ELSEVIER

Cardiovascular Revascularization Medicine 12 (2011) 20–24

CARDIOVASCULAR REVASCULARIZATION MEDICINE

Platelet reactivity in diabetic patients subjected to acute exercise stress test

Mickey Scheinowitz, Rajbabu Pakala, Itsik Ben-Dor, Gilles Lemesle, Rebecca Torguson, Augusto D. Pichard, Joseph Lindsay, Ron Waksman*

Division of Cardiology, Washington Hospital Center, Washington, DC

Received 11 January 2010; accepted 11 January 2010

Abstract

Background: Previous studies have reported ambiguous results regarding the effect of acute exercise on platelet reactivity in healthy and cardiac patients.

Objectives: We aimed to assess platelet reactivity among diabetic patients before and immediately after an acute exercise stress test.

Methods: Patients (controls: mean age 53.1±12.1 years; four males; body mass index 27.0±5.7 kg/m^2; HbA_{1c} 6.0±1.1%, n=8) and diabetic patients (52.9±11.3; six males; body mass index 30.7±2.2 kg/m^2; HbA_{1c} 7.8±1.7%, n=8) referred for diagnostic nuclear exercise stress test were recruited. Blood samples obtained at rest and immediately post-exercise were stimulated with adenosine diphosphate (ADP), collagen and arachidonic acid. Expression of CD41 (pan-platelet marker) and CD62p (platelet stimulation marker) were measured by flow cytometry. Aspirin responsiveness was measured using VerifyNow.

Results: Although peak systolic blood pressure was significantly higher in the diabetics compared with nondiabetics (186.3±25.4 vs. 157.1±19.1, respectively, P=.028), peak exercise heart rate was similar (156.5±8.3 vs. 155.5±12.1 for diabetics and nondiabetics, respectively). No differences were observed between groups for aspirin resistance. Platelet stimulation with ADP exhibited significantly lower CD62p-positive cell population (%) in the diabetic patients both prior to and following the exercise stress test (P=.03). In addition, although not significant, platelet stimulation was higher post-exercise in the diabetic patients (6.3±4.7% vs. 12.0±5.6%, for pre- and post-exercise, respectively, P=.2) with no difference in controls (9.2±5.5% vs. 8.9±5.9%).

Conclusion: Platelet stimulation in diabetic patients is blunted and might be explained by the prolonged exposure of platelets to multiple diabetic risk factors.

Keywords: Flow cytometer; Exercise stress test; VerifyNow

1. Introduction

It has been shown that intense physical activity is associated with acute coronary thrombosis and arterial occlusion in sedentary, unfit individuals or in those with preexisting vascular disease [1]. In both cases, exercise results in disruption of coagulation and the fibrinolytic systems that up-regulate platelet activity. A recent systematic review of the association between acute exercise and hemostasis suggests that the risk of thrombosis is increased shortly after the exercise ends [2]. However, the paradox between the ability of exercise to prevent or to stimulate coronary thrombosis is controversial and depends on exercise intensity, baseline fitness level, acute vs. chronic exercise, and others factors [3]. In sedentary individuals, platelet aggregation [induced by adenosine diphosphate (ADP) stimulation] was increased post-maximal exercise and was significantly reduced following moderate types of exercise [4]. Furthermore, reduced shear-induced platelet

* Corresponding author. Washington Hospital Center, 110 Irving Street NW, Suite 4B-1, Washington, DC 20010. Tel.: +1 202 877 2812; fax: +1 202 877 2715.

E-mail address: ron.waksman@medstar.net (R. Waksman).

1553-8389/10/$ – see front matter © 2011 Elsevier Inc. All rights reserved.
doi:10.1016/j.carrev.2010.01.005

aggregation was reported in subjects participating in regular physical activity compared with their sedentary counterparts. This effect was reversed after deconditioning [5]. Furthermore, higher platelet reactivity was demonstrated in cardiac [6] and chronic heart failure [7] patients undergoing an exercise stress test with reduced platelet aggregation [8]. Aspirin therapy seems to have no impact on platelet aggregability following exercise among aspirin responders [9] but is associated with increased platelet reactivity among nonresponders [10]. In contrast with the conflicting reports among cardiac patients, there is consensus among patients with metabolic syndrome [11] and diabetics [12] who exhibit metabolic alterations, increased oxidative stress, and inflammation, all of which contribute to increased thromboxaneA_2 synthesis and platelet activation under resting conditions [13,14]. An increased prothrombotic state, which is associated with higher platelet reactivity among diabetic patients, makes those patients prone to increased risk of acute myocardial infarction and sudden death [15]. However, little is known about platelet function in diabetic patients following acute exercise. Therefore, the purpose of the present study was to assess platelet reactivity in type 2 diabetic patients subjected to acute exercise stress test.

2. Methods

The experimental protocol was approved by the Washington Hospital Center Institutional Review Board Committee (approval no. 2008-124). Patients referred for diagnostic nuclear exercise stress test participated in this study after signing an informed consent form. Patients were instructed to fast for 12 h prior to the stress test and to continue all medications while on the stress test.

Symptom-limited or age-predicted maximal heart rate (85%) exercise stress test was performed using the Bruce protocol. A 12-lead electrocardiogram was taken at rest, during exercise, and during a 5-min recovery period. Blood pressure (systolic and diastolic) was measured at rest, at the end of each stage of the Bruce protocol, and during the recovery period post-exercise. Maximal metabolic equivalent (MET) was extrapolated from the maximum time achieved on the Bruce protocol.

Blood samples were collected prior to the stress test from an antecubital vein using a 21-gauge needle for measuring glucose, insulin, HbA_{1c} levels, and for platelet function analysis. Blood was collected in 3.2% citrate tubes. At peak exercise (within 1 min during the recovery period), a second blood sample was collected for platelet function only. The first 2 ml of each blood sample drawn was discarded to minimize platelet aggregates. Platelet function analysis was performed within 1 h of blood collection. Platelet activation was determined from whole blood following stimulation with ADP, collagen, and arachidonic acid (AA), using flow cytometry by measuring the percentage of platelets expressing P-selectin.

Platelets were stimulated with different concentrations of ADP, collagen, and AA. Sample platelets were identified and gated using a phycoerythrin-conjugated mouse anti-human CD-41a antibody (BD Pharmingen, San Diego, CA, USA) as pan-platelet marker. The percentage of platelets expressing P-selectin in the gated cells was determined by using a mouse anti-human fluorescein isothiocyanate-conjugated CD62p antibody (BD Pharmingen). A minimum of 5000 positive events were determined in each sample.

Aspirin responsiveness in both pre- and post-exercise samples was determined using the point-of-care assay VerifyNow (Accumetrics, San Diego, CA, USA) with aspirin cartridge, which uses AA to stimulate platelets to aggregate. Aspirin resistance is defined as aspirin reaction units $\geq$550. Data were compared between exercise and resting conditions (diabetics vs. nondiabetics). Mean±S.D. was calculated for each parameter and one-factor analysis of variance was used to test for differences in platelet stimulation between groups both at rest and following exercise. A P value $\leq$.05 was considered significant.

3. Results

Diabetics and nondiabetic patients were similar in age: 52.9±11.3 and 53.1±12.1 years, respectively. Body mass index was nonsignificantly higher in diabetic patients (30.7±2.2 kg/m^2 vs. 27.0±5.7 kg/m^2 for the nondiabetics, P=.104). Additional patient characteristics are shown in Table 1. Exercise parameters are shown in Table 2. Peak systolic blood pressure was significantly higher in diabetic patients (186.3±25.4 vs. 157.1±19.1 mmHg, respectively, P=.028), and maximal METs were insignificantly lower compared to nondiabetics (21.6±4.9 vs.

Table 1
Characteristics, medication, and blood work for both nondiabetic and diabetic patients

	Nondiabetic (n=8)	Diabetic (n=8)
Age (years)	53.1±12.1	52.9±11.3
Men	4 (50%)	6 (75%)
White	4 (50%)	2 (25%)
Black	4 (50%)	6 (75%)
Height (cm)	170.5±8.8	169.8±10.6
Weight (kg)	78.6±16.5	89.0±13.2
Body mass index (kg/m^2)	27.0±5.7	30.7±2.2
Medications		
Aspirin (325 mg)	6 (75%)	2 (25%)
Antihypertensive	4 (50%)	4 (50%)
Hypercholesterolemic	1 (12.5%)	2 (25%)
Hyperglycemic	0	4 (50%)
Blood work		
Glucose (mg/dl)	96.7±17.3	154.6±65.7 #
HbA_{1c} (%)	6.0±1.1	7.8±1.7 *
Insulin (U/ml)	10.5±7.3	7.7±7.5

P=.042.
* P=.034.