Pharmacodynamic Effects of Different Aspirin Dosing Regimens in Type 2 Diabetes Mellitus Patients With Coronary Artery Disease

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Background—Patients with type 2 diabetes mellitus (T2DM) have reduced aspirin-induced pharmacodynamic effects. This may be attributed to increased platelet turnover rates resulting in an increased proportion of non–aspirin-inhibited platelets during the daily dosing interval. The hypothesis of this study was that an increase in the frequency of drug administration [twice daily (bid) versus once daily (od)] may provide more effective platelet inhibition in T2DM patients.

Methods and Results—T2DM patients with stable coronary artery disease were prospectively recruited. Patients modified their aspirin regimen on a weekly basis according to the following scheme: 81 mg/od, 81 mg/bid, 162 mg/od, 162 mg/bid, and 325 mg/od. Pharmacodynamic assessments included light-transmittance aggregometry after arachidonic acid, collagen and adenosine diphosphate stimuli; VerifyNow-Aspirin assay; and serum thromboxane B2 (TXB2) levels. Twenty patients were analyzed. All patients were sensitive and compliant to aspirin irrespective of dose, as assessed by arachidonic acid–induced aggregation. When aspirin was administered once daily, there was no significant effect on platelet reactivity by increasing the once-daily dosing using aspirin-sensitive assays (collagen-induced aggregation and VerifyNow-Aspirin). An increase in aspirin dose by means of a second daily administration was associated with a significant reduction in platelet reactivity assessed by collagen-induced aggregation and VerifyNow-Aspirin between 81 mg/od and 81 mg/bid (P<0.05 for both assays) and between 81 mg/od and 162 mg/bid (P<0.05 for both assays). There was no impact of aspirin dosing regimens on adenosine diphosphate–induced aggregation. A dose-dependent effect of aspirin was observed on serum TXB2 levels (P=0.003).

Conclusions—Aspirin dosing regimens are associated with different pharmacodynamic effects in platelets from T2DM patients and stable coronary artery disease, with a twice-daily, low-dose aspirin administration resulting in greater platelet inhibition than once-daily administration as assessed by aspirin-sensitive assays and a dose-dependent effect on serum TXB2 levels. The clinical implications of a modified aspirin regimen tailored to T2DM patients warrant further investigation.

Clinical Trial Registration—URL: http://www.clinicaltrials.gov. Unique identifier: NCT01201785.


Key Words: aspirin • platelets • diabetes mellitus
ing platelets.\textsuperscript{8,9} Overall, this may explain why the “aspirin resistance” phenomenon is a common feature in T2DM patients and contributes to their increased risk of adverse cardiovascular events.\textsuperscript{1,2,10,11}

**Clinical Perspective on p 118**

The dose of aspirin recommended for secondary prevention of ischemic events in practice guidelines and accordingly used in clinical practice ranges between 75 and 325 mg/daily.\textsuperscript{12} Although in vitro studies demonstrate that doses of aspirin even below this range are already sufficient to achieve full COX-1 inhibition,\textsuperscript{7} ex vivo pharmacodynamic studies have suggested that patients at high risk, such as those with T2DM, may have improved pharmacodynamic responses with higher doses of aspirin.\textsuperscript{11} However, the aspirin dosing regimen leading to the best pharmacodynamic effects in T2DM still remains matter of continuous debate. Because diabetic platelets are characterized by an enhanced turnover rate, it may be hypothesized that an increase in the frequency rather than the dose of drug administration may be a more effective strategy to inhibit platelet reactivity in diabetic patients because this may enable COX-1 blockade of newly generated platelets. However, how different dosing regimens affect the pharmacodynamic effects of aspirin selectively in T2DM has been poorly explored. Therefore, the aim of the present pilot investigation was to evaluate how increasing the frequency of aspirin administration, remaining within the daily recommended therapeutic doses, affects antiplatelet responsiveness in T2DM patients with coronary artery disease (CAD).

**Methods**

**Subject Population and Study Design**

This was a prospective, open-label study in which platelet function was assessed in medically treated (taking oral hypoglycemic medication and/or insulin) T2DM patients 18 to 75 years of age with stable CAD. T2DM was defined according to the World Health Organization Report.\textsuperscript{14} All patients had angiographically documented CAD. Patients were considered as stable in their clinical presentation because they had not had any changes in their ischemic symptoms or required hospitalization or coronary revascularization (percutaneous or surgical) in the past 12 months. The study cohort was recruited at the outpatient clinic of the Division of Cardiology at Shands Jacksonville Hospital.

Patients were eligible for the study if they were taking aspirin 81 mg/daily for at least 7 days. Patients on a different dose of aspirin were switched to aspirin at the dose of 81 mg/daily for at least 7 days before pharmacodynamic assessments were performed. After having been on aspirin 81 mg/daily for at least 1 week, patients switched their aspirin regimen on a weekly basis according to the following scheme: aspirin 81 mg twice daily (bid) for 1 week; aspirin 162 mg once daily (od) for 1 week; aspirin 162 mg/bid for 1 week; aspirin 325 mg/od for 1 week. Pharmacodynamic assessments were made after each sequence (5 time points). Afterward, patients resumed the dose of aspirin that they were on before entering the study.

Exclusion criteria included blood dyscrasia or bleeding diathesis; oral anticoagulation therapy with a Coumadin derivative; recent antipleatlet treatment (<30 days) with a glycoprotein IIb/IIIa antagonist, thienopyridine (ticlopidine, clopidogrel), cilostazol, or dipryidamole; platelet count <100×10\(^6\)/μL; history of gastrointestinal bleed within the last 6 months; history of cerebrovascular accident within last 3 months; history of hospitalization for an acute coronary event or coronary revascularization (percutaneous or surgical) in the past 12 months; active bleeding or hemodynamic instability; any active malignancy; serum creatinine >2 mg/dL; baseline ALT >2.5 times the upper limit of normal; pregnant women; HbA1C >10%; and use of nonsteroidal anti-inflammatory drugs in the past 10 days.

The study complied with the Declaration of Helsinki and was approved by the Institutional Review Board of the University of Florida College of Medicine–Jacksonville. All subjects provided written informed consent. An independent data safety monitoring committee was instituted for adjudication of adverse clinical events.

**Blood Sampling**

Blood sampling for pharmacodynamic testing was performed at the end of each of the 5 different treatment regimens as follows: (1) after being on aspirin 81 mg/od for at least 1 week (V1); (2) after aspirin 81 mg/bid for 1 week (V2); after aspirin 162 mg/od for 1 week (V3); after aspirin 162 mg/bid for 1 week (V4); and after aspirin 325 mg/od for 1 week (V5). Patients on a once-daily regimen were instructed to take aspirin between 8 AM and 10 AM. Patients on a twice-daily regimen were instructed to take aspirin between 8 AM and 10 AM and 8 PM and 10 PM. On the day of the scheduled visit, patients would withhold aspirin intake until blood sampling was performed. Compliance was assessed by interview and pill counting. Blood sampling for platelet function analyses was collected from an antecubital vein with a 21-gauge needle the morning (between 8 AM and 10 AM) of the scheduled visit. The first 2 to 4 mL of blood was discarded to avoid spontaneous platelet activation, and samples were processed within 1 hour. Samples were processed by laboratory personnel blinded to treatment. Platelet function assays included light transmission aggregometry (LTA), VerifyNow Aspirin (VN-ASA), and serum thromboxane B\(_2\).

**Light Transmission Aggregometry**

LTA was performed according to standard protocols as previously described.\textsuperscript{15,16} In brief, platelet aggregation was assessed using platelet-rich plasma and platelet-poor plasma by the turbidometric method in a 2-channel aggregometer (Chrono-Log 490 Model, Chrono-Log Corp, Havertown, PA). Light transmission was adjusted to 0% for platelet-rich plasma and to 100% for platelet-poor plasma for each measurement. Maximal platelet aggregation (MPA) was measured after stimuli with arachidonic acid (1 mmol/L), collagen (2 μg/mL), and adenosine diphosphate (ADP) (5 μmol/L and 20 μmol/L).

**VerifyNow-Aspirin (VN-ASA) Assay**

The VN-ASA assay is a rapid whole-blood point-of-care device and was used according to the instructions of the manufacturer (Accumetrics, Inc, San Diego, CA), as previously described.\textsuperscript{16} In brief, VN-ASA assay mimics turbidometric aggregation and uses disposable cartridges containing arachidonic acid. The VN-ASA assay reports the results as aspirin reaction units (ARU). ARU values decrease with enhanced aspirin-induced platelet inhibition.

**Serum Thromboxane B\(_2\)**

The concentration of serum thromboxane B\(_2\) (TXB\(_2\)) was measured by using the TXB\(_2\) ELA kit (Cayman Chemical Company, Ann Arbor, MI) according to the instructions of the manufacturer.\textsuperscript{17} Briefly, samples were diluted with ELA buffer to bring their concentrations within the range of the standard curve. No other purification was performed on any of the samples. A standard curve was established by serial dilution of TXB\(_2\) between 1000 pg/mL and 7.8 pg/mL using ELA buffer as the matrix. The concentration of TXB\(_2\) in the samples was calculated from a logistic 4-parameter fit of the standard concentrations versus percent-age bound/maximum bound.

**Statistical Analysis**

Continuous variables are presented as mean±SD and were compared using ANOVA for repeated measures. Normal distribution was evaluated for continuous variables with the Shapiro-Wilk test. Categorical variables are presented as counts and percentages and were compared using the χ\(^2\), Fisher, or Cochran Q tests, as appropriate. \(P<0.05\) was considered statistically significant. All data were processed using the Statistical Package for Social Sciences, version 16 (SPSS, Chicago, IL).
Collagen-induced platelet aggregation was chosen for estimation of the sample size of this study. In fact, although LTA using arachidonic acid as a stimuli is the most COX-1 specific, it is well established that in compliant patients there is very limited variability in pharmacodynamic measures as COX-1 activity is fully suppressed thus not making this a suitable parameter to test for aspirin induced effects on platelet function profiles.\textsuperscript{15,16,19,20} Arachidonic acid induced platelet aggregation was used as a marker to guarantee that patients were compliant to aspirin therapy, defined as <20% MPA.\textsuperscript{15,16,19,20} Collagen activates phospholipase A\textsubscript{2}, allowing release of arachidonic acid from membrane phospholipids, the first step in the pathway toward the release of thromboxane A\textsubscript{2}.\textsuperscript{20,21} Collagen-induced aggregation has been therefore broadly used as a marker to define aspirin induced antiplatelet effects and accordingly considered for the power calculation of our pilot investigation.\textsuperscript{11,13,20,22} A 20% standard deviation for the true difference between collagen-induced platelet aggregation by LTA in patients on aspirin 81 mg/od versus 81 mg/bid was assumed, and calculations were based on a power of 80% and a significance level of 0.05. Based on these values, we determined that a sample population of 20 patients would be sufficient to demonstrate a 20% difference in collagen-induced aggregation between patients on aspirin 81 mg/od versus 81 mg/bid.

Results

From January 2009 to April 2010, a total of 82 T2DM patients were screened. Of these, 34 presented study exclusion criteria, whereas the remaining 48 where eligible to participate in the study. Of the latter, 36 agreed to provide their written consent to participate in the study. Among patients providing their written informed consent, 16 did not complete all 5 treatment regimens. Therefore, a total of 20 completed all the study phases. Patient disposition is illustrated in Figure 1. Baseline demographic and clinical characteristics of the study cohort are shown in Table 1.

Light Transmittance Aggregometry

Arachidonic-Induced Platelet Aggregation

Arachidonic acid–induced aggregation was low at each time point of the study, and no significant effect of increasing a single (Table 2) or a double dose (Table 3) was observed. There was also no effect when staggering the aspirin dose (Table 4). All MPA values at each time point and for each dosing regimen were <20%, confirming that patients were compliant to aspirin therapy.

Collagen-Induced Platelet Aggregation

Given as a single administration, aspirin did not exert any dose-dependent effect on 2 µg/mL collagen-induced LTA (Table 2). However, when the dosing was augmented by means of a second daily administration, a significant effect was found between 81 mg/od and 81 mg/bid (primary end point) (Figure 2A), between 81 mg/od and 162 mg/bid (Figure 2B) and between 162 mg/od and 162 mg/bid (Figure 2C). There was no difference between the antiplatelet effect exerted by twice daily doses of 81 mg or 162 mg (Table 3). Further, no effect of staggering a 162 mg or 325 mg daily dose was noted (Table 4).

ADP-Induced Platelet Aggregation

When aspirin was administered as a single dose, there was no dose-dependent effect on 5 µmol/L ADP-induced LTA (Table 2). Similarly, there was no dose-dependent effect on platelet aggregation with a once daily administration after challenge with 20 µmol/L ADP, except when patients received aspirin 162 mg/od compared with 81 mg/od. When the dosing was augmented by means of a second daily administration of aspirin, no significant effect on 5- or 20 µmol/L-induced platelet aggregation was observed between 81 mg/od and 81 mg/bid (49±13 versus 54±13, \(P = 0.203\) and 66±7 versus 69±10, \(P = 0.339\)), between 81 mg/od and 162 mg/bid (49±13 versus 50±15, \(P = 0.507\) and 66±7 versus 67±14, \(P = 0.847\)), and between 162 mg/od and 162 mg/bid (54±10 versus 50±15, \(P = 0.247\) and 71±11 versus 67±14, \(P = 0.225\)). There was no difference between the antiplatelet effect exerted by twice daily doses of 81 mg or 162 mg (Table 3). Further, no effect of staggering a 162 mg or 325 mg daily dose was noted (Table 4).

VerifyNow-Aspirin Assay

Given once daily, aspirin did not exert any dose-dependent effect on VN-ASA (Table 2). When the dosing was augmented by means of a second daily administration, a significant effect was found between 81 mg/od and 81 mg/bid (Figure 3A) and between 81 mg/od and 162 mg/bid (Figure 3B). No significant effect was seen by doubling the aspirin dose from 162 mg/od to 162 mg/bid (Figure 3C). No difference was noted between the antiplatelet effect exerted by twice-daily doses of 81 mg or 162 mg (Table 3). No effect of staggering a 162 mg or 325 mg daily dose was observed (Table 4).

Serum Thromboxane

TX\textsubscript{B\textsubscript{2}} levels significantly reduced in a dose-dependent manner (\(P = 0.003\)) across different aspirin regimens are shown in
Table 1. Baseline Demographic and Clinical Characteristics

<table>
<thead>
<tr>
<th></th>
<th>n=20</th>
<th>Age, y, ±SD</th>
<th>59±7</th>
<th>Male, n (%)</th>
<th>10 (50)</th>
<th>BMI, kg/m², ±SD</th>
<th>33±9</th>
<th>Risk factors, n (%)</th>
<th>6 (30)</th>
<th>Hypertension</th>
<th>19 (95)</th>
<th>Dyslipidemia</th>
<th>17 (85)</th>
<th>Insulin-treated</th>
<th>8 (40)</th>
<th>Medical history, n (%)</th>
<th>5 (25)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Medical therapy, n (%)</td>
<td></td>
<td>β-blockers</td>
<td>11 (55)</td>
<td>ACE inhibitors</td>
<td>18 (90)</td>
<td>Ca²⁺ antagonists</td>
<td>11 (55)</td>
<td>Lipid-lowering agents</td>
<td>8 (40)</td>
<td>Non-CYP 3A4</td>
<td>0 (0)</td>
<td>Proton pump inhibitors</td>
<td>5 (25)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Laboratory data</td>
<td></td>
<td>Platelet count (1000/mm³, ±SD)</td>
<td>241±66</td>
<td>Hematocrit, %, ±SD</td>
<td>42±4</td>
<td>HbA1C, %, ±SD</td>
<td>7.1±1.3</td>
<td>Creatinine, g/dL, ±SD</td>
<td>1.0±0.3</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

ACE indicates angiotensin-converting enzyme; BMI, body mass index; CABG, coronary artery bypass graft surgery; CAD, coronary artery disease; CYP 3A4, hepatic cytochrome P450 3A4; MI, myocardial infarction; MPV, mean platelet volume; and HbA1C, glycated hemoglobin A1C.

Figure 4. In particular, serum TXB₂ levels decreased with increasing aspirin daily doses (Table 2). When the dosing was augmented by means of a second daily administration, a significant effect was also found between 81 mg/od versus 81 mg/bid (107±143 versus 34±50, \( P=0.048 \)) and 81 mg/od versus 162 mg/bid (107±143 versus 19±21, \( P=0.017 \)). Serum TXB₂ levels reduced, although not significantly, between 162 mg/od and 162 mg/bid (41±79 versus 19±21, \( P=0.213 \)). There was no significant difference in TXB₂ levels between twice-daily doses of 81 mg or 162 mg (Table 3). Further, no effect of staggering a 162 mg or 325 mg daily dose was noted (Table 4).

Discussion

The optimal dose of aspirin required for secondary prevention of ischemic events is a topic of controversy. In fact, the need for different dosing regimens tailored according to the thrombotic risk of the patient has been suggested. Patients with T2DM have specific abnormalities in platelet function, which therefore make them a target population to assess if modified dosing regimens of aspirin may impact pharmacodynamic response profiles. Of note, there is accumulating evidence demonstrating that pharmacodynamic markers used to measure antiplatelet drug effects, including aspirin, have important prognostic implications underscoring the need for achieving optimized antiplatelet effects. The present investigation was designed with the aim of understanding how different aspirin dosing regimens affect antiplatelet effects as measured by numerous pharmacodynamic parameters in a cohort of T2DM patients with stable CAD. The present study demonstrates that the addition of a second staggered 81 mg dose is associated with reduced platelet reactivity compared with patients who assume a single 81 mg dose as assessed by assays sensitive to aspirin-induced effects (collagen-induced aggregation using LTA and VN-ASA). Importantly, using the above-mentioned assays, there was no dose effect on platelet reactivity when the aspirin dose was doubled from 81 mg to

Table 2. Dose Comparison in Once-Daily Administration

<table>
<thead>
<tr>
<th>Assay</th>
<th>81 mg/od</th>
<th>162 mg/od</th>
<th>325 mg/od</th>
<th>81 mg/od Versus 162 mg/od</th>
<th>162 mg/od Versus 325 mg/od</th>
<th>81 mg/od Versus 325 mg/od</th>
<th>162 mg/od Versus 325 mg/od</th>
</tr>
</thead>
<tbody>
<tr>
<td>Arachidonic acid, 1 mmol/L, %</td>
<td>2±0.9</td>
<td>2±0.7</td>
<td>2±0.7</td>
<td>1.000</td>
<td>1.000</td>
<td>1.000</td>
<td>1.000</td>
</tr>
<tr>
<td>Collagen, 2 μg/mL, %</td>
<td>44±23</td>
<td>39±14</td>
<td>35±15</td>
<td>0.285</td>
<td>0.083</td>
<td>0.374</td>
<td>0.157</td>
</tr>
<tr>
<td>ADP, 5 μmol/L, %</td>
<td>49±13</td>
<td>54±10</td>
<td>54±11</td>
<td>0.111</td>
<td>0.851</td>
<td>0.612</td>
<td>0.192</td>
</tr>
<tr>
<td>ADP, 20 μmol/L, %</td>
<td>66±7</td>
<td>71±11</td>
<td>68±7</td>
<td>0.033</td>
<td>0.459</td>
<td>0.145</td>
<td>0.109</td>
</tr>
<tr>
<td>VN-ASA, ARU</td>
<td>455±51</td>
<td>432±62</td>
<td>431±58</td>
<td>0.087</td>
<td>0.126</td>
<td>0.922</td>
<td>0.121</td>
</tr>
<tr>
<td>Serum TXB₂, pg/mL</td>
<td>107±143</td>
<td>41±79</td>
<td>22±21</td>
<td>0.008</td>
<td>0.030</td>
<td>0.328</td>
<td>0.008</td>
</tr>
</tbody>
</table>

Data are expressed as mean±SD. ADP indicates adenosine diphosphate; VN-ASA, VerifyNow-Aspirin assay; and TXB₂, thromboxane B₂.
162 mg, given as a single administration. Overall, these findings suggest that increasing the frequency of aspirin administration yields more potent antiplatelet effects than simply increasing the dose. These pharmacodynamic observations may therefore substantiate the hypothesis that a second staggered aspirin dose is likely to inhibit newly generated platelets and thus improve aspirin-induced antiplatelet effects.

A number of different mechanisms have been advocated to explain why individuals with T2DM have an increased risk of cardiovascular events compared with the counterpart without diabetes.1,2 Increased platelet turnover contributes to the hyperreactive status of T2DM platelets,3,4 In normal conditions, approximately 10% to 15% of circulating platelets are replaced every day. Diabetic patients have a greater number of large and hypersensitive younger platelets and a more abundant fraction of small exhausted platelets compared to nondiabetics.5,6 Aspirin irreversibly acetylates and inactivates COX-1 in circulating platelets and has only a 20-minute half-life.7 Therefore, there is the theoretical possibility that a single daily dose could not be sufficient to exert a full inhibitory effect on the new platelets generated and released by the bone marrow during the course of the 24 hours after aspirin intake8 (Figure 5). In addition, acetylated platelets remain sensitive to other stimuli generated by nonacetylated platelet, including thromboxane.3,4,10 On this background, we tested the hypothesis that increasing the frequency of drug administration might be effective in inhibiting the newly generated platelets and thus improving aspirin-induced pharmacodynamic effects.

In the present investigation, a gradient of different aspirin doses and dosing regimens within the range recommended by clinical guidelines was used. We found that all the aspirin doses and regimens were effective in achieving effective inhibition of COX-1, as measured by arachidonic acid–induced LTA. These findings are in line with previous pharmacodynamic investigations demonstrating that low doses of aspirin, even lower than doses used in clinical practice, are sufficient to fully inhibit COX-1 activity.7,15,16,18,19 In addition, this was also an indicator that our study patients were compliant to aspirin therapy.7,15,16,18,19 Because of this high anticipated platelet responsiveness to arachidonic acid, our study was powered to detect more meaningful differences elicited by tests that are sensitive to aspirin-induced effects albeit non–COX-1–specific (ie, collagen-induced LTA), which may show a variable range in response profile.11,13,19,22 This choice was driven by the notion that aspirin has shown to play an important role in the modification of the platelet response to stimuli other than arachidonic acid.11,13,19,22 The latter has also led to suggest that aspirin may have COX-1–independent effects, which may be more susceptible to dosing than that needed to fully block COX-1 activity, as also shown in our study. The activation of platelets by collagen, in particular, is known to activate the phospholipase A2 and the liberation of arachidonic acid from membrane phospholipids, the first step in the pathway toward the release of TXA2.20,21 In our study, we found that increasing the dose of aspirin is the most successful strategy in achieving more pronounced platelet inhibitory effects in T2DM patients with stable CAD when a twice-daily rather than once-daily regimen is used when using aspirin-sensitive tests (collagen-induced LTA and VN-ASA). In particular, in contrast to single dosing, a significant decrease in

### Table 4. Comparison of Single Versus Staggered Daily Administration of the Same Aspirin Dose

<table>
<thead>
<tr>
<th>Assay</th>
<th>162 mg/od</th>
<th>81 mg/bid</th>
<th>P</th>
<th>325 mg/od</th>
<th>162 mg/bid</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Arachidonic acid, 1 mmol/L, %</td>
<td>2±0.7</td>
<td>2±0.5</td>
<td>0.772</td>
<td>2±0.7</td>
<td>2±1.4</td>
<td>0.094</td>
</tr>
<tr>
<td>Collagen, 2 μg/mL, %</td>
<td>39±14</td>
<td>32±14</td>
<td>0.060</td>
<td>35±15</td>
<td>33±14</td>
<td>0.490</td>
</tr>
<tr>
<td>ADP, 5 μmol/L, %</td>
<td>54±10</td>
<td>54±13</td>
<td>0.857</td>
<td>54±11</td>
<td>50±15</td>
<td>0.273</td>
</tr>
<tr>
<td>ADP, 20 μmol/L, %</td>
<td>71±11</td>
<td>69±11</td>
<td>0.343</td>
<td>68±7</td>
<td>67±14</td>
<td>0.751</td>
</tr>
<tr>
<td>VN-ASA, ARU</td>
<td>432±62</td>
<td>420±41</td>
<td>0.345</td>
<td>431±58</td>
<td>423±52</td>
<td>0.551</td>
</tr>
<tr>
<td>Serum TXB2, pg/mL</td>
<td>41±79</td>
<td>34±50</td>
<td>0.716</td>
<td>22±21</td>
<td>19±21</td>
<td>0.579</td>
</tr>
</tbody>
</table>

Data are expressed as mean±SD. ADP indicates adenosine diphosphate; VN-ASA, VerifyNow-Aspirin assay; and TXB2, thromboxane B2.

![Figure 2](http://circinterventions.ahajournals.org/Downloaded from http://circinterventions.ahajournals.org/)

Figure 2. Comparison of different aspirin regimens [A, 81 mg once daily [od] versus 81 mg twice daily [bid]; B, 81 mg once daily [od] versus 162 mg twice daily [bid]; C, 162 mg once daily [od] versus 162 mg twice daily [bid)] by collagen-induced light transmission aggregometry (LTA). Values are expressed as percentage (% of platelet aggregation. Error bars indicate standard deviations.
platelet reactivity may be obtained by adding a second administration, regardless of whether an additional 81 mg or a 162 mg are given. The lack of any changes in ADP-induced aggregation in our study patients using different aspirin dosing regimens is in line with prior investigations in diabetic patients.13 The poor sensitivity of aspirin to modulate ADP-mediated platelet activation and aggregation processes and the pivotal role of this signaling pathway, which has also been suggested to be upregulated in T2DM patients, may explain the greater benefit of the ADP P2Y12 receptor antagonist clopidogrel therapy over aspirin in diabetic patients.27

Our study findings using aspirin-sensitive tests are in contrast with a subgroup analysis of DM patients enrolled in the ASPECT (Aspirin-Induced Platelet Effect) study. This study showed enhanced inhibitory effects on collagen-induced aggregation when increasing the dose of aspirin from 81 mg/od to 162 mg/od or 325 mg/od, without differences between the latter 2 doses.19 In addition, ARU values were decreased only when increasing the dose of aspirin to 325 mg/od, with no effects compared with 81 mg/od when using 162 mg/od. The post hoc nature of this assessment and the fact that the study was limited only to a once-daily regimen and did not assess how dosing frequency affects aspirin-induced effects may contribute to these conflicting findings. To the best of our knowledge, our study is the first evidence specifically designed in T2DM patients showing that a second daily dose of aspirin is associated with enhanced pharmacodynamic effects.

A further finding from our study was a dose-dependent effect of aspirin on thromboxane generation as detected by serum TXB2 levels. Aspirin doses of 162 mg and 325 mg significantly affected TXB2 levels compared with an 81 mg dose, regardless of whether these doses were given staggered or as once daily administrations. This disparity compared with the pharmacodynamic data provided by collagen induced aggregation and VN-ASA could indicate a different effect of aspirin on platelets according to their stages of maturation. It has been suggested that aspirin may accumulate in the bone marrow and exert COX-1–inhibitory effects on megakaryocytes.28 As a consequence, a cumulative effect of aspirin on megakaryocytes located in the bone marrow may lead to a dose-dependent decrease in TXB2 levels. On the contrary, this does not occur when evaluating mature circulating platelets subject to more rapid turnover rates.

The optimal dose of aspirin is still not fully defined from large, randomized, controlled trials. Large-scale registry data from secondary prevention studies have shown that aspirin doses in the lowest range (75 to 150 mg) are associated with the greatest risk reduction in ischemic events.29,30 In addition, high aspirin dosing is associated with an increased risk of bleeding, therefore limiting its ischemic benefit.29–31 Further, it has been argued that the ischemic benefit of high-dose aspirin may be offset by the fact that this may also lead to COX-2 inhibition with resultant decreases in prostaglandin I2 production.32 The recently reported CURRENT-OASIS (Clopidogrel Optimal Loading Dose Usage to Reduce Recurrent Events/Optimal Antiplatelet Strategy for Interventions) trial was the first large-scale clinical investigation to compare high (300 to 325 mg daily) versus low (75 to 100 mg daily) dose aspirin, which showed no differences in efficacy end points at 30 days between the 2 aspirin regimens, and a trend toward increase risk of gastrointestinal bleeds was observed with the higher dosage.33 Although our findings support the hypothesis that twice-daily administration of low-dose aspirin is associated with enhanced platelet inhibition, probably by overcoming increased platelet turnover in T2DM patients and potentially provide more cardiovascular protection, large-scale clinical trials are warranted to support the safety and efficacy of this dosing regimen.
Figure 5. Schematic of circadian release of platelets into bloodstream from bone marrow and impact of a single daily dose of aspirin on newly generated platelets in type 2 diabetes mellitus. Platelets from patients with type 2 diabetes mellitus (T2DM) have a reduced life-span and increased turnover rates, leading to enhanced bone marrow megakaryocyte generation and release of new and hyperreactive platelets into the bloodstream. Aspirin has only a 20-minute half-life and therefore the accelerated thrombopoiesis, which characterizes T2DM patients and does not allow newly generated platelets entering the circulation to be sufficiently exposed to aspirin if given once daily. This may lead to a considerable proportion of circulating platelets with uninhibited cyclooxigenase-1 (COX-1) activity that continue to generate high levels of serum thromboxane and therefore promote activation of circulating platelets (acetylated and non-acetylated) via thromboxane receptors (TP) on the platelet surface. A twice-daily administration of aspirin may allow newly generated platelets released into the bloodstream to be COX-1 inhibited, thus achieving more optimal blockade of platelet activation processes in T2DM.

Study Limitations
The present pilot investigation was conducted in a small sample size, which limited adjustment for multiple comparisons. Because 5 paired groups were tested and 9 of 10 potential comparison were performed, a more conservative $\alpha$ value (ie, 0.05/10=0.005) would have been more indicated to reduce the risk of type I error (eg, accepting false-positive results). However, given the exploratory nature of our investigation, adjustment for multiple comparisons was admittedly not planned to avoid increasing the risk of type II error (eg, accepting false-negative results), thereby missing important pilot information of potential value to be replicated and confirmed in larger-scale datasets. In addition, our investigation assessed 5 different aspirin dosing regimens in a sequential manner in the same cohort of patients without washout phases, raising concerns on the potential for carry-over effects. However, the selection of the same cohort of patients to conduct this pharmacodynamic study obviates for the multiple and unpredictable individual characteristics that may affect individual response to antiplatelet agents, which must be minimized in similar pilot investigations. Further, the duration of treatment with each aspirin dosing regimen was sufficient to allow turnover of circulating platelets with newly generated ones, allowing them to be exposed to the new regimen thus limiting carry-over effects. Finally, it may be argued that a twice-daily administration may be of more benefit in selected patients with baseline turnover rates above a certain threshold. However, cutoff values to identify these patients are not established and warrant dedicated experiments. In addition, how this may be affected by how glycemic control is achieved among T2DM patients (insulin-treated versus non-insulin-treated) also deserves further investigation. Indeed, our pilot study supports the rationale for a twice-daily aspirin regimen in T2DM patients and sets the basis for further investigation in the field.

Conclusions
Aspirin dosing regimens are associated with different pharmacodynamic effects in platelets from T2DM patients and stable CAD. In particular, a twice-daily low-dose aspirin administration is associated with greater platelet inhibition than a once-daily administration, as assessed by aspirin-sensitive assays, and a dose-dependent effect is observed on serum TXB2 levels. The clinical implications of a modified aspirin regimen tailored to T2DM patients warrant further investigation.

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References
The present pilot investigation was designed with the aim of understanding how different aspirin dosing regimens affect platelet response as measured by pharmacodynamic parameters in a cohort of twenty patients with type 2 diabetes mellitus (T2DM) and stable coronary artery disease. The study hypothesis was that an increase in the frequency of aspirin administration may provide more effective platelet inhibition in T2DM patients. The working hypothesis was developed on the basis that patients with T2DM have increased platelet turnover rates resulting in an increased proportion of non-aspirin-inhibited platelets with a once-daily dosing regimen. The present investigations showed that a twice-daily low-dose aspirin administration results in greater platelet inhibition than a once-daily administration. The clinical implications of such modified aspirin treatment regimen in T2DM patients warrant further investigation.
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