Platelet Tests and Antiplatelet Drugs in Coronary Artery Disease

Auke C. de Boer, M.D., Ping Han, M.B., Ph.D., Alexander G. G. Turpie, M.B., Rodney Butt, R.T., Michael Gent, M.Sc., and Edward Genton, M.D.

SUMMARY This study was designed to clarify discrepancies in the literature concerning platelet survival time and β-thromboglobulin (βTG) levels in patients with coronary artery disease (CAD) and the effect of platelet-suppressant drugs on these tests. Platelet survival time and plasma βTG levels were determined in 48 patients with angiographically documented CAD. The effect of sulfipyrazone or aspirin/dipyridamole on these measurements was investigated in a double-blind, crossover trial that included a placebo phase. In patients with CAD, the mean plasma βTG concentration was significantly elevated, but the mean platelet survival time was not significantly different from that in controls. Treatment with sulfipyrazone or aspirin/dipyridamole did not produce changes in platelet survival time or plasma βTG concentration that were significantly different from the values during the placebo phase. This study demonstrates that compared with the spontaneous variation in platelet survival time or βTG concentration, there was no measurable effect of sulfipyrazone or aspirin/dipyridamole on the results of the tests.

CONSIDERABLE evidence has been accumulated from animal studies and clinical observations to establish an important role for blood platelets in atherosclerosis and thrombotic complications associated with the process.1,2 Numerous laboratory tests have been developed to detect and quantify responses that platelets undergo when they are stimulated, including adhesion, aggregation, release and consumption. Platelet tests that measure platelet utilization and release are, respectively, estimation of platelet survival of isotopically labeled autologous platelets and measurement in plasma or urine by radioimmunoassay of β-thromboglobulin (βTG), a platelet-specific protein. Platelet survival time and βTG concentration have been studied in patients with coronary artery disease (CAD), but the results of the studies have been conflicting. However, in several reports, platelet survival has been found to be decreased3,4 and plasma βTG levels increased5-7 in CAD patients compared with controls. These studies have led to the consideration that these tests may prove useful not only in confirming the role of platelets in CAD, but also in studying the effect of drugs that alter platelet reactivity on platelet behavior in vivo and possibly to monitor the administration of these drugs. However, none of the studies of platelet survival time or βTG levels have been sufficiently well designed that the results can be considered conclusive. Because of the significance and current interest in this subject, and because of the importance of the areas and questions about the conflicting data, the performance of a methodologically sound trial is important to resolve the outstanding differences.

This study was therefore designed to determine the platelet survival time and plasma βTG concentration in a group of patients with documented CAD, the correlation and reproducibility of these measurements and, from a randomized, double-blind, crossover trial, the effects of sulfipyrazone and aspirin/dipyridamole on these indicators of platelet activation.

Methods

Forty-nine patients under 65 years of age with ischemic heart disease documented by coronary angiography (greater than 50% stenosis in at least one coronary artery) who were to be treated medically agreed by written consent to participate in the study.

Baseline estimation of platelet survival time and plasma βTG levels were obtained 1-4 weeks after angiography. Patients received each of the following treatment regimens in three consecutive 4-week periods according to a prescribed randomized arrangement: sulfipyrazone, 200 mg four times daily; dipyridamole, 50 mg, in combination with aspirin, 300 mg, four times daily; and placebo. Randomization was done before baseline estimation of platelet survival time. The medications for each of the three treatment regimens appeared identical, allowing the study to be conducted in a double-blind manner. Platelet survival time and plasma βTG were measured in the final week of each phase. Platelet counts in all patients in each phase of the study were normal (> 150 x 10^9/L) and did not vary between phases of the study.

During the study all but four of the patients were treated with nitrates and propranolol. None of the patients was taking aspirin or related drugs during the study, except when prescribed, or in the 2 weeks before the baseline platelet survival time. The patients were followed clinically at monthly intervals to assess symptoms of coronary artery disease, changes in antianginal medications and adverse effects of the treatments. Compliance with the drug therapy was assessed by history and pill count.

Platelet Survival Time

Platelet survival time was determined with autol-
ogous $^{51}$Cr-labeled platelets using the method recommended by the International Committee for Standardization in Hematology (ICSH). Platelet-rich plasma was prepared from 350 ml of blood drawn into acid citrate dextrose (ACD) by differential centrifugation, and the red cells were retransfused. A platelet pellet was produced by centrifugation and resuspended in 5 ml of platelet-poor plasma and incubated with 250 $\mu$Ci of $^{51}$Cr for over 30 minutes. The labeled platelets were washed, separated, resuspended in platelet-poor plasma and re injected into the patients. The degree of red cell contamination was measured, and a correction factor was applied in the estimation of platelet survival. The mean in vivo platelet recovery was 69.9 ± 14% (± SD). There was no correlation between in vivo platelet recovery and the mean platelet survival time ($r = -0.13$).

The platelet survival time was calculated from the radioactivity in seven blood samples collected daily over 7 days, based on the least-square regression technique with computer assistance using three mathematical functions: linear, exponential and gamma. All survivals were calculated from duplicate samples collected over 7 days to avoid variability in the calculations, particularly when the gamma or multiple-hit model was used. In an earlier study, we demonstrated that the survival time, calculated as exponential or as linear functions, correlated closely ($r = 0.84$), but the gamma function calculation correlated poorly with the exponential or linear measurement ($r = 0.3$).

**Plasma $\beta$TG Concentration**

Plasma $\beta$TG concentration was measured by a radioimmunoassay, as previously described. Blood was collected through a 19-gauge needle into a plastic syringe and 2.7 ml were immediately transferred into precooled tubes containing 0.3 ml of an anticoagulant mixture to prevent in vitro release; the anticoagulant mixture contained 0.1 ml of EDTA, 0.219 $M$, 0.1 ml of heparin, 30 mM, and 0.1 ml of prostaglandin $E_1$, 10 $\mu$M. Platelet-poor plasma was prepared by centrifugation at 2000 g for 60 minutes at 4°C within 1 hour of collection and stored at -70°C until assayed. The sensitivity of the assay was 3 ng/ml. The mean $\beta$TG concentration in 80 normal volunteers was 27 ng/ml (geometric mean); the 95% probability range was 12–54 ng/ml. With this technique, it has been demonstrated in normal volunteers that $\beta$TG levels do not vary significantly in serial samples. In the present study, samples for $\beta$TG determination were drawn 2 hours apart in 23 coronary patients, and no significant differences were observed.

Platelet survival data were expressed as mean ± SEM. Because of the skewed distribution of the $\beta$TG data, the results were expressed as geometric mean with 95% probability ranges. Analysis of variance procedures were used to determine the statistical significance of differences in outcomes between the treatment groups.

**Results**

Forty-nine patients were entered into the study and baseline platelet survival times were obtained from 48. Reported drug compliance was greater than 90% in all patients. Thirty-eight patients completed all four phases of the protocol, remained stable throughout the course of the study and did not require a change in medical treatment. Of the 11 patients who did not complete all phases, one patient died of acute myocardial infarction; three patients had coronary bypass surgery because of unstable angina, five patients withdrew for nonmedical reasons, and two patients did not complete the protocol because of technical difficulties with the collection of blood for performance of platelet survival time. No patients withdrew because of side effects of treatment. The clinical characteristics of the patients are shown in table 1.

<table>
<thead>
<tr>
<th>TABLE 1. Clinical Characteristics of the 49 Patients with Coronary Artery Disease Entered into the Study</th>
</tr>
</thead>
<tbody>
<tr>
<td>Completed study (n = 38)</td>
</tr>
<tr>
<td>Age (mean ± SD)</td>
</tr>
<tr>
<td>Female/male</td>
</tr>
<tr>
<td>1-vessel disease</td>
</tr>
<tr>
<td>2-vessel disease</td>
</tr>
<tr>
<td>3-vessel disease</td>
</tr>
<tr>
<td>History of myocardial infarction</td>
</tr>
<tr>
<td>Smokers</td>
</tr>
<tr>
<td>Diabetes</td>
</tr>
<tr>
<td>Hyperlipoproteinemia</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>TABLE 2. Platelet Survival Time in Normal Controls, Patients with a Normal Coronary Angiogram and Patients with Coronary Artery Disease</th>
</tr>
</thead>
<tbody>
<tr>
<td>Linear</td>
</tr>
<tr>
<td>Controls (n = 14)</td>
</tr>
<tr>
<td>Normal angiogram (n = 19)</td>
</tr>
<tr>
<td>CAD (n = 48)</td>
</tr>
</tbody>
</table>

All values are mean ± SEM (days). There were no significant differences by analysis of variance. Abbreviation: CAD = coronary artery disease.
lation between the platelet survival time and the severity of the clinical symptoms, family history of coronary artery disease, smoking history, response to medical therapy or angiographic severity of CAD.

The platelet survival times of the 38 patients who completed the trial, calculated by the three mathematical models for each phase of the trial with antiplatelet drugs, are shown in table 3. In individual patients there was a wide range in platelet survival time between phases. Analysis of variance showed that there was no significant difference in the mean platelet survival time for the groups between phases of the study.

Because it was possible that the effect of the antiplatelet agents tested would only be evident in the patients with the shortest baseline platelet survival, they were examined separately. Ten of the 48 patients had baseline survival times calculated by the gamma function of less than 7 days, which was more than 1 standard deviation below the mean of normal controls. Eight of these patients completed all phases of the study. The mean platelet survival time in these patients was significantly longer in the sulfinpyrazone (7.3 ± 0.6 days), aspirin/dipyridamole (8.5 ± 0.6 days) and, in particular, the placebo phase of the study (8.1 ± 0.5 days) than the baseline survival time (5.4 ± 0.5 days).

**Table 3. Effect of Antiplatelet Drugs on Platelet Survival Time in 38 Patients with Coronary Artery Disease**

<table>
<thead>
<tr>
<th></th>
<th>Baseline</th>
<th>Placebo</th>
<th>Sulfinpyrazone</th>
<th>Aspirin/dipyridamole</th>
</tr>
</thead>
<tbody>
<tr>
<td>Linear</td>
<td>9.2 ± 0.1</td>
<td>9.5 ± 0.1</td>
<td>9.5 ± 0.1</td>
<td>9.7 ± 0.2</td>
</tr>
<tr>
<td>Exponential</td>
<td>4.8 ± 0.1</td>
<td>5.1 ± 0.1</td>
<td>5.1 ± 0.1</td>
<td>5.3 ± 0.2</td>
</tr>
<tr>
<td>Gamma</td>
<td>7.8 ± 0.2</td>
<td>8.3 ± 0.2</td>
<td>7.6 ± 0.3</td>
<td>8.6 ± 0.3</td>
</tr>
</tbody>
</table>

All values are mean ± SEM (days).

Importantly, there was no significant difference between the placebo phase and the two drug phases of the trial.

When the exponential model was used to calculate platelet survival times, 11 patients had baseline survival times of less than 4.5 days, which is more than 1 standard deviation below the normal mean of 5.0 days. Platelet survival times in these patients were significantly longer during each of the placebo (4.5 ± 0.2 days), sulfinpyrazone (4.8 ± 0.2 days) and aspirin/dipyridamole (4.9 ± 0.4 days) phases than the baseline survival time (3.9 ± 0.1 days). Again, there was no significant difference between the placebo phase and the sulfinpyrazone or aspirin/dipyridamole phases of the study. A further demonstration of the fluctuation of platelet survival time that can occur in patients with CAD was seen in five patients with normal baseline platelet survival time calculated by the gamma model who had short platelet survival times (< 7 days) during the placebo phase of the study.

Complete βTG data for all four phases of the study were obtained from 28 patients. Baseline βTG concentrations were significantly increased compared with normal controls (p < 0.002) and elevated in 29% of the patients (fig. 2). The βTG concentration did not correlate with the severity of CAD either clinically or angiographically. Normal βTG levels were observed throughout all phases in 57% of patients (table 4). In the 12 patients with elevated baseline values, only three had persistently increased levels, and the results in the remaining patients varied between normal and abnormal values. Mean values did not differ significantly from baseline during the drug or placebo phase of the study. There was no correlation between the
TABLE 4. Effect of Antiplatelet Drugs on Plasma $\beta$-thromboglobulin Levels in 28 Patients with Coronary Artery Disease

<table>
<thead>
<tr>
<th></th>
<th>Baseline</th>
<th>Placebo</th>
<th>Sulfinpyrazone</th>
<th>Aspirin/dipyridamole</th>
</tr>
</thead>
<tbody>
<tr>
<td>Geometric mean</td>
<td>43</td>
<td>43</td>
<td>43</td>
<td>36</td>
</tr>
<tr>
<td>95% range</td>
<td>14–127</td>
<td>11–172</td>
<td>12–151</td>
<td>11–125</td>
</tr>
</tbody>
</table>

Values are in ng/ml.

baseline platelet survival time and plasma $\beta$TG concentration ($r = -0.04$).

**Discussion**

The results of this study indicate that platelet survival times varied widely, but there was no significant difference in the mean values between normal volunteers, patients with chest pain and normal coronary angiograms, or patients with documented CAD. Platelet survival time did not correlate significantly with clinical variables; drugs considered to be platelet suppressants were not shown to significantly alter platelet survival time. Plasma $\beta$TG levels were elevated and abnormally high in about one-third of the patients with CAD. Serial measurements of $\beta$TG levels fluctuated without apparent relationship to clinical variables, and platelet-suppressant drugs tested did not significantly affect the plasma $\beta$TG concentration.

The finding that the mean platelet survival time was not significantly different from normal in patients with documented CAD is in agreement with studies by several authors, but is in disagreement with others. Although it is not possible to identify specific factors that differ between the studies reporting conflicting results, differences in the patient populations studied or in technical variables associated with the performance of the tests may be involved. In determining platelet survival times, it is especially important that technical variables be controlled, especially the sampling method and the number of days over which samples are collected. To limit the interassay variation in our study, we used the standard method recommended by the ICSH. The platelet survival times were calculated using linear, exponential and gamma mathematical functions. Controversy remains as to which of these methods is most suitable for this type of study, because linear models tend to overestimate and exponential models underestimate the real values for platelet survival time and, therefore, minimize differences between the patient groups.

The gamma function, or multiple-hit model, was introduced as a method of estimating platelet survival regardless of the disappearance of platelets, and has been recommended by the ICSH as the standard model for calculating platelet survival time. In our experience, however, the multiple-hit model lacks robustness, which results in minor variations in the data points, producing marked differences in estimated platelet survival time. Because of the limitations of each of the methods of calculation, the data in this study was calculated using all three mathematical functions.

In this study, with each of the methods of data calculation, there was a wide range of calculated platelet survival time in the normal population and in the population with CAD, but there was no significant differences between the mean of the groups. In addition, when the results from the baseline phase were compared with those obtained during the placebo administration in individual patients, the calculated platelet survival time varied widely. These changes did not appear to reflect fluctuations in the course of the CAD, for there was no significant correlation of the platelet survival time with the clinical or therapeutic variables that were monitored.

Earlier studies reported lengthening of shortened platelet survival in patients with CAD after treatment with antiplatelet drugs. These studies were, however, open, uncontrolled trials, and the observations were made in patients with short platelet survival at baseline measurement. Because the platelet survival times from the baseline and placebo phases did fluctuate in this study, meaningful results of the effect of drug treatment on platelet-survival time requires a randomized, blinded trial with a placebo group. Using this approach, we could not demonstrate that either sulfinpyrazone or aspirin and dipyridamole caused a significant change in platelet survival time. Even in patients with relatively short platelet survival at baseline, treatment with the platelet-suppressant drugs failed to prolong the survival time compared with that during the placebo phase. We therefore conclude that the wide variability in platelet survival time in patients with CAD makes it difficult to demonstrate prolongation of platelet survival by drugs, using this study design, unless the effect is large or many more patients are studied. However, we cannot exclude the possibility that the drugs had a small effect on platelet survival time that was not detected by this experimental design.

The mean plasma $\beta$TG concentration was elevated in patients with CAD, as previously reported, which indicated that the platelet release reaction occurred in these patients but did not conclusively document that the site of platelet activation was in the coronary circulation. Again, as with platelet survival time, the plasma $\beta$TG values fluctuated widely, often between normal and abnormal in a given patient. Treatment with sulfinpyrazone produced no detectable effect on plasma $\beta$TG concentration, and although mean plasma $\beta$TG concentration with aspirin/dipyridamole was lower, the difference was not statistically significant. This finding is in agreement with the findings of others. There was no apparent correlation between plasma $\beta$TG concentration and the platelet survival time, in contrast to a recent report, although in that study the correlation was relatively low ($r = 0.39$).

**References**

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Platelet Function, Thromboxane Formation and Blood Pressure Control During Supplementation of the Western Diet with Cod Liver Oil

REINHARD LORENZ, M.D., ULLRICH SPENGLER, M.D., SVEN FISCHER, PH.D., JOCHEN DUHM, M.D., AND PETER C. WEBER, M.D.

SUMMARY Epidemiologic and experimental data suggest an antiatherothrombotic potential of ω-3 polyunsaturated fatty acids. Therefore, the Western diet, which supplies predominantly ω-6 polyunsaturated fatty acids, was supplemented with 40 ml/day of cod liver oil, which provides about 10 g of ω-3 polyunsaturated fatty acids daily, for 25 days in eight volunteers. The ω-3 polyunsaturated fatty acids were incorporated in platelet and erythrocyte membrane phospholipids at the expense of ω-6 polyunsaturated fatty acids. Bleeding time increased (p < 0.01) and platelet count (p < 0.05), platelet aggregation upon ADP and collagen (p < 0.01–0.05), and associated thromboxane B2 formation (p < 0.01) decreased. Blood pressure (p < 0.05) and blood pressure response to norepinephrine (p < 0.01) and angiotensin II (NS) fell, without major changes in plasma catecholamines, renin, urinary aldosterone, kallikrein, prostaglandins E2 and F2α, and red cell cation fluxes. Biochemical and functional changes were reversed 4 weeks after cod liver oil was discontinued. Formation of prostaglandins derived from eicosapentaenoic acid and interference of eicosapentaenoic acid with formation and action of prostaglandins derived from arachidonic acid were evident in vitro. Whatever the mechanism, this moderate supplement of ω-3 polyunsaturated fatty acids markedly changed membrane phospholipids, which was associated with a shift toward less reactive platelets and a blunted circulatory response to pressure hormones.

SEVERAL independent lines of evidence suggest a protective potential of dietary eicosapentaenoic acid (all cis C20:5ω3) against cardiovascular disease. Greenland Eskimos and, to a lesser extent, some Japanese, have a high dietary intake of long-chain ω-3 polyunsaturated fatty acids from seafood1 and a low incidence of cardiovascular disease, even compared with their Westernized ethnic counterpart.2 A low prevalence of hypertension in Eskimos,3 a favorable pattern of serum lipids,4 and especially a hemostatic "defect" evidenced by a bleeding tendency and reduced platelet aggregability,5 have been invoked as underlying protective mechanisms. Furthermore, ω-3 polyunsaturated fatty acid-enriched diets have been shown to reduce the size and sequelae of cerebral6 and myocardial7 infarction in experimental animals.

On a normal Western diet, in which ω-6 polyunsaturated fatty acids predominate, the prostanoids of the two series, derived from membrane-bound arachidonic acid (all cis C20:4ω6) prevail (fig. 1). The balance

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