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Regular Article

Effect of intensive glycemic control on platelet reactivity in patients with long-standing uncontrolled diabetes

Joelle Singer [a,1], Adaya Weissler Snir [b,1], Dorit Leshem-Lev [c], Merav Rigler [a], Ran Kornowski [b,c], Eli I. Lev [b,c,*]

[a] Endocrinology Department, Rabin Medical Center, Petah-Tikva, Israel
[b] Cardiology Department, Rabin Medical Center, Petah-Tikva, the Sackler Faculty of Medicine, Tel-Aviv University, Israel
[c] The Felsenstein Medical Research Institute, Petah-Tikva, Israel

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ABSTRACT

Background: It has been previously shown that platelets of patients with diabetes are more reactive and less responsive to anti-platelet drugs compared with platelets from subjects without diabetes. Studies examining the effect of glycemic control on platelet reactivity have yielded conflicting data. Thus, in this study, we sought to explore the effect of tight glycemic control on platelet reactivity in patients with long standing uncontrolled diabetes.

Methods: The study included 30 patients with long-standing treated diabetes and a baseline HbA1c level of ≥ 8.5%. All patients were treated with aspirin and statins. Patients were tested at baseline and after 3 months of intensive glycemic and metabolic control. The treatment goal was to achieve a HbA1c level of ≤7%. Platelet reactivity was assessed by light transmission aggregation in response to 5 and 10 μM ADP and to 0.5 mg/ml arachidonic acid (AA). Additonally, platelet activation was assessed by plasma levels of soluble P-selectin using an enzyme-linked immunosorbent assay.

Results: The mean duration of diabetes from the time of diagnosis was 20.46 ± 9.31 years. Baseline HbA1c was 9.4 ± 0.8%. Following the intensive glycemic control period, the HbA1C level decreased to 8.1 ± 0.8% ($P < 0.0001$). Other laboratory parameters did not change significantly except for triglyceride levels, which decreased. None of the platelet aggregation studies nor P-selectin levels differed between baseline and after 3 months of intensive glycemic control.

Conclusions: Intensive glycemic control in patients with longstanding uncontrolled diabetes does not seem to result in a reduction in platelet reactivity.

Introduction

Diabetes mellitus (DM) is a strong risk factor for the development and progression of atherosclerotic cardiovascular disease and its thrombotic complications [1]. Moreover, cardiovascular disease is the leading cause of morbidity and mortality in patients with DM [2]. In acute coronary syndrome (ACS) setting, DM is a strong independent predictor of worse short-term and long-term outcomes including mortality [3,4]. Platelets play a pivotal role in atherogenesis and ACS [5–9]. It has been previously shown that platelets of patients with diabetes are larger and more reactive compared with platelets from subjects without diabetes. In addition, platelets from patients with diabetes demonstrate increased adhesion, aggregation, expression of activation markers (e.g. GPIIb/IIIa and ADP receptors), and platelet-dependent thrombin generation [10–16]. Moreover, DM is also associated with hypo-responsiveness to antiplatelet drugs [12,17,18] and high platelet reactivity is more prevalent in patients with type 2 DM compared with nondiabetic patients, even when treated with dual antiplatelet therapy [19, 20]. Several mechanisms have been proposed to contribute to the increased platelet reactivity including hyperglycemia, insulin resistance, associated metabolic conditions such as obesity and inflammation and other cellular abnormalities [21, 22].

Previous studies have demonstrated independent positive associations between glycosylated hemoglobin (HbA1c) and vascular complications, including cardiovascular complications [23,24]. However, while glycemic control has been shown to delay the progression of macrovascular and microvascular complications of DM [25–28], studies examining the effect of glycemic control on platelet reactivity have

Abbreviations: DM, Diabetes mellitus; ACS, acute coronary syndrome; HbA1c, glycosylated hemoglobin; AA, Arachidonic acid.

* Corresponding author at: Department of Cardiology, Rabin Medical Center, Petah-Tikva, Tel-Aviv University, Israel. Tel.: +972 3 9372251; fax: +972 3 9231016.
E-mail addresses: elil@clalit.org.il, elev@tmhs.org (E.I. Lev).

[1] Drs. Singer and Weissler Snir contributed equally to this article.

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yielded conflicting data [29–35]. Thus, in this study, we sought to explore the effect of tight glycemic control on platelet reactivity in patients with long standing uncontrolled diabetes.

Methods

Patients and Eligibility Criteria

The study included 30 patients with diabetes, aged 20-75 years who were at least 10 years from the initial diagnosis. Only patients with treated diabetes (with insulin and/or oral hypoglycemic medications) and a baseline **HbA1c level of ≥8.5%** were included. All patients were treated with aspirin and statins. Exclusion criteria were renal insufficiency (creatinine ≥ 2.5 mg/dL), hepatic dysfunction (alanine aminotransferase ≥2.5 times the upper limit of normal), thrombocytopenia ($\leq 100x10^3$ cells/mm^3), anemia (hemoglobin ≤ 10 g/dl) or specific contraindications to oral hypoglycemic medications or insulin therapy. Patients with a history of acute coronary syndrome or coronary revascularization in the past three months, malignant diseases or hematologic disorders were also excluded. The study was approved by the Investigational Review Board and Ethics Committee of the Rabin Medical Center, and all subjects provided written informed consent.

Intensive Glycemic Control

The goal of treatment was to achieve an **HbA1c level of ≤7%** over a period of 3-4 months. Patients were examined periodically by a multidisciplinary team composed of a physician (JS), nurse and dietician, all specializing in management of diabetes, at the following time intervals: baseline and weekly examinations during the first 4 weeks, bi-weekly visits during the latter 8 weeks and weekly telephone reviews between the visits in the latter 8 weeks. In addition, open access via the phone was maintained throughout the study. Patients were treated with oral pharmacological treatment and/or insulin, based on regular home glucose monitoring and according to guidance from the study team. Topics of intervention for all patients also included healthy lifestyle, self-monitoring of blood glucose, diet, physical activity reports and carbohydrate counting. Aside from the diabetes management, other medications (including statins, aspirin and antihypertensives) were unchanged for the duration of the study. This was verified at the 3-4 month visit.

Blood Sampling

Patients were tested twice: at baseline and after 3-4 months of intensive glycemic control. Following overnight fasting, venous blood was drawn in heparinized tubes from an anticubital vein for platelet activation and reactivity testing. All samples were processed within one hour of blood collection

Platelet Aggregation

Light transmittance (turbidimetric) platelet aggregation was performed in platelet-rich plasma. Platelets were stimulated with 0.5 mg/ml (1.6 mmol/l) Arachidonic acid (AA) and 5 **μM** and 10 **μM** ADP. Aggregation was performed with a BioData PAP-4 platelet aggregometer (BioData, Horsham, Pennsylvania). The extent of aggregation was defined as the maximal light transmission at least 6 min after addition of the agonist, with platelet-poor plasma used as reference.

Platelet Activation

Plasma levels of soluble P-selectin were measured from plasma stored at -70 °C, in duplicate, using an enzyme-linked immunosorbent assay, according to the manufacturer's instructions and using commercial reagents and recombinant human antibodies as standard(R&D Systems, Minneapolis, Minnesota)

Statistical Analysis

AA aggregation test results were not normally distributed, determined by the Kolmogorov-Smirnov normality test. Therefore, AA aggregation test data are presented as median (interquartile range (IQR)), and comparisons were performed by two-tailed Wilcoxon matched-pair signed rank test. Other parameters in the study and clinical variables were normally distributed and therefore, are presented as mean ± standard deviation (SD). Comparisons of the continuous normally distributed variables were performed by paired Student's t-tests. Analyses were performed using SPSS v.21 (SPSS Inc., Chicago, IL) and $P \leq 0.05$ was considered statistically significant.

Results

Clinical characteristics and baseline medications of the patients are presented in Table 1. Specific disease-related characteristics and diabetic treatment are presented in Table 2. The mean duration of diabetes from the time of diagnosis was 20.46 ± 9.31 years. 20% of the patients had type I diabetes. There were no major differences in the diabetic treatment at baseline and at the end of the glycemic control period, except from a small relative increase in the proportion of short acting to long acting insulin use (Table 2). However, in addition to medications, the glycemic control program consisted of changes in diet, carbohydrate counting, physical activity and close monitoring of glucose. At baseline patients had a mean HbA1C level of 9.4 ± 0.8%. Following the intensive glycemic control period, the HbA1C level decreased to 8.1 ± 0.8% ($P < 0.0001$, Table 3). Although the target HgA1C of 7% was attained only in the minority of patients (10%), *a decrease in HgA1C was achieved in all patients*. No major hypoglycemia events (requiring intervention of a third party to treat hypoglycemia) were observed during the intervention period. Other laboratory parameters did not change significantly from baseline to follow-up measurement, except for triglyceride levels, which decreased (190 ± 166 mg/dL vs 146 ± 95 mg/dL, $P = 0.02$). (Table 3). Of note, the mean weight did not change significantly after the control period.

Table 1
Baseline Characteristics.

Demographic characteristics	
Age (years)	59.9 ± 13.3
Women	7 (23.3%)
BMI (kg/m^2)	32.21 ± 8.04
Hypertension	21 (70%)
Dyslipidemia†	28 (93.3%)
Current smoking	4 (13.3%)
Prior MI	14 (46.6%)
Prior PCI	21 (70%)
Prior CABG	7 (23.3%)
Prior stroke	3 (10%)
Baseline Medications	
Aspirin	30 (100%)
Statins	30 (100%)
Clopidogrel	6 (20%)
Beta blockers	22 (73.3%)
ACE inhibitors/ARB	27 (90%)

BMI = body mass index; MI = myocardial infarction; PCI = percutaneous coronary intervention; CABG = coronary artery bypass graft; ACE = angiotensin converting enzyme inhibitors; ARB = angiotensin receptor blockers.
† Diagnosis previously made by physician or receiving lipid-lowering therapy.