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Platelet Dysfunction in Type 1 Diabetes: Stressing the Thromboxanes



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Overall death from cardiovascular disease (CVD) has declined over the past 20 years; yet, the population with diabetes has seen minimal benefit (1,2). When broken out by diabetes type, individuals with type 1 diabetes mellitus (T1DM) have approximately a 10-fold age-adjusted increased risk of CVD, even higher than that observed in those with type 2 diabetes mellitus (T2DM), with women being more affected than men (3,4). This is in the face of drastically improved insulin therapy regimens over the past decades, suggesting that hyperglycemia itself may not explain this increased CVD. Paradoxically, though, high-quality clinical studies regarding the risks of CVD in T1DM and the potential mechanisms underlying this pathophysiology remain woefully underrepresented as compared with similar studies related to individuals with T2DM, who are known to have persistently elevated platelet reactivity and a depressed inhibitory response to aspirin (5,6). Furthermore, the published studies that do exist are performed variably on pediatric and adult T1DM patients and show highly conflicting results (7–14). Taken together, the current state of the field is a hindrance to the proper care and treatment of individuals with T1DM.

A number of risk factors have been proposed to elevate CVD risk in T1DM patients, including hyperglycemia, dyslipidemia, inflammation, oxidative stress, and genetics, among others (3). A significant contributing factor to the diabetic prothrombotic state is the aberrant regulation of antiplatelet-activating mechanisms that normally maintain high levels of inhibitory cAMP to prevent aggregation (1). Molecules directly affecting platelet cAMP production are the arachidonic acid metabolites thromboxane A₂ (TXA₂) and prostacyclin (PGI₂). TXA₂ is produced in the platelets themselves and is a positive-feedback mediator of platelet activation, while PGI₂ is produced in endothelial cells and is an inhibitor of platelet aggregation (see Fig. 1 for a summary of TXA₂ and PGI₂ synthetic and signaling pathways in the platelet).

In this issue of *Diabetes*, Zaccardi et al. (15) aim to definitively determine the state of platelet reactivity in

adult T1DM patients, the responsiveness of their platelets to aspirin prophylaxis, and the potential mechanisms mediating any platelet dysfunction in T1DM. Study subjects were adult T1DM patients with well-controlled diabetes (mean age 37 years) without any poorly controlled comorbid conditions or pharmaceuticals that could confound interpretation of the results. Baseline characteristics were essentially identical among all of the groups, except for reticulated (immature) platelets, which were slightly lower in the T1DM subjects as compared with the healthy control subjects.

The authors found that T1DM subjects, particularly females, had significantly higher thromboxane metabolite (TXM) excretion, a by-product of TXA₂ released from activated platelets. The oxidative stress urinary marker, 8-iso-PGF_{2α}, and the vascular endothelial cell dysfunction urinary marker, proteinuria, were also elevated in T1DM patients as compared with healthy individuals. Both of these markers correlated strongly and directly with TXM excretion. The metabolite of endothelial cell-derived antiplatelet PGI₂, 2,3-dinor-6-keto-PGF_{1α}, was comparable between T1DM and healthy subjects. Taken together, T1DM subjects show indications of excess TXA₂ production, oxidative stress, and endothelial cell dysfunction, potentially setting up an environment that promotes platelet activation.

As the platelet activator TXA₂ is synthesized by cyclooxygenase 1 (COX-1) in the platelets, the authors wanted to determine if inhibition of COX-1 with aspirin, which is irreversible and semi-selective, could improve T1DM subjects' platelet reactivity. Following once-daily 100 mg aspirin consumption for 21 days, sera from healthy and T1DM subjects were analyzed for TXB₂, a readout of maximal biosynthetic capacity of platelet COX-1, which showed a comparable reduction at 12 and 24 h after treatment in both T1DM and healthy control subjects. Although the 7-day recovery kinetics of serum TXB₂ and the baseline-adjusted urinary TXM from T1DM and healthy subjects were comparable, the amount of TXM in the urine of T1DM patients remained elevated compared with healthy control

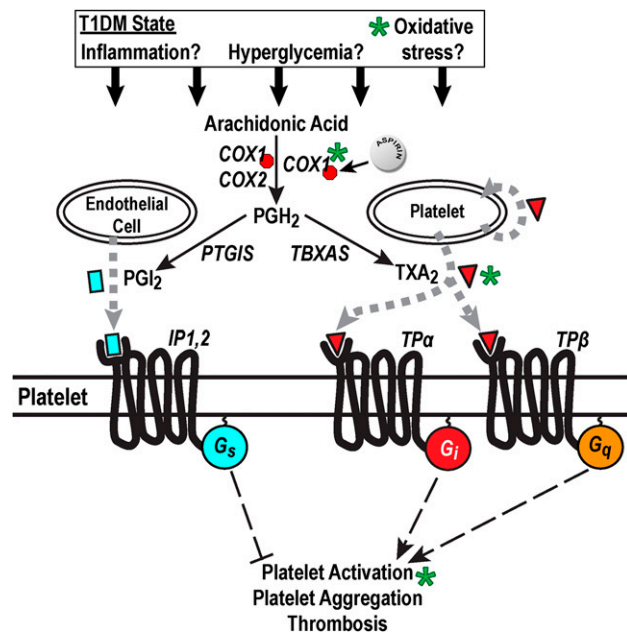


Figure 1—Summary of the platelet activation pathways addressed in Zaccardi et al. (15) and how these may be impacted by factors in the T1DM state and aspirin prophylaxis. The arachidonic acid metabolites PGI₂ and TXA₂ have opposing impacts on platelet activation, with PGI₂ acting through a pair of G_s-coupled G-protein-coupled receptors (GPCRs), IP1 and IP2, to inhibit downstream platelet activation. PGI₂ is produced from endothelial cells in the vascular wall from the unstable intermediate, PGH₂, by the enzyme prostaglandin I synthase (PTGIS). TXA₂, on the other hand, is produced from PGH₂ by thromboxane A synthase (TBXAS) in the platelets themselves and works through a pair of G_i- and G_q-coupled GPCRs, TP α and TP β , to promote downstream platelet activation. PGH₂ is produced by both COX-1 and COX-2, with COX-1 being of primary importance in the platelet. Zaccardi et al. (15) showed that platelet TXM is persistently elevated in adult T1DM subjects and that this correlates with increased oxidative stress but not inflammation or hyperglycemia and that production of TXA₂ metabolites can be effectively ameliorated by treatment with low-dose aspirin, an irreversible and semi-selective inhibitor of COX-1. Proteins and enzymes are shown in boldface italic type and steps that are specifically revealed to be important in this T1DM population by the current study are identified by green asterisks. Further mechanistic studies will be important to confirm the specific molecular signaling pathways at play in this population.

subjects. This suggests that T1DM platelets are still responsive to aspirin.

This well-designed, well-conducted, and interesting study has many strengths. First, although relatively small, it was adequately powered based on a pilot study found in the Supplementary Data online (see ref. 15) and by using stringent inclusion and exclusion criteria, allowing the authors to make some definitive conclusions without certain confounders. Second, the T1DM and healthy control groups were very well matched for a number of clinical and biometric parameters that could have significantly impacted results, such as age, sex distribution, and BMI. Third, the authors used a number of different clinical and research laboratory tests to evaluate each of their hypotheses. Finally, in the aspirin intervention study, subject adherence was determined not

only by pill counts but by direct measurement of serum TXB₂ levels at two different time points. These latter two strengths add significantly to the authors' ability to make strong conclusions based on the results of their study.

There are some limitations to study by Zaccardi et al. (15) that need to be considered, though. First and foremost is a lack of clinical end points. The authors show persistently high TXA₂ and TXB₂ levels in T1DM versus healthy control subjects, as well as the strong ability to blunt platelet activity with aspirin; yet, there is no correlation with the progression to CVD or the ability to prevent this progression with aspirin prophylaxis. Second, the stringent inclusion and exclusion criteria, while necessary to decrease confounders, may limit the overall relevance of this study to all T1DM patients, particularly those that are obese or have already been diagnosed with CVD. Third, other signaling pathways that impact on platelet activation were not considered in the current study. Finally, the specific molecular mechanisms behind increased oxidative stress in T1DM individuals and how this impacts on TXA₂ production and how signaling pathways downstream in the platelet are being affected were not elucidated in this study. Addressing these limitations will make for interesting future directions.

In conclusion, the article by Zaccardi et al. (15) has helped to resolve controversies in the literature regarding platelet reactivity in T1DM. It also distinguishes the state of platelet reactivity in T1DM from T2DM by showing that T1DM individuals have a strong and appropriate response to aspirin prophylaxis. This work provides the rationale for a larger, randomized controlled trial of aspirin prophylaxis in a broader range of T1DM individuals, where the clinical end points should be protection from or amelioration of CVD.

Duality of Interest. No potential conflicts of interest relevant to this article were reported.

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