Platelet activation in CAD patients with type-2 diabetes mellitus: insights into their prothrombotic propensity

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Diabetic patients show an increased risk of developing coronary artery disease (CAD), even if they are in primary prevention with aspirin. Type 2 diabetes mellitus (T2DM)-related metabolic unbalance is associated with haemostatic alterations; several evidences indicate that T2DM enhances platelet reactivity (1, 2).
Our group has recently shown that platelet-associated Tissue Factor (pTF) is increased in CAD patients compared to healthy subjects (3).
No information is still available on pTF expression in CAD patients with T2DM.
Our aim is to provide insight into the enhanced risk of thrombotic complications associated with T2DM in order to clarify pathways altered by diabetes and to find new targets to modulate.
In this study we assessed whether T2DM affects platelet activation markers, including pTF expression and platelet transcriptome profile in CAD patients.
We enrolled 30 CAD patients with T2DM and 30 CAD patients without T2DM; patients from both group were treated with low-dose aspirin.
Assessment of platelet activation markers, such as activated GpIIbIIIa, P-selectin, percentage of monocyte-platelet aggregates and, in particular, pTF was performed by whole blood flow cytometry, both under resting and upon ADP-stimulated conditions.
The global haemostatic function of the two groups of patients was evaluated by thromboelastometry (ROTEM), a technique that assesses in whole blood the kinetics of clot formation as well as the size of the clot, taking into account two important and opposing components of coagulation, thrombus formation and clot lysis.
Moreover platelet transcriptome profiles were studied using Illumina BeadChip Human HT-12 v4 microarray in order to find differences in transcript expression levels between the 2 groups of patients (4).
No differences in GPIIbIIIa and P-selectin expression as well as in the number of platelet-monocyte aggregates were observed in CAD patients with T2DM compared to patients without T2DM; we observed only a trend toward an higher percentage of P-selectin-positive platelets in T2DM patients compared to patients without T2DM. By contrast, T2DM patients showed significantly higher number of TF positive platelets compared to patients without T2DM (3.7±1.2% vs. 2.4±0.5%). Moreover, the TF fold induction upon ADP stimulation was significantly higher in diabetic patients compared to patients without diabetes (30.5±11.7% vs. 17.6±3.9%).
Maximum Clot Firmness, Thrombodynamic Potential Index, and Maximum Velocity of clot formation assessed by ROTEM were all significantly increased in diabetic patients.
Microarray analysis showed that 23 transcripts are differentially expressed in platelets from the 2 groups of patients and that two molecular functions appeared significantly altered by T2DM in CAD patients: alternative splicing and nucleotide binding.
The higher amount of TF positive platelet and the increased global haemostatic function found in T2DM patients further extend our knowledge on the potential mechanism responsible for the T2DM-associated prothrombotic phenotype. The differentially expressed transcripts in T2DM platelets may provide insights into the mechanisms underlying the increased platelet reactivity and/or represent biomarkers for thrombotic risk or targets for developing new drugs.

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