Measurements of thromboxane production and their clinical significance in coronary heart disease

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Thromboxane A2 is the main product of arachidonic acid (AA) metabolism through the action of cyclooxygenase (COX)-1 in platelets and COX-2 in monocytes and other nucleated cells (including endothelial cells) where COX-2 can be expressed in response to inflammatory and mitogenic stimuli. Both cyclooxygenases convert AA to prostaglandin (PG) G2 through their COX activity, and then reduce PGH2 to PGH2 through their peroxidase activity. From PGH2, different eicosanoids are synthesised through specific isomerases: thromboxane (TX) A synthase, mostly in platelets, specifically isomerises PGH2 to TXA2. TXA2 is a potent vasoconstrictor and platelet agonist, with a central role in platelet aggregation and thrombosis. Therefore, the inhibition of TX biosynthesis, throughout the inactivation of platelet COX activity by low-dose aspirin, is a mainstay of antiplatelet therapy in coronary heart disease. COX-1 inhibition by low-dose aspirin accounts for about 25% of risk reduction in the secondary prevention of vascular disease.

However, several patients continue to experience occlusive vascular events despite aspirin therapy. It has been proposed that some of such thrombotic events occurring despite aspirin may be partly explained by an incomplete suppression of TX biosynthesis, contributing to the so-called phenomenon of “aspirin resistance” (1, 2). For this reason, beside platelet function tests, the measurement of residual TX production despite the use of aspirin has been widely used in research as a tool to assess aspirin complete or incomplete pharmacological efficacy.

The first approaches to measure eicosanoids date from the 1970s, when researchers attempted at identifying the biologic actions of these autacoids and their role in human physiology and disease (3). Some eicosanoids are however extremely evacuated in biologic media. TXA2, specifically, has an estimated half-life of 30 seconds in plasma, due to chemical instability of the oxane ring. In aqueous media, TXA2 is rapidly converted to the chemically stable and biologically inactive hydration product TXB2 (4). While this is the final end product in vitro and ex vivo, TXB2 is further metabolised through β-oxidation in vivo, resulting in the formation of 2,3-dinor-TXB2 and, through dehydrogenation, in a series of metabolites among which 11-dehydro-TXB2 has a longer plasma half-life and is excreted at higher levels than the others (5). Gas chromatography/mass spectrometry (GC), radioimmunoassay (RIA), and – more recently – enzyme immunoassays (EIA) are the most commonly analytic techniques used to measure TX metabolites in biologic fluid. The choice of the appropriate target for biochemical analysis is not, however, simple, and has been the object of numerous investigations. Because of its short half-life, TXA2 cannot be easily measured. TXB2 should reflect the generation of its active precursor. However, the capacity of platelets to synthesise TXA2 greatly exceeds the actual production in vivo; since platelets are highly susceptible to activation ex vivo during and after blood sampling, plasma concentration of TXB2 as revealed by any assays, are by far higher than the reported in vivo levels. Indeed, it has been argued that as little as 0.1% platelet activation would lead to apparent plasma concentration of 200–400 pg/ml, compared with estimated basal circulating concentration ≤2 pg/ml (6). For this reason, TXB2 concentrations in plasma are thought by many to be an extremely unreliable index of TX generation in the circulation.

Attention has consequently focused on the measurement of 2,3-dinor-TXB2 and 11-dehydro-TXB2 in plasma or urine. Metabolic transformation of TXB2 occurs predominantly in the liver rather than in the bloodstream, and measurements of levels of such metabolites would thus bypass the problem of ex vivo platelet activation: because of its lesser polarity and longer half-life, 11-dehydro-TXB2 is currently the metabolite of choice to monitor steady state TX production in vivo, with plasma concentrations in the order of 1–2 pg/ml (7). The urinary excretion of 11-dehydro-TXB2 has been indeed shown to increase linearly as a function of the rate of TX entry into the circulation (8). Even this method, however, is not specific for the platelet source of production of TX, and it has been argued that about 30% of urinary 11-dehydro-TXB2 derives from extra-platelet sources, with the fraction of extra-platelet production likely increasing with the degree of inflammation (9). Finally, a common method to evaluate the production of platelet TXA2 is the measurement of TXB2 generated in clotted blood after 1 hour incubation in glass tubes at 37°C, with a production of TXB2 here in the order of 300–400 ng/ml (10). This method has been proven to be extremely valuable in characterising the dose-response relationship between platelet production capacity and the dose of inhibitors, such as aspirin; and has been used to detect the possible failure of these drugs in suppressing antiplatelet activity.

Besides a physiologic function of platelets in primary haemostasis and endothelial repair, platelets participate in athero-
sclerosis releasing many inflammatory mediators and interacting with endothelial cells and leukocytes (11). TX is one of the main actors in such processes because it is a targetable mediator of platelet aggregation and vasoconstriction, and it is also a marker of disease activity. Indeed, a residual TX production despite aspirin therapy has been implicated as a cause of enhanced platelet function, and elevated urinary excretion of 11-dehydro-TXB₂ despite aspirin has been related to a higher risk of cardiovascular events (12). The mechanisms underlying the escape of TX production despite the almost complete (>95%) suppression of platelet-derived serum TX are not entirely identified. Residual TX production, as revealed by different methods, may derive from COX-1 or COX-2. Increased platelet turnover, occurring in particular clinical conditions of increased platelet-vessel wall interactions, such as diabetes or extensive peripheral arterial disease, or in conditions of primarily increased platelet production, such as in polycthemia vera, may partly explain the apparent failure or limited efficacy of aspirin to reduce major cardiovascular events in such conditions, as a faster renewal of platelet COX-1 may overcome the effectiveness of the usual once-daily low-dose aspirin in inhibiting TX biosynthesis (13). On the other hand, COX-2, transiently expressed in newly formed platelets or megakaryocytes (14), and scarcely sensitive to low-dose aspirin, may contribute to enhanced TX production in this setting.

In addition, extra-platelet sources may contribute to aspirin-insensitive TX generation: monocytes/macrophages and vascular endothelial cells express COX-2 in response to inflammatory stimuli, and the up-regulation of COX-2 activity may account for a TX biosynthesis not sensitive to once daily low-dose aspirin (11). These observations are in agreement with a recent study showing that serum TXB₂ levels and platelet aggregation, evaluated by AA-induced light transmittance aggregometry, after administration of aspirin are related with platelet count, inflammatory markers, current smoking and diabetes mellitus (15). Increased TX production despite aspirin therapy may also occur because of genetic polymorphisms of genes involved in TX biosynthesis (16), probably predicting the future risk of cardiovascular events (17). Finally, drug interaction, the most well documented of which is the reduced platelet inhibition observed during concomitant administration of non-steroidal anti-inflammatory drugs (NSAIDs) (18, 19), has been advocated as a mechanism of aspirin failure, but observational studies and post-hoc analyses have provided neither consistent nor definitive evidence that this interaction is clinically important (20).

In this issue of Thrombosis and Haemostasis, Niccoli et al. (21) investigated the relationship between angiographic coronary atherosclerotic burden and residual platelet activation, assessed by baseline plasma levels of TXB₂, in patients with non-ST-elevation acute coronary syndromes treated with low-dose aspirin. The investigators measured plasma concentration of TXB₂, and divided patients into quartiles on the basis of TX levels. They observed that the severity and extent of coronary artery disease, as well as platelet count, independently predicted the residual production of TX in patients treated with aspirin after an acute coronary syndrome, thus identifying the extent of atherosclerosis as a potential cause of residual TX production.

The present study has some limitations, which should be clearly outlined. First, the measurement of plasma TXB₂ does not specifically reflect the in vivo production of TXA₂ as highlighted above, and is highly affected by in vitro platelet activation. The authors apparently excluded such possibility of an artefactual increase of TX by the parallel use of another assay measuring platelet-derived thrombospondin-1 levels; but still, the small contribution of true in vivo TX production compared with the huge potential of TX production by platelets under maximal stimulation remains a source of concern. In addition, since there was no control group, it is not possible to compare the reported levels of TXB₂ in patients with those of stable patients, or of control subjects, treated with aspirin. Finally, the authors did not substantiate their findings by the measurements of urinary 11-dehydro-TXB₂, or platelet functional tests, which would have been desirable.

Despite such limitations, however, the authors’ findings are of interest because they suggest that, in a clinical setting characterised by inflammatory activation, elevated platelet turnover and platelet-mono cyte interactions, as may occur in patients with acute coronary syndromes, everything else being equal, the extent of coronary atherosclerosis may explain the higher production of TX, either by COX-1– or COX-2-related mechanisms. This may partially overcome aspirin inhibition and, conceivably, aspirin antiplatelet efficacy. Higher plasma TXB₂ levels in their patient cohort indeed appear to be related to the extent and severity of underlying coronary atherosclerosis. Also referring to previous data from several years ago demonstrating high levels of urinary TX metabolites in patients not treated with aspirin and with severe atherosclerosis (7), these data indicate that residual TX is a marker of disease severity. Of note, the same investigators have previously reported that plasma levels of TXA₂ on admission are associated with no-reflow after primary percutaneous coronary intervention (22).

In principle – and this is an important practical implication – residual TX is also possibly the target of antiplatelet therapies or administration schemes better than currently done with the dogma of once daily low-dose aspirin. This latter has recently been challenged, as twice daily dosing of aspirin has been shown to improve platelet inhibition in whole blood in patients with type 2 diabetes mellitus and micro- or macrovascular complications (23). Also, once daily aspirin does not provide stable 24-hour antiplatelet protection in a significant proportion of patients with coronary artery disease (15). Indeed, the study by Niccoli et al. (21) provides additional data that residual TX should be a focus of better strategies to improve cardiovascular outcomes.

Conflicts of interest

None declared.

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References


