Platelet Function Studies in Coronary Artery Disease

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SUMMARY
Platelets have been implicated and abnormal platelet function proposed in the pathogenesis of coronary artery disease (CAD). This investigation examines platelet function in men with stable, arteriographically defined CAD and correlates results with lipid pattern, history of angina or myocardial infarction and smoking habits. Platelet survival (51Chromium method), adhesiveness, and aggregation were measured in 21 men with CAD. Twelve patients had Type IV hyperlipoproteinemia and nine patients had normal lipoprotein level. Eighteen had angina and 13 had had infarction.

The average platelet survival for the total group was normal (6.8 ± 0.24 days, avg ± SEM), but survival was shortened in 11 and normal in ten. There was no significant difference between: (1) patients with hyperlipoproteinemia (6.8 ± 0.30 days) and those with normal lipoproteins (6.9 ± 0.41 days); (2) patients with angina (6.8 ± 0.28 days) and without (6.6 ± 0.26 days); (3) patients with infarction (6.8 ± 0.26 days); and without (6.8 ± 0.50 days); (4) patients with varying patterns of arteriographic involvement; and (5) with varying smoking histories. Adhesiveness was normal in all. Aggregation was normal in 14. Neither adhesiveness nor aggregation correlated with platelet survival or defined the subgroups of CAD. Results suggest an abnormal short platelet survival frequently occurs in patients with CAD, but there is no discernible difference in platelet survival among the various clinical subgroups of CAD.

Additional Indexing Words:
Thrombosis Atherosclerosis Hyperlipoproteinemia Myocardial infarction Angina Cigarette smoking

The cause of atherosclerotic coronary artery disease remains unknown. In recent years, attention has returned to the hypothesis of Duguid,1 who presented evidence that microthrombi in various stages of organization can be found on the aortic intima of autopsied patients; he was able to establish that mural thrombi on the arterial intima can become covered by endothelium. This latter point answered the major criticism of the thrombogenetic theory of atherosclerosis first advanced by Rokitansky in 1842.2 Further, Duguid demonstrated that repeated deposition of mural thrombi may lead to narrowing of the arterial lumen, and that both the material deposited in the arterial wall by the thrombus and the intima undergo degeneration and cellular organization, thus explaining the apparent incorporation into the arterial wall of the thrombus and the absence of identifiable cellular constituents.

During the past two decades, considerable additional data have accumulated to support the hypothesis that thrombosis may contribute to the development of atherosclerosis and its complications and establishing that the platelet plays a primary role in arterial thrombosis.3 In these studies, the presence of coronary artery disease was determined solely on clinical grounds and there have been few attempts to relate the findings to the clinical subgroupings of the coronary artery disease spectrum. Further, evidence has been presented to suggest that alterations of lipid metabolism can affect platelet function.4 Thus, the two major hypotheses of the pathogenesis of atherosclerosis, thrombosis and altered lipid metabolism, may be interrelated.

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Supported by research funds of the Veterans Administration, by a grant from the Colorado Heart Association, and by grant (RR-51) from the General Clinical Research Centers Program of the Division of Research Resources, National Institutes of Health, and by grant (HL 10290) from the National Institutes of Health. Presented in part at the 44th Annual Meeting of the Central Society for Clinical Research, November, 1971, Chicago, Illinois.

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Received May 2, 1973; revision accepted for publication July 25, 1973.

1194 Circulation, Volume XLVIII, December 1973
PLATELET FUNCTION IN CAD

The purpose of this investigation was to assess platelet function in a population of men with arteriographically defined coronary artery disease and evaluate results with respect to the lipid, lipoprotein pattern, the presence or absence of angina or myocardial infarction, the smoking histories and the arteriographic patterns of coronary involvement.

Methods and Patients

Platelet function studies were performed on 21 men (ages 39–64, average 50 years). All had undergone selective coronary arteriography for evaluation of coronary artery disease and consented to undergo the platelet function studies. Coronary arteriography revealed disease in all cases with at least one area of greater than 50% narrowing of at least one of the major coronary arteries in all patients. No patient had valvular heart disease.

Platelet survival time was performed by the 51Chromium method of Aster and Jandl. Autologous platelets from 400–450 ml of blood were labelled with 100–150 μCi of 51Chromium. Blood samples for measurement of radioactivity in platelet concentrate were obtained beginning three hours following reinfusion of labelled platelets and daily for seven days. A single exponent was fitted to the data by computer-assisted least squares analysis (normal, avg ± SEM = 6.7 ± 0.21 days, N = 16).

Platelet adhesiveness was measured by a modification of the glass bead method of Salzman. Two ml of venous blood was drawn into plastic syringes wetted with heparin and passed through a standard glass bead column in a constant time within two minutes of collection (normal, 30–60%).

Platelet aggregation dose-response curves induced by adenosine diphosphate (ADP) and collagen were obtained by a modification of the turbidimetric method of Bonn. To stirred citrated platelet-rich plasma was added ADP (0.5–20 μg/ml) or collagen suspension (undiluted and in titer of 1:1 to 1:8). Platelet adhesiveness and aggregation tests were performed coincident with the platelet survival study.

During the course of the platelet function studies, serum was obtained for determination of cholesterol, triglyceride, and the lipoprotein electrophoretic pattern. Serum was obtained in the morning after a 14-hour fast and while patients were ingesting a standard American diet. Serum cholesterol was measured by the method of Henry and triglyceride by the method of Fletcher. Lipoprotein electrophoresis on paper was performed by the method of Frederickson et al. using albumin-containing buffer.

Patients were classified as Type IV hyperlipoproteinemia when hypertriglyceridemia was present with increased staining of the pre-beta migrating lipoprotein band. Invariably, a distinct separation of the pre-beta from the beta band is present in the true Type IV, which is an aid in the recognition of this pattern.

Patients were further defined with respect to the symptom of angina pectoris. Angina was typical in most respects in the 18 patients who had this symptom, occurred at least five times per week, and had been clinically stable for at least three months. Patients who were considered angina-free denied episodes in at least the three months prior to study.

Acute myocardial infarction had occurred in 13 patients in whom an appropriate clinical history was supported by evolutionary changes and initial force deformity (Q waves) on the electrocardiogram. Infarcts occurred at least four months prior to study.

Smoking histories were obtained and the patients classified as actively smoking, having stopped smoking, or having never smoked. Six men were smoking cigarettes (and had been for at least one year) at the time of study. All had smoked heavily. Five patients had never smoked cigarettes.

During the course of these studies and for at least three weeks before, no medication known to affect platelet function was ingested by these patients.

Student's t-test was used to analyze the data. Informed consent was obtained from all patients.

Results

Normal subjects consisted of 14 men and two women, aged 30–45 years, without clinical evidence of atherosclerotic vascular disease. Average platelet survival was 6.7 ± 0.24 days (fig. 1). Twelve had normal survival time and two were shortened.

Average platelet survival time for these 21 men with arteriographically established coronary artery disease was within the normal range (avg ± SEM, 6.8 ± 0.24 days) (fig. 1). Eleven patients (52%) had abnormally shortened platelet survival time (less than 6.8 days) and ten patients (48%) were normal.

Nine patients (ages 39–57, avg 50 years) had normal lipids and lipoproteins (table 1). Serum triglyceride was clearly normal; that is, less than 150 mg% in four of these patients. Five men had

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Figure 1

Bar graphs comparing the average platelet survival time ± standard error of the mean for the men with coronary artery disease with normals.
The average normal lipoproteins (CD, triglyceride ranged within the normal range). Five patients (HF, MT, VB, CD, HL: table 1) had shortened platelet survival.

Twelve patients (ages 41–64, avg 51 years) had Type IV hyperlipoproteinemia (table 2). Serum triglyceride ranged from 183 to 650 mg%. One patient (GJ) had triglyceride less than 210 mg%, but he had a definite increase in pre-beta lipoproteins on paper and this band was clearly separated from the beta band. Average platelet survival time for the 12 men with Type IV hyperlipoproteinemia was within the normal range (6.8 ± 0.30 days) and not significantly different from the average of the patients with normal lipoproteins (fig. 2). Six men (EC, RP, AF, RA, JG, DG, table 2) had shortened platelet survival times and six had normal survival times. In neither the normal lipoprotein group nor the Type IV group was a relationship between serum cholesterol or triglyceride and platelet survival time apparent.

Average platelet survival times for the subgroupings of patients were all normal—angina (6.8 ± 0.28 days), no angina (6.6 ± 0.26 days), infarction (6.8 ± 0.26 days), no infarction (6.8 ± 0.50 days), actively smoking (7.0 ± 0.66 days), stopped smoking (6.6 ± 0.20 days), and never smoked (7.7 ± 0.58 days). In each of the subgroups, approximately half of the patients had abnormally shortened platelet survival time (tables 1 and 2).

The distribution of coronary arterial abnormalities (vessels with at least one area of greater than 50% occlusion) in respect to platelet survival time is seen in figure 3. The most common pattern, involvement of the right coronary artery and the left anterior descending, was present in eight patients. Four patients had triple vessel involvement, and single vessel involvement was present in seven patients. Platelet survival time did not discriminate the site or the extent of atherosclerotic involvement. Five of the eleven patients with abnormal platelet survival time had double vessel involvement, two had triple vessel involvement, and four had single vessel involvement.

Platelet survival was remeasured in six patients (MW, GJ, RP, JM, RA, DG) an average of eight months (range 3–12 months) after the first study. The second study was performed when the patients

Table 1

<table>
<thead>
<tr>
<th>Age</th>
<th>Serum cholesterol (mg %)</th>
<th>Serum triglyceride (mg %)</th>
<th>Platelet survival time (days)</th>
<th>Platelet adhesiveness (%)</th>
<th>Platelet aggregation</th>
<th>Angina</th>
<th>Myocardial infarction</th>
<th>Smoking history*</th>
</tr>
</thead>
<tbody>
<tr>
<td>1)</td>
<td>JH 51</td>
<td>244</td>
<td>77</td>
<td>7.2</td>
<td>58</td>
<td>Borderline</td>
<td>0</td>
<td>Smoke</td>
</tr>
<tr>
<td>2)</td>
<td>HF 57</td>
<td>157</td>
<td>80</td>
<td>6.4</td>
<td>56</td>
<td>Borderline</td>
<td>0 +</td>
<td>Quit</td>
</tr>
<tr>
<td>3)</td>
<td>MT 39</td>
<td>193</td>
<td>100</td>
<td>5.8</td>
<td>36</td>
<td>Normal</td>
<td>+</td>
<td>Smoke</td>
</tr>
<tr>
<td>4)</td>
<td>FD 55</td>
<td>147</td>
<td>118</td>
<td>7.2</td>
<td>41</td>
<td>Normal</td>
<td>0</td>
<td>Never</td>
</tr>
<tr>
<td>5)</td>
<td>VB 48</td>
<td>180</td>
<td>152</td>
<td>6.4</td>
<td>38</td>
<td>Normal</td>
<td>0 +</td>
<td>Quit</td>
</tr>
<tr>
<td>6)</td>
<td>JB 53</td>
<td>207</td>
<td>153</td>
<td>9.4</td>
<td>49</td>
<td>Normal</td>
<td>+</td>
<td>Smoke</td>
</tr>
<tr>
<td>7)</td>
<td>CD 47</td>
<td>213</td>
<td>164</td>
<td>6.0</td>
<td>26</td>
<td>Normal</td>
<td>+</td>
<td>Quit</td>
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<tr>
<td>8)</td>
<td>HL 52</td>
<td>215</td>
<td>175</td>
<td>5.4</td>
<td>27</td>
<td>Normal</td>
<td>+</td>
<td>Smoke</td>
</tr>
<tr>
<td>9)</td>
<td>MW 50</td>
<td>275</td>
<td>187</td>
<td>8.2</td>
<td>40</td>
<td>Normal</td>
<td>+</td>
<td>Smoke</td>
</tr>
</tbody>
</table>

Avg ± SEM

6.6 = 0.14 41% ± 3.8

*Smoke = actively smoking; quit = quit smoking; and never = never smoked.
were stable, and in no instance had there been any clinical change in their course in the interval between studies. Platelet survival time at the second study did not vary more than 0.2 days from that of the first study and in no instance did the second study change survival time from abnormal to normal or vice versa.

Platelet adhesiveness was normal, i.e., less than 60%, in all men (tables 1 and 2). Three men had adhesiveness less than 20% and an additional patient had adhesiveness less than 30%. There were no significant differences in platelet adhesiveness between those with Type IV lipoproteinemia (33 ± 3.8%) and those with normal lipoproteins (41 ± 3.8%). Neither the presence of angina or its absence nor the occurrence of myocardial infarction or its absence yielded significant differences in platelet adhesiveness. Smokers had adhesivity no different from nonsmokers or from those who had quit smoking. Platelet adhesiveness did not discriminate the site and extent of coronary atherosclerosis as determined arteriographically. Platelet adhesiveness did not correlate with platelet survival time in these 21 patients. Average platelet adhesiveness of the 11 patients with shortened platelet survival time (35 ± 3.8%) was not significantly different from the average adhesiveness of the ten patients with normal survival time (38 ± 4.4%).

Platelet aggregation dose-response curves to ADP and collagen were constructed for these 21 patients. Platelet aggregation in response to ADP was similar to that induced by collagen. Platelet aggregation was normal in 14 of 21 patients (tables 1 and 2), a ratio which is not different from normals. Two patients (HL, GJ) had abnormally high platelet aggregation and five (JH, HF, EC, RA, JC) had borderline high aggregation responses. In the 12 patients with the Type IV patterns, platelet aggregation was normal in eight, borderline high in three and clearly abnormally high in one. In the nine patients with a normal lipoprotein pattern, platelet aggregation was normal in six, borderline high in two and clearly high in one. There was no correlation between platelet aggregation and serum cholesterol or triglyceride in these patients.

**Figure 3**

Average platelet survival times in respect to the arteriographic distribution of obstructive lesions. LAD = left anterior descending, LCX = left circumflex, RCA = right coronary artery.
was no significant difference in platelet aggregation between patients with Type IV hyperlipoproteinemia and those with normal lipoproteins.

Similarly, platelet aggregation failed to discriminate patients in the various subgroups analyzed above and did not correlate with either platelet survival time or platelet adhesiveness.

Discussion

Our data suggest that platelet survival time is frequently abnormally shortened in coronary artery disease, although the average platelet survival time is within the normal range. As pointed out by Murphy and Mustard and by Chakrabarti, the common occurrence of atherosclerotic vascular disease in the population makes the designation of a nonaffected control group difficult. We cannot be certain that our normal subjects are free of coronary artery disease, as defined by coronary arteriography. We have measured platelet survival in four patients who have completely normal coronary arteriograms. Two of these patients have Type IV hyperlipoproteinemia and platelet survival was normal in both (6.8 and 7.0 days). The other two patients had a normal lipoprotein pattern, and their survival was also normal (6.8 and 7.4 days). These four patients are included in the 16 normal subjects discussed above.

Thus, our data suggest that there is an increased frequency of abnormal platelet survival in coronary artery disease and that platelets may play a role in some patients with coronary disease.

We have shown that platelet survival time is abnormally shortened in patients with mitral prosthetic valves of older design, valves associated with high rates of thromboembolism. Further, platelet survival is normal in patients with newer mitral valves (Beall, series 6300 Starr-Edwards), valves with lower rates of thromboembolism. In patients with mitral stenosis the platelet survival time has been shown to distinguish patients with a history of thromboembolism from those without such a history, as well as patients with substitute cardiac valves. Thus, in valvular heart disease, shortened platelet survival time seems to correlate with the occurrence of thromboembolism.

Murphy and Mustard found shortened mean platelet survival (DF32P method) in 31 men with clinical evidence of stable atherosclerotic vascular disease including men with angina and myocardial infarction. O'Neill and Firkin studied five patients with established peripheral vascular disease and found platelet survival time (51Chromium method) normal in all. In addition, five subjects with hypercholesterolemia and symptoms suggesting coronary artery disease had normal platelet survival times. Abrahamsen noted shortened platelet survival time (51Chromium method) only in the acute phase of myocardial infarction and found that of 36 patients studied two weeks following infarction, survival time was normal. Interestingly, in that study, 18 patients with established peripheral vascular disease had shortened mean platelet survival time. None of our patients have clinical evidence of peripheral or cerebral vascular disease.

Platelet adhesiveness has been extensively studied in patients with coronary artery disease. Multiple techniques have been used which confuse the results as the relationships between the different methods have not been established. Most authors have noted increased platelet adhesiveness in coronary artery disease. Five other studies in addition to ours have shown normal platelet adhesiveness, and the preponderance of recent studies have shown normal results.

We could find no instances of increased platelet adhesiveness in our patients; in fact, we noted decreased adhesiveness (less than 20%) in three patients. We found no relationship between platelet survival time and adhesiveness.

We found increased platelet aggregation induced by ADP and collagen in only two of these 21 men. An additional five men had platelet aggregation that was borderline between normal and high. Goldenfarb et al. found significantly increased platelet aggregation induced by ADP in men with a history of myocardial infarction. Renaud et al. found increased platelet aggregation induced by thrombin, but not by ADP or collagen, in five men with signs of coronary artery disease.

Recently, Hampton and Gorlin reported increased platelet electrophoretic response to ADP in 32 of 34 patients with clinical evidence of coronary artery disease and in 17 of 29 relatives of young men with atherosclerotic vascular disease. In an additional 12 patients who had coronary arteriography, an increased sensitivity of platelets to ADP was demonstrated in seven of the eight with coronary structural abnormalities. In the four patients with normal coronary arteriograms, one patient had abnormally sensitive platelets. Ruhento-Bauer and Grotten have noted normally sensitive platelets assessed by electrophoretic mobility in coronary artery disease.

Circulation, Volume XLVIII, December 1973
PLATELET FUNCTION IN CAD

Most studies of platelet function in coronary disease have presented the results of one test of platelet reactivity. In our study, we have used three tests of platelet reactivity—survival time, adhesivity, aggregation—and results fail to yield any correlation between these tests. This is not unexpected as each of these tests probably measures different properties of the platelet. Platelet survival time was correlated with thromboembolism in patients with valvular heart disease and this test may be more predictive of proneness to thrombosis than other available methods.

The men in our study with abnormal platelet survival time are scattered throughout the various subgroups of coronary disease that we have employed. We could find no differences in platelet survival time between men with the Type IV hyperlipoproteinemia pattern and those with normal lipoproteins. Differences in platelet survival time were not apparent when patients were divided as to the presence or absence of angina (although only three patients did not have angina) and a history of myocardial infarction. The smoking history of these men did not correlate with platelet survival time. In addition, there were no differences in platelet survival time with respect to the extent and distribution of coronary disease as judged by coronary arteriography.

Platelet survival time measures the interaction of platelets with the vascular surface. Abnormal platelet survival in coronary artery disease could occur as a response to plaque rupture and the release of thrombogenic material into the lumen or as a response to turbulence induced by the irregular lumen or due to excessive circulating catecholamines. Platelet aggregation induced by these mechanisms, or by other mechanisms, is theoretically harmful through the formation of thrombi on the vascular surface leading to further narrowing of the lumen, through the formation of thrombi on ruptured plaques leading to myocardial infarction, through distal microembolism of these aggregates producing myocardial ischemia, and through further vascular injury induced by these aggregates.

Sulfinpyrazone14 and dipyridamole15 have been shown to increase platelet survival time in patients with prosthetic heart valves and dipyridamole has decreased the incidence of thromboembolism in patients with these valves. Therapy with platelet inhibitors may be of value in patients with coronary artery disease to reduce platelet reactivity and thereby decrease the likelihood of micro- or macrothrombi in coronary vessels.

Acknowledgments
The authors acknowledge the expert technical assistance of Mrs. Ann Burns, Mrs. Gloria Smith, Miss Jean Baughman and Mr. William Harryman.

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Circulation. 1973;48:1194-1200
doi: 10.1161/01.CIR.48.6.1194
Circulation is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
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Print ISSN: 0009-7322. Online ISSN: 1524-4539

The online version of this article, along with updated information and services, is located on the World Wide Web at:
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