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Full Length Article

Meal-induced platelet activation in diabetes mellitus type 1 or type 2 is related to postprandial insulin rather than glucose levels☆

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ABSTRACT

Aim: Postprandial platelet activation was related to postprandial insulin rather than glucose levels in a previous meal insulin study in type 2 diabetes mellitus (T2DM). We therefore compared postprandial platelet activation in type 1 (T1DM) patients without insulin secretion and T2DM patients with high postprandial insulin levels.
Material and methods: Patients with T1DM ($n = 11$) and T2DM ($n = 12$) were studied before and 90 min after a standardized meal without premeal insulin. Five T1DM patients volunteered for a restudy with their regular premeal insulin. Platelet activation was assessed by flow cytometry, with and without the thromboxane analogue U46619 or ADP, and by whole blood aggregometry (Multiplate®). Effects of insulin (100 μU/mL) in vitro were also studied.
Results: Before the meal, glucose, insulin and platelet activation markers other than platelet-leukocyte aggregates (PLAs) were similar in T1DM and T2DM; PLAs were higher in T1DM. Postprandial glucose levels increased more markedly in T1DM (to 22.1 ± 1.4 vs. 11.2 ± 0.6 mmol/L) while insulin levels increased only in T2DM (from 24.4 ± 4.4 to 68.8 ± 12.3 μU/mL). Platelet P-selectin expression, fibrinogen binding and PLA formation stimulated by U46619 were markedly enhanced (approximately doubled) and whole blood aggregation stimulated by U46619 was increased ($p < 0.05$ for all) after the meal in T2DM patients but not in T1DM patients. The pilot study with premeal insulin in T1DM patients showed postprandial platelet activation when postprandial insulin levels increased. In vitro insulin mildly activated platelets in both groups.
Conclusion: Postprandial platelet activation via the thromboxane pathway is related to postprandial hyperinsulinemia and not to postprandial hyperglycaemia in patients with diabetes.

1. Introduction

Platelets play a key role in the pathogenesis of arterial occlusive disorders, and platelet activity is increased among diabetic patients [1,2]. Postprandial hyperglycemia (PPH) is thought to be an independent risk factor for cardiovascular complications [3,4] and is associated with increased production of free radicals, endothelial dysfunction and platelet activation [5–8].

Postprandial platelet activation does not occur in healthy volunteers [7] but such activation has been seen in T2DM and was believed to be related to their PPH [6–8]. However, our study of premeal insulin in T2DM challenged this hypothesis and suggested that postprandial platelet activation is related to postprandial insulin rather than glucose levels [9]. Thus, the postprandial platelet activation was further enhanced by premeal insulin compared to placebo, i.e., when the postprandial glucose levels were lower and insulin levels were higher in the T2DM patients. This postprandial platelet activation was mainly seen via the thromboxane pathway and was correlated directly to insulin but inversely to glucose levels in plasma [9]. Since T1DM patients lack insulin secretion, we designed the present study to compare postprandial platelet activation in T1DM and T2DM patients and highlight the role of insulin secretion in relation to postprandial platelet activation. We also performed a pilot study with premeal insulin in T1DM patients who were willing to be restudied.

2. Material and methods

The study compared postprandial platelet activation in T1DM and T2DM patients. It was performed according to good clinical practice and the protocol (and an amendment to recall T1DM patients who were willing to repeat the study with their regular premeal insulin)

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was approved by the regional Ethical Review Board. All patients gave written informed consent.

2.1. Patients

Outpatients with diabetes aged 40–76 years without a history of cardiovascular disease were included. T2DM patients were treated with insulin and/or metformin. All patients had HbA1c levels ≤90 mmol/mol (10.5% by the DCCT method) and antecubital forearm veins allowing technically good sampling for platelet function studies. Exclusion criteria were: use of antiplatelet or anticoagulant drugs, thrombocytopenia ($<150 \times 10^9$/L), and acute or chronic kidney or liver diseases. Additional exclusion criteria for T1DM were BMI above 26 kg/m^2 (to avoid including T1DM patients likely to be insulin resistant) and C peptide ≥0.3 nmol/L (to avoid confounding by residual insulin secretion).

2.2. Study design

The study conditions were identical to the ones previously used [9]. Patients taking an evening dose of basal insulin took half of this evening dose and no glucose lowering medications in the morning. They should abstain from tobacco, nicotine and caffeine on the day of the experiment (however, all were non-smokers in this study). The patients arrived at the laboratory fasting, and rested in a semi-reclined position during 30 min before blood sampling for glucose, insulin and platelet function tests. They then ingested a standardized meal, consisting of 54% carbohydrates, 30% fat and 16% protein, during 15 min [6,7,9]. Plasma glucose and plasma insulin were measured every 15 min after the meal and platelet function tests were repeated after 90 min.

2.3. Platelet function tests

2.3.1. Flow cytometry

Platelet P-selectin expression and fibrinogen binding, and platelet-leukocyte aggregates (PLA) were measured by whole blood flow cytometry as described previously, [10–12] and are reported as percentages of positive cells in the platelet or leukocyte populations. Briefly, 5 μl of citrated whole blood was within 3 min after sampling added to hepes buffered saline containing agonists or vehicle and appropriately diluted fluorescent monoclonal antibodies and incubated at RT for 20 min before fixation with 0.5% (*v*/*v*) formaldehyde saline. Platelet function was measured at rest (with no agonist) or following stimulation by the thromboxane A_2 analogue U46619 and ADP at submaximal concentrations of 0.3 μmol/L and 1 μmol/L, respectively. Measurements were also performed after preincubation of blood samples with insulin (final concentration 100 μU/ml) or vehicle for 20 min in vitro before further handling as described above.

2.3.2. Platelet aggregation in whole blood (Multiplate®)

Platelet impedance aggregation in whole blood anticoagulated by hirudin (i.e., with normal extracellular calcium levels) was assessed by multiple electrode aggregometry (Multiplate®, Roche Diagnostics International, Rotkreuz, Switzerland). Agonists used were: ADP (6.5 μmol/L; ADPtest®), arachidonic acid (0.5 mmol/L; ASPItest®), collagen (3.2 μg/mL; COLtest®), and U46619 (0.3 μmol/L).

2.4. Other tests

Plasma glucose (glucose oxidase technique; Hemocue Glucose 201 +, Hemocue AB, Ängelholm, Sweden) and insulin (radioimmunoassay [6]). Complete blood cell counts were measured in samples anticoagulated with EDTA using a MICROS 60 cell counter (ABX Diagnostics, Montpellier, France).

2.5. Statistics

Differences between T1DM and T2DM patients were analysed by Student's unpaired *t*-test for continuous variables, or the chi square test for non-continuous variables. Responses to the meal were analysed by 2-factor repeated measures ANOVAs yielding group effect, meal effect and an interaction term for group × meal. When responses were non-existent in the T1DM group we analysed changes within the T2DM group by Student's *t*-test or (for skewed variables) Wilcoxon's matched pairs test. Mean values and SEMs are given unless otherwise stated. Correlations between platelet activation (P-selectin) and age, BMI, fasting triglycerides or BMI were estimated using Pearson correlation for normally distributed variables and the Spearman rank test for skewed variables. Analyses were performed using STATISTICA 12 software (StatSoft, Tulsa, OK) and probability (P) values are given.

3. Results

Out of 14 T1DM patients screened 11 were enrolled, and out of 15 T2DM patients, 12 were enrolled. Reasons for exclusion were too high HbA1c levels ($n = 3$), thrombocytopenia ($n = 1$), and unwillingness to participate ($n = 2$). T1DM patients were younger, had longer durations of known diabetes, lower BMIs and fasting triglyceride levels, and higher HDL levels than T2DM patients (Table 1).

3.1. Glucose and insulin levels

Patients with T1DM and T2DM had similar fasting glucose and insulin levels (Fig. 1a-b). Glucose levels increased more markedly after the meal in T1DM (from 10.0 ± 0.9 to 22.1 ± 1.4 mmol/L) compared to T2DM patients (from 7.5 ± 0.5 to 11.2 ± 0.6 mmol/L; $p < 0.001$ for group difference) (Fig. 1a). Insulin levels increased from 24.4 ± 4.4 to 68.8 ± 12.3 mU/mL in T2DM ($p = 0.002$) but did not change in T1DM ($p = 0.8$) ($p < 0.001$ for group difference) (Fig. 1b).

Table 1
Patient characteristics.

	Type 1 $n = 11$	Type 2 $n = 12$	*p* value
Male	8	7	0.77
Age, years Median (range)	65 (40–72)	65 (60–76)	0.05
Duration of known diabetes, years Median (range)	32 (10–66)	13.5 (2 − 32)	0.005
Microvascular complications (n)*	7	6	0.7
Hypertension (n)**	5	6	1.0
Hyperlipidemia (n)**	5	7	0.7
Current smokers	0	0	
BMI, kg/m^2 Mean (range)	23.3(18–25.5)	29.7(22.7–35.9)	<0.001
HbA1c, mmol/mol Mean (range)	67.5 (51–90)	59.1(50–67)	0.09
Cholesterol, mmol/L Mean ± SD			
Total	4.9 ± 0.5	4.8 ± 1.0	0.8
LDL	2.8 ± 0.4	2,8 ± 0.8	0.98
HDL	1.8 ± 0.4	1.3 ± 0.4	0.008
Triglycerides	0.7 ± 0.1	1.5 ± 0.8	0.005
Medications			
Metformin	0	10	<0.001
Insulin	11	7	0.04
Statin	4	8	0.2
ACE inhibitor/ARB	3	5	0.7

* Microvascular complications are retinopathy, neuropathy or nephropathy.
** Defined as drug treatment for hypertension and hyperlipidemia.