patients [6]. It has been demonstrated that fibrin accumulation occurs within and on the surface of diseased valves in AS patients, and it is associated with thrombin generation and fibrin turnover in circulating blood [7]. Moreover, tissue factor (TF) is abundantly expressed in human stenotic aortic valves in association with thrombin formation in circulating blood [8,9]. The loss of high

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molecular weight multimers of von Willebrand factor (vWF) owing to high shear stress lesion and vWF-mediated platelet dysfunction can also be observed in patients with severe AS and predispose to bleeding [10]. Recently, we have reported that hypofibrinolysis is more common in AS patients than in controls [11].

It is known that exercise can induce a prothrombotic state [12]. Beneficial effects of physical activity on the risk of coronary artery disease (CAD) and cardiovascular mortality may result at least in part from increased fibrinolysis following exercise [12,13]. To our knowledge, there have been no studies on the effect of exercise on blood coagulation and fibrinolysis in AS patients. The present study was performed to evaluate potential differences in the hemostatic response to exercise test in AS patients vs. controls.

Materials and methods

Patients

Thirty-three consecutive adult patients with asymptomatic moderate-to-severe AS [defined as transvalvular maximal velocity (V_{max}) $\geq$3 m/s] were recruited from March 2011 to June 2012.

The exclusion criteria were: history of angina, dizziness, syncope, another cardiac valve disease of more than a moderate degree, left ventricular (LV) ejection fraction (EF) <50%, history of or current atrial fibrillation, hyper- or hypothyroidism, diabetes treated with insulin, renal or hepatic dysfunction, lung disease, oral anticoagulant therapy, use of thienopyridine or nonsteroidal anti-inflammatory drugs other than aspirin, known cancer, bleeding tendency, autoimmune disorders, a history of myocardial infarction, stroke, or venous thromboembolism. Patients who were not able to perform exercise testing were also excluded from the study. Sex- and age-matched individuals recruited from the families of hospital personnel served as controls. The study protocol was approved by the University Bioethical Committee, and each patient provided written, informed consent to participate in the study.

To evaluate the extent of atherosclerotic vascular disease, which coexists in about 50% of AS patients [14], we measured intima–media thickness (IMT) in both right and left common carotid artery in accordance with the Mannheim IMT consensus [15]. The ankle brachial pressure index (ABI) was measured using an arterial pressure sphygmomanometer and a continuous wave Doppler ultrasound blood flow detector and values of 0.9–1.15 were considered normal. Hypertension was diagnosed based on a history of hypertension or antihypertensive treatment. Hyperlipidemia was diagnosed based on medical records, statin therapy, or total cholesterol of $\geq$5.0 mmol/L.

Echocardiography

Transthoracic echocardiography was performed in all enrolled subjects using Philips iE33 (Philips Electronics, Andover, MA, USA). LV volumes and EF were measured by the biplane Simpson's method. The aortic valve area (AVA) was calculated using the standard continuity equation. V_{max}, peak pressure gradient (PPG), and mean pressure gradient (MPG) were calculated using the modified Bernoulli equation.

A symptom-limited exercise stress echocardiography was performed on a bicycle ergometer (Ergoline, Bitz, Germany) in a semisupine position with a continuous echocardiographic examination by an experienced cardiologist. After 3 min of the initial workload of 25 W, the workload was increased every 3 min by 25 W [16]. Electrocardiogram was monitored and blood pressure was measured every 3 min during exercise. Exercise was stopped in case of typical chest pain, breathlessness, dizziness, muscular exhaustion, hypotension, ventricular arrhythmia, when age-related maximum heart rate was reached, or on the patient's demand. The test was performed at rest and peak exercise.

Laboratory tests

Fasting blood samples were drawn from the antecubital vein between 07.00 h and 10.00 h. Fibrinogen was measured by the von Clauss method. High-sensitivity C-reactive protein was determined using immunoturbidimetry (Roche Diagnostics, Mannheim, Germany). Blood samples to determine hemostatic parameters were drawn four times: at rest, at peak exercise, and 1 h and 24 h after exercise and then centrifuged at 2500 $\times$ *g* at 20 °C for 10 min and stored at −80 °C until analysis. Technicians were blinded to the origin of the samples.

Thrombin generation

Measurement of the thrombin endogenous potential was performed using calibrated automated thrombography (Thrombinoscope BV, Maastricht, Netherlands) according to the manufacturer's instructions in the 96-well plate fluorometer (Ascent Reader, Thermolabsystems OY, Helsinki, Finland). Eighty microliters of platelet-poor plasma was diluted with 20 μL of the reagent containing 5 pmol/L recombinant TF, 4 μM phosphatidylserine/phosphatidylcholine/phosphatidylethanolamine vesicle, and 20 μL of FluCa solution (HEPES, pH 7.35, 100 nmol/L $CaCl_2$, 60 mg/mL bovine albumin, and 2.5 mmol/L Z-Gly-Gly-Arg-AMC). Samples were analyzed in duplicate; intra-assay variability was 5.7%. We analyzed the maximum concentration of thrombin generated.

Endothelial cell activation markers

Plasma soluble thrombomodulin (TM) (Diagnostica Stago, Asniéres, France) and tissue plasminogen activator (tPA) antigen (Hyphen BioMed, Neuville-Sur-Oise, France) were determined by enzyme-linked immunosorbent assays (ELISA).

Fibrinolysis

Measurement of thrombin activatable fibrinolysis inhibitor (TAFI) antigen was performed with an ELISA (Chromogenix, Lexington, MA, USA). The activity levels of plasminogen activator inhibitor-1 (PAI-1) and TAFI were measured using a chromogenic assay (Chromolize PAI-1, Trinity Biotech, Bray, County Wicklow, Ireland; and ACTICHROME® Plasma TAFI Activity Kit, American Diagnostica, Greenwich, CT, USA, respectively).

PAI-1 antigen was determined in citrated plasma using commercially available ELISAs according to the manufacturer's instructions (American Diagnostica).

Plasminogen and α2-antiplasmin (α2-AP) were measured by chromogenic assays (Diagnostica Stago).

Plasma D-dimer was determined by immunoturbidimetry (Innovance D-dimer, Siemens, Erlangen, Germany). Intra-assay and inter-assay coefficients of variation were <8%.

Clot lysis time (CLT) was measured as previously described [17] with some modifications. Briefly, citrated plasma was mixed with Tris buffer (1:1) containing 15 mmol/L calcium chloride, 0.6 pmol/L human TF (Innovin, Siemens, Marburg, Germany), 12 μmol/L phospholipid vesicles (Avanti Polar Lipids, Alabaster, AL, USA), and 60 ng/mL recombinant tPA (Boerhinger Ingelheim, Ingelheim, Germany). Turbidity was measured at 405 nm at 37 °C. CLT was defined as the time from the midpoint of the clear-to-maximum-turbid transition, which represents the clot formation, to the midpoint of the maximum-turbid-to-clear transition, representing the lysis of the clot. Intra-assay and inter-assay coefficients of variation were 6.0 and 7.4%, respectively.