COX-1 sensitivity and thromboxane A₂ production in type 1 and type 2 diabetic patients under chronic aspirin treatment

Fabio M. Pulcinelli¹*†, Luigi M. Biasucci²†, Silvia Riondino¹, Simona Giubilato², Andrea Leo², Livia Di Renzo¹, Elisabetta Trifirò¹, Teresa Mattiello¹, Dario Pitocco³, Giovanna Liuzzo², Giovanni Ghirlanda³, and Filippo Crea²

¹Department of Experimental Medicine, 'Sapienza' University of Rome, Viale Regina Elena 324, 00161 Rome, Italy; ²Institute of Cardiology, Catholic University, Rome, Italy; and ³Department of Internal Medicine, Catholic University, Rome, Italy

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Aims
Although aspirin treatment is useful in reducing ischaemic events in diabetic patients, recent studies suggest that it is less effective when compared with non-diabetics (ND). We sought to evaluate COX-1 sensitivity and thromboxane A₂ (TxA₂) production in type 1 (T1DM) and type 2 diabetic (T2DM) patients under chronic aspirin treatment, and also evaluate the association between thromboxane A₂ (TxA₂) production and markers of inflammation and metabolic control, such as high-sensitivity C-reactive protein, fasting blood glucose, and haemoglobin A₁c (HbA₁c).

Methods and results
Agonist-induced platelet aggregation (PA) and TxB₂, a stable metabolite of TxA₂, production, serum TxB₂, and platelet COX-1 and COX-2 expression were studied in T2DM patients, T1DM patients, and high-risk ND subjects, all receiving a low dose of aspirin. TxB₂ formation was studied in platelets treated in vitro with aspirin alone or with a COX-2 inhibitor (NS-398). PA, collagen-induced TxB₂ production, and serum TxB₂ were higher in T1DM and T2DM patients than in ND subjects. TxB₂ production was reduced in diabetic patients by in vitro treatment with aspirin. COX-2 was expressed in all diabetic patients but only in 46% of ND patients. In diabetic patients significant correlations were observed between TxB₂ production and both fasting plasma glucose and HbA₁c.

Conclusion
COX-1 sensitivity and TxB₂ production is similarly reduced in both T1DM and T2DM patients under chronic aspirin treatment. The association between TxB₂ production and either fasting plasma glucose and HbA₁c levels suggests that in diabetic patients hyperglycaemia is a determinant of the reduced platelet sensitivity to aspirin.

Keywords
Diabetes • Aspirin • Cyclooxygenase • Platelets • Thromboxane

Introduction
The benefit of aspirin in diabetic patients has been consistently documented in several trials.¹² Yet, in the meta-analysis of the Antithrombotic Trialists’ Collaboration, the event rate of diabetic patients on treatment was similar to that of non-diabetic (ND) patients off treatment.³ In the Primary Prevention Project Trial⁴ aspirin treatment reduced cardiovascular events and deaths in high-risk ND patients, but not in patients with type 2 diabetes mellitus (T2DM). Furthermore, in the recent Japanese Primary Prevention of Atherosclerosis With Aspirin for Diabetes Study⁵ a low dose of aspirin in primary prevention did not reduce the risk of cardiovascular events at 4 years in diabetic patients. Subset analyses of other studies in secondary prevention similarly suggested that aspirin might be less effective in T2DM,⁶⁷ especially in patients with poor metabolic control, than in ND patients, the underlying mechanism being still largely debated.⁸ It has been proposed that reduced sensitivity to aspirin in diabetic patients might be owing to accelerated thrombopoiesis⁹ or to reduced platelet permeability to aspirin caused by membrane glycosylation.¹⁰

* Corresponding author. Tel: +39 (0) 6 49973002, Fax: +39 (0) 6 4454820, Email: fabio.pulcinelli@uniroma1.it
† The first two authors contributed equally to the study.

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Because of the different pathogenic mechanisms, patients with type 1 diabetes (T1DM) were included to test the effects of hyperglycaemia in the presence of different degrees of activation of inflammatory cells, as shown in previous studies. In order to study the platelet sensitivity to aspirin in diabetic patients, we designed a prospective case–control study to compare platelet aggregation (PA) and thromboxane B₂ (TxB₂) production, a stable metabolite of thromboxane A₂ (TxA₂), in patients with T2DM, T1DM, and in high-risk ND subjects under chronic aspirin treatment. Correlations between fasting blood glucose, haemoglobin A₁c (HbA₁c), high-sensitivity C-reactive protein, and TxB₂ production were performed in order to assess the relative role of hyperglycaemia and inflammation with COX1 sensitivity and TxB₂ production. We also performed, in a subgroup of our population, further in vitro analyses to assess the molecular mechanisms responsible for the reduced sensitivity of diabetic patients to aspirin.

Methods

From May 2005 to October 2007 we enrolled in our hospital 150 diabetic patients, 120 with T2DM and 30 with T1DM, under chronic treatment with low dose of aspirin (100–160 mg) for primary or secondary prevention. Overall, 20 T2DM patients and 4 T1DM patients were excluded from the study, owing to use of other drugs, apart from aspirin, known to interfere with platelet function (non-steroidal anti-inflammatory drugs, ticlopidine, clopidogrel, cilostazol) in the previous 10 days (T2DM, n = 10; T1DM, n = 3); use of oral anticoagulant or heparin (previous two days; T2DM, n = 3), chronic or acute inflammatory diseases (T2DM, n = 4), or plasma salicylate concentrations <0.02 μg/mL, as a marker of lack of compliance to aspirin treatment (T2DM, n = 3; T1DM, n = 1). Thus, a total of 100 consecutive T2DM patients and 26 T1DM patients were included in the study. They were compared with a CHARISMA-like population of 100 high-risk ND subjects without history of diabetes on aspirin for primary or secondary prevention, followed-up in our outpatient clinic in order to monitor antiplatelet treatment, as recommended by their family doctor. In all of the control population, a screening for diabetes was performed measuring fasting blood glucose. All controls had a threshold salicylate level for patient enrolment was fixed at 200 μg/dL. No data were found on TxB₂. Therefore, we used the data by Watala and with this assumption we have calculated a population of at least 25 patients to be sufficient for our aim. The study was approved by the Ethics Committees of the Catholic and of ‘La Sapienza’ University, and all patients gave their consent to use part of their blood sample for scientific purpose. All patients underwent the laboratory assays planned for the study.

Blood sampling

Three blood samples were obtained by clean venipuncture after overnight fasting. The first sample was drawn in test tubes containing one-tenth volume of Na-citrate 3.8% and used to prepare platelet-rich plasma (PRP); the second and the third were drawn in test tubes without anticoagulant and used for serum measurements (see below).

Sample preparation

PRP was prepared by centrifugation at 200 g for 15 min at room temperature. In order to minimize the presence of white blood cells (WBC), PRP was further centrifuged at 180 g for 5 min, and the platelet count was adjusted to 2.5 × 10⁹/mL with Tyrode’s buffer at pH 7.35. Patients with WBC count above 0.1 × 10⁹ cells/µL were excluded from the study. Cell count was analysed on a Hemalaser II SEBIA routine haematology blood counter (Sebia, Italy).

Platelet-poor plasma was obtained after further centrifugation of PRP at 2000 g for 10 min and used for the measurement of plasma salicylate level. Peripheral blood mononuclear cells (PBMC) were prepared on a Ficoll hypaque density gradient.

Glycaemic control

Both glycated haemoglobin (HbA₁c), as a long-term monitor of average glycaemia, and daily control of fasting plasma glucose levels were evaluated. For each patient, HbA₁c mean levels were obtained using HPLC and analysis performed using Diamat BioRad (BioRad, Milan, Italy). The HbA₁c reference range for healthy subjects was 4.3–5.9%. Fasting plasma glucose levels were analysed by the glucose oxidase method.

C-reactive protein measurement

Serum high-sensitivity C-reactive protein levels were measured using a high-sensitivity nephelometric system (Latex BNII, Dade-Behring, Glasgow, Delaware, NJ, USA) with a detection limit of 0.1 mg/L.

Plasma salicylate levels

In all patients quantitative analysis of plasma concentrations of salicylate was performed using HPLC according to Cerletti et al. The threshold salicylate level for patient enrolment was fixed at 0.02 μg/mL.

Platelet aggregation

PA (Born’s Method) was evaluated on PRP in an AggRAM (Helena Biosciences, Sunderland, UK) aggregometer as previously described. The results are reported as the maximal percentage of aggregation (Mx%) observed after 4 min stimulation in response to collagen (4 μg/mL), arachidonic acid (AA; 1 mM), and adenosine diphosphate (ADP; 2 μM) (all from Helena Laboratories). Concurrent controls were performed to ensure that all agonists retained the same level of activity during the whole study.
Thromboxane B2 production

TxB2 production was evaluated by blocking collagen-induced platelet activation after 4 min with indomethacin (10 μM); samples were then spun at 8000 g for 1 min. TxB2 was measured in the sample supernatants as well as in serum samples using an EIA commercial kit (Cayman Chemical Company, Ann Arbor, MI, USA). Collagen was selected as platelet agonist as it has been used to identify residual platelet activation, both TxA2-dependent and -independent.20,21 In vitro substudies were performed in 50 T2DM and all (n = 100) T1DM patients. Platelets of these patients already on aspirin treatment were further pre-incubated in vitro (10 min at 37°C prior to agonist addition) with aspirin (Sigma Chemicals Co., St Louis, MO, USA) at a concentration (100 μM) required to completely abolish AA-induced TxB2 production22,23 with or without 10 μM of the COX-2 inhibitor NS-398 (Sigma Chemicals), as COX-2-dependent TxB2 production can be studied only in cells pre-treated with aspirin.24 Control platelets were treated with the inhibitor solvent (dimethyl sulfoxide).

COX-1 and COX-2 assessment

Cells (1.5 × 10⁷ platelets for COX-1 studies and 7.5 × 10⁶ platelets for COX-2 studies; 0.5–1.5 × 10⁷ PBMC) from 50 T2DM, all (n = 26) T1DM patients, and 50 controls were lysed using RIPA buffer and resolved using 10% sodium dodecyl sulfate–polyacrylamide gel electrophoresis. Following electrophoresis proteins were transferred to Immobilon-P (Millipore, Bedford, MA, USA) membranes and identified with anti-COX-1 or anti-COX-2 monoclonal antibodies (Cayman Chemical), followed by horseradish peroxidase-conjugated secondary antibody and detected with ECL chemiluminescence reaction reagent (both from Amersham Pharmacia, Biotech, Little Chalfont, UK) and Kodak X-ray film (X-OMAT AR). For COX-2 detection, lysates of PBMC cells (7.5 × 10⁶) activated with lipopolysaccharide were used as positive controls.25

Statistical analysis

As the distributions of TxB2 and high-sensitivity C-reactive protein values were non-normal according to Kolmogorov–Smirnov, the differences between groups were analysed using non-parametric tests—Kruskal–Wallis or Wilcoxon signed rank—as appropriate. The remaining continuous variables were compared using analysis of variance. Corrections for multiple comparisons were performed using Dunnett’s or Dunn’s test as appropriate. Categorical data were compared using χ² test. Non-parametric Spearman’s correlation

### Table 1 Baseline characteristics of patients

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Type 1 (n = 26)</th>
<th>Type 2 (n = 100)</th>
<th>Non-diabetics (n = 100)</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (mean ± SD, years)</td>
<td>59 ± 8</td>
<td>68 ± 8</td>
<td>64 ± 9</td>
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<tr>
<td>Males, n (%)</td>
<td>12 (46)</td>
<td>57 (57)</td>
<td>50 (50)</td>
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<tr>
<td>Clinical conditions</td>
<td></td>
<td></td>
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<td></td>
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<tr>
<td>Hypertension, n (%)</td>
<td>21 (80)</td>
<td>74 (74)</td>
<td>67 (67)</td>
<td>ns</td>
</tr>
<tr>
<td>Hypercholesterolaemia, n (%)</td>
<td>16 (61)</td>
<td>52 (52)</td>
<td>33 (33)</td>
<td>ns², 0.01⁴, 0.01⁴</td>
</tr>
<tr>
<td>Smoke, n (%)</td>
<td>8 (31)</td>
<td>22 (22)</td>
<td>15 (15)</td>
<td>ns</td>
</tr>
<tr>
<td>Previous MI, n (%)</td>
<td>5 (19)</td>
<td>48 (48)</td>
<td>20 (20)</td>
<td>0.008⁸, &lt;0.0001⁹, ns¹⁰</td>
</tr>
<tr>
<td>Previous stroke, n (%)</td>
<td>0 (0)</td>
<td>10 (10)</td>
<td>5 (5)</td>
<td>ns</td>
</tr>
<tr>
<td>Previous PAD, n (%)</td>
<td>1 (0.4)</td>
<td>10 (10)</td>
<td>0 (0)</td>
<td>ns², 0.002⁹, ns¹⁰</td>
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<td>HbA1c %</td>
<td>7.72 ± 1.06</td>
<td>7.0 ± 1.21</td>
<td></td>
<td>ns¹</td>
</tr>
<tr>
<td>Fasting plasma glucose (mg/dL)</td>
<td>183.6 ± 38.22</td>
<td>170.5 ± 39.98</td>
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<td>ns³</td>
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<td>Medications</td>
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<tr>
<td>Beta-blocking agents, n (%)</td>
<td>1 (4)</td>
<td>25 (25)</td>
<td>18 (18)</td>
<td>ns</td>
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<td>Calcium antagonist, n (%)</td>
<td>7 (27)</td>
<td>34 (34)</td>
<td>11 (11)</td>
<td>ns², &lt;0.0001⁷, 0.05⁷</td>
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<td>ACE-inhibitors/ARBs, n (%)</td>
<td>19 (73)</td>
<td>72 (72)</td>
<td>53 (53)</td>
<td>ns², 0.008⁸, ns¹⁰</td>
</tr>
<tr>
<td>Nitrates, n (%)</td>
<td>0 (0)</td>
<td>8 (8)</td>
<td>8 (8)</td>
<td>ns</td>
</tr>
<tr>
<td>Statin, n (%)</td>
<td>16 (61)</td>
<td>52 (52)</td>
<td>24 (24)</td>
<td>ns², &lt;0.0001⁷, 0.001⁸</td>
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<td>Diuretics, n (%)</td>
<td>5 (19)</td>
<td>28 (28)</td>
<td>6 (6)</td>
<td>ns², &lt;0.0001⁷, 0.049⁸</td>
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<td>Insulin, n (%)</td>
<td>26 (100)</td>
<td>20 (20)</td>
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<tr>
<td>Metformin, n (%)</td>
<td>0 (0)</td>
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<td>Sulfonylureas, n (%)</td>
<td>0 (0)</td>
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<tr>
<td>Repaglinide, n (%)</td>
<td>0 (0)</td>
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<td>Acarbose, n (%)</td>
<td>0 (0)</td>
<td>3 (3)</td>
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<tr>
<td>Aspirin dosage</td>
<td></td>
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<tr>
<td>100 mg/day, n (%)</td>
<td>24 (93)</td>
<td>76 (76)</td>
<td>77 (77)</td>
<td>ns</td>
</tr>
<tr>
<td>160 mg/day, n (%)</td>
<td>2 (7)</td>
<td>24 (24)</td>
<td>23 (23)</td>
<td>ns</td>
</tr>
</tbody>
</table>

MI, myocardial infarction; PAD, peripheral artery disease; HbA1c, haemoglobin A1c; ACE-inhibitor, angiotensin-converting enzyme-inhibitor; ARBs, angiotensin-receptor blockers.

¹T1DM vs. T2DM.
²T2DM vs. ND.
³T1DM vs. ND.
⁴P-value.
coefficient was used to assess the relationship between collagen-induced TxB2 formation and serum TxB2 levels. Tests for linear trends were computed using an ordinal variable for biomarker quartiles.

Significance was accepted at the \( P < 0.05 \) level. Data are reported as mean \( \pm \) SD and, when appropriate, as medians and ranges. All tests were two-tailed and the data were analysed using Stata version 6.0 (College Station, TX, USA; Stata Corporation, 1999).

### Results

#### Baseline measurements

Both T2DM and T1DM patients displayed a significantly greater Max% PA, compared with ND subjects, following activation by ADP (\( P = 0.01 \) for both comparisons), by collagen (\( P = 0.02 \) for both comparisons), or by AA (\( P = 0.01 \) for both comparisons). Of note, all patients showed AA-induced PA \(<20\%\), except one who had an AA-induced PA of 24%. In contrast, no difference was found between T1DM and T2DM patients (Figure 1).

Serum TxB2 levels were significantly higher in T2DM patients than in ND subjects (\( P = 0.001 \)). Although serum TxB2 levels were markedly increased in T1DM, this difference was not significant (\( P = 0.32 \)), and no significant difference was found between T1DM and T2DM patients (\( P = 0.29 \); Figure 2A). Similarly, collagen-induced TxB2 production was significantly higher in T2DM patients than in ND subjects (\( P = 0.001 \)). Although collagen-induced TxB2 in T1DM were markedly increased compared with ND, this difference was not significant (\( P = 0.7 \)), and no difference was found between T1DM and T2DM patients (\( P = 0.6 \); Figure 2B).

In each group a statistically significant correlation was found between serum TxB2 levels and both collagen-induced PA (\( R = 0.796, P = 0.03 \) in T1DM, \( R = 0.654, P = 0.04 \) in T2DM and \( R = 0.776, P = 0.03 \) in ND) and collagen-induced TxB2 formation (\( R = 0.878, P = 0.005 \) in T1DM, \( R = 0.579, P = 0.04 \) in T2DM and \( R = 0.809, P = 0.04 \) in ND). No differences were found between patients under aspirin treatment for primary or secondary prevention in terms of PA (Max%: 57.9 \( \pm \) 12.4 vs. 56.5 \( \pm \) 14.1 for collagen, \( P = 0.28 \); 16 \( \pm \) 4 vs. 14 \( \pm \) 1 for AA, \( P = 0.89 \); 41.7 \( \pm \) 10.2 vs. 39.3 \( \pm \) 13.3 for ADP, \( P = 0.59 \)) and in terms of collagen-induced TxB2 production (113 \( \pm \) 407 pg/10⁸ cells in primary prevention vs. 705 \( \pm \) 255 pg/10⁸ cells in secondary prevention, \( P = 0.3 \)).

We did not find any treatment-dependent differences in our patients, particularly between insulin and oral hypoglycaemic agents, in terms of PA (data not shown). In diabetic patients with fasting plasma glucose \(<126 \text{ mg/dL} \), serum TxB2 production was 625 \( \pm \) 182 pg/mL, while in those with fasting plasma glucose \( \geq 126 \text{ mg/dL} \), serum TxB2 production was 1793 \( \pm \) 203 pg/mL (\( P = 0.04 \)). Weak but significant correlations were observed between TxB2 production and either plasma glucose (\( R = 0.25, P = 0.014 \)) or HbA1c levels (\( R = 0.22, P = 0.026 \)). Furthermore, by dividing patients into quartiles defined by the distribution of fasting plasma glucose (Figure 3A) or of HbA1c levels (Figure 3B), we observed significantly higher TxB2 production from bottom to top quartile (\( P \) for trend = 0.009 and 0.012, respectively). Serum high-sensitivity C-reactive protein levels were significantly higher in T2DM patients than in T1DM patients and in ND subjects (2.40 mg/L, range 0.15–18.8 mg/L vs. 0.15 mg/L, range 0.15–10.7 mg/L and 1.53 mg/L, range 0.15–12.4 mg/L, respectively, \( P = 0.01 \) for both). No correlation was found between serum high-sensitivity C-reactive protein levels and serum TxB2 production (\( R = –0.3, P = 0.1 \)). Furthermore, we did not find any correlation between serum TxB2 production and plasma salicylate levels (\( R = 0.14, P = 0.7 \)).

#### In vitro studies

In platelets from T1DM patients, in vitro incubation with 100 \( \mu \text{M} \) aspirin significantly reduced collagen-induced TxB2 production, compared with untreated platelets (44.6 \( \pm \) 12.7 pg/10⁸ cells vs. 845.4 \( \pm \) 381.9 pg/10⁸ cells, \( P = 0.003 \)). The incubation with 100 \( \mu \text{M} \) aspirin and 10 \( \mu \text{M} \) NS-398 caused a further reduction of TxB2 production that was not significant, however, vs. aspirin alone (34.3 \( \pm \) 10.1 pg/10⁸ cells vs. 845.4 \( \pm \) 381.9 pg/10⁸ cells, and 34.3 \( \pm \) 10.1 pg/10⁸ cells vs. 44.6 \( \pm \) 12.7 pg/10⁸ cells; \( P = 0.0012 \) vs. non-treated and \( P = 0.71 \) vs. acetylsalicylic acid (ASA) alone, respectively). Similarly, in platelets obtained from 50 T2DM patients, in vitro incubation with 100 \( \mu \text{M} \) aspirin significantly reduced collagen-induced TxB2 production, compared with untreated platelets (61.4 \( \pm \) 11.5 pg/10⁸ cells vs. 896.4 \( \pm \) 232.3 pg/10⁸ cells, \( P = 0.0014 \)). The incubation with both 100 \( \mu \text{M} \) aspirin and 10 \( \mu \text{M} \) NS-398 caused a further reduction of TxB2 production that was not significant when compared with aspirin alone (48.1 \( \pm \) 9.2 pg/10⁸ cells vs. 896.4 \( \pm \) 232.3 pg/10⁸ cells, and 48.1 \( \pm \) 9.2 pg/10⁸ cells vs. 61.4 \( \pm \) 11.5 pg/10⁸ cells; \( P = 0.001 \) vs. non-treated and \( P = 0.26 \) vs. ASA, respectively, Figure 4).

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**Figure 1** Platelet aggregation (PA) is higher in both type 1 (T1DM) and type 2 (T2DM) patients than in non-diabetic (ND) subjects. PA in ND (closed columns), T1DM (open columns), and T2DM (dotted columns) patients under chronic treatment with aspirin. PA was analysed as maximal aggregation (Max%) in response to adenosine diphosphate (ADP; 2 \( \mu \text{M} \)), collagen (4 \( \mu \text{g/mL} \)), and arachidonic acid (AA; 1 mM).
COX-1 and COX-2 expression

COX-1 was highly expressed in all groups. Conversely, COX-2 was expressed in all diabetic patients analysed (n = 26 T1DM; n = 50 T2DM), but in only 23 out of 50 ND subjects (P = 0.03). However, it is worth noting that in all patients the amount of COX-2 was markedly lower than that of COX-1, in spite of the smaller amount of platelet proteins loaded for COX-1 assessment (1.5 x 10⁷ platelets) with respect to those used for COX-2 detection (7.5 x 10⁷ platelets). Figure 5 reports a representative western blot with samples from three T2DM and two ND subjects. The possibility of WBC-dependent COX-2 contamination in the platelet suspension was evaluated by analysing COX-2 expression in lysates of PBMC from the same patients, loaded up to a concentration five times higher (1.5 x 10⁵ PBMC) than the upper cut-off of total WBC for patient enrolment (see Methods). COX-2 was undetectable in such PBMC lysates (data not shown), demonstrating that the COX-2 detected in platelet lysates from our patient populations was of intra-platelet origin only.

Figure 2 Platelet and serum thromboxane B₂ (TxB₂) production in type 1 (T1DM), type 2 (T2DM) diabetic patients, and in non-diabetic (ND) subjects. Serum TxB₂ concentration (expressed as picograms/millilitre) (A) and collagen-induced (4 µg/mL) TxB₂ production (expressed as pg/10⁸ cells) (B) in ND (dark grey columns), T1DM (white columns), and T2DM (light grey columns) patients under chronic treatment with aspirin.

Figure 3 Association between serum thromboxane B₂ (TxB₂) production and glycaemic control. Comparison between quartiles of fasting glucose plasma levels (expressed in mg/dL) (A) or haemoglobin A1c (HbA1C, expressed as %) (B), and TxB₂ production. P for trend = 0.009 and 0.012, respectively.
Discussion

Our study demonstrates a reduced platelet sensitivity to the inhibitory action of aspirin assessed by higher PA and TxB2 production in both T1DM and T2DM patients, when compared with high-risk ND subjects. Thus, they provide an explanation for the lower sensitivity to the beneficial effects of aspirin in diabetic patients observed in clinical trials\(^1\)\(^-\)\(^5\) and might account for the higher platelet reactivity associated with enhanced risk for adverse cardiovascular events demonstrated in T2DM.\(^26\)

Of note, the higher TxB2 production in our patients cannot be ascribed to lack of compliance to the antiplatelet treatment, as all our patients had detectable salicylate plasma levels.

In this study among diabetic patients, TxB2 production following platelet stimulation with collagen, ranged from 18 pg/10\(^8\) cells to 7344 pg/10\(^8\) cells, and correlated with serum levels of TxB2. More importantly, TxB2 production was much greater in diabetic patients than in ND. Incomplete inhibition of platelet COX-1 by oral aspirin in diabetic patients is supported, in our study, by the evidence that further in vitro addition of aspirin markedly reduced TxB2 production. This finding is in accordance with previous observations of our group demonstrating that among high-risk ND patients under chronic aspirin treatment, in vitro addition of aspirin reduced TxB2 formation in those with baseline TxB2 levels above the median value, while no additional inhibitory effect was obtained in those with baseline TxB2 levels below the median.\(^22\)

Furthermore, in the present study all diabetic patients expressed platelet COX-2, although in aspirin-treated diabetic patients only a small proportion of TxB2 produced following platelet stimulation was COX-2-dependent. Indeed, in vitro platelet incubation with aspirin plus the COX-2 inhibitor NS-398 resulted in a further, albeit not significant, reduction in TxB2 production, when compared with aspirin alone. We can exclude that these findings are because of platelet contamination by PBMC, as no COX-2 was detectable in lysates of PBMC from the same patients.

Regardless of the mechanism, higher platelet TxB2 production in diabetic patients on aspirin may be clinically relevant, as TxB2 production must be reduced by at least 95% in order to achieve platelet inhibition.\(^27\)

Taken together, our findings confirm and expand those of previous studies showing enhanced excretion of urinary 11-dehydro-TxB2 in diabetic patients, even when on aspirin.\(^28,29\)

The reduced sensitivity to aspirin in T2DM might be related to hyperglycaemia or to associated inflammation.\(^8\) The demonstration in our study of a correlation between in vitro TxB2 production and systemic levels of fasting glucose or HbA1c suggests that hyperglycaemia per se might play an important role, beyond that played by inflammation. This notion is confirmed in our study by the demonstration that sensitivity to aspirin was equally reduced in T1DM and T2DM patients, while high-sensitivity C-reactive protein levels were higher in T2DM.

The molecular mechanisms by which hyperglycaemia might reduce the sensitivity to the antiplatelet action of aspirin are probably multiple and cannot be deduced from the results of our study. The reduced inhibitory action of aspirin on platelet COX-1, observed in our study, might be ascribed either to glycation-induced conformational changes of platelet membranes with resulting impaired aspirin entrance\(^10\) and/or to a less-efficient acetylation previously demonstrated in platelets resuspended in high glucose medium.\(^30\)

The presence of platelet COX-2 observed in all diabetic patients might contribute to the reduced sensitivity of platelets to aspirin. The persistence of COX-2 expression might be consequent either to glucose-induced megakaryocyte COX-2 expression, similar to that observed in monocytes,\(^31\) and/or to the presence of a larger number of newborn platelets found in diabetic patients,\(^9\) since newborn platelets express COX-2.\(^32\)
Limitations of the study

Our study has some limitations. First, the sample size of T1DM is small, therefore our population is large enough to detect differences between ND and T1DM in aggregrometric tests, but might be too small to detect lack of statistical difference in TxB2 production between T1DM patients and ND. Secondly, in this study we did not evaluate clinical endpoints. Further larger studies are needed to determine the clinical implication of a reduced platelet sensitivity to aspirin in the diabetic population.

Conclusion

This study shows for the first time that the reduced sensitivity of diabetic patients to the beneficial effects of aspirin on cardiovascular risk might be caused by both less-efficient inhibition of platelet COX-1 and enhanced COX-2 expression. Furthermore, the similar findings in T1DM and T2DM, as well as the association between TxA2 production and systemic levels of fasting glucose and HbA1c suggests that hyperglycaemia per se is likely to play an important role in determining the sensitivity of diabetic patients to the beneficial effects of aspirin.

It also remains to establish whether an optimal glycaemic control might restore the sensitivity of diabetic patients to the beneficial effects of aspirin and whether other drugs, able to specifically inhibit TxA2 receptors, might be more effective in diabetic patients alone or in addition to aspirin. All of these hypotheses need to be tested in further larger prospective studies.

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Aspirin treatment in diabetic patients
Aortopulmonary paraganglioma is a rare tumour of the mediastinum of neural crest origin arising in the chromaffin tissues, making up less than 1% of mediastinal tumours. Unlike pheochromocytomas, they are seldom if ever functional, and clinical presentation is frequently delayed until compression of adjacent structures causing angina or dyspnoea. The only effective treatment is complete resection, which may result in a surgical challenge because of its proximity to the heart, great vessels, and trachea, often rendering a complete resection difficult to achieve. We report a 40-year-old male patient with sudden effort dyspnoea and recurrent cervical paraganglioma manifestations in the past history. The urgent transthoracal echocardiography showed normal contractility, enhanced right ventricular load, and a 46 × 57 mm soft tissue mass partially impressing the pulmonary trunk and left atrial roof. Cardiac magnetic resonance imaging revealed a tumour body of 382 mL in the aortopulmonary window (Panel A) causing approximately 50% reduction of RVOT. Coronarography excluded ischaemic heart disease although disclosed the multiple vascular alimentation of the mass partially originating from the coronary system (Panels B and C). The tumour was excised under cardiopulmonary bypass completely intact and was a benign non-catecholamine-secreting paraganglioma with chromogranin (Panel D), Protein S-100 and CD56 positivity, and also showed a very low mib-1-indicated proliferation activity. The patient’s dyspnoea was relieved and the control computed tomography scan showed no evidence of recurrent tumour mass after 6 months.

Panel A. T1 magnetic resonance image of the tumour mass, letter P indicates compressed hilar structures.
Panel B. Mammarian artery supplies the paraganglioma
Panel C. Collaterals from the right coronary artery
Panel D. Chromogranin-stained histological image

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