Platelet Function and Survival in Patients with Severe Hypercholesterolemia

Laurence Corash, Judith Andersen, Betty J. Poindexter, and Ernst J. Schaefer

Platelet aggregation and survival were measured in twelve subjects with severe hypercholesterolemia. There was a small increase in sensitivity to epinephrine and adenosine diphosphate among the most hypercholesterolemic patients, but this did not correlate with reduced platelet lifespan. Platelet survival was normal or only moderately reduced in the markedly hypercholesterolemic homozygous subjects. However, the incidence of reduced platelet survival was significantly increased ($p < 0.05$) among the older patients with more extensive atherosclerotic vascular disease compared to the younger patients with limited vascular disease. Marked hypercholesterolemia in the absence of atherosclerosis does not appear to accelerate platelet destruction, although a modest increased aggregability is present.


During the past decade, several laboratories have published data supporting the concept that platelets play a critical role in the pathogenesis of atherosclerosis. Decreased platelet survival, an indication of platelet involvement in atherosclerotic vascular disease, has been reported in coronary artery disease with and without hyperlipoproteinemia. Steele and Rainwater found an increased incidence of reduced platelet survival among males with coronary artery disease associated with hyperprothromboproteinemia and hyperbetalipoproteinemia compared with normolipemic subjects. Correction of the lipid abnormalities resulted in improved platelet survival for some patients.

In view of these studies and others that identified elevated serum lipids as a specific risk factor in the development of vascular disease, it seemed reasonable to examine platelet function in hyperlipemic subjects. Carvalho et al. reported that platelets from hypercholesterolemic patients showed increased sensitivity to common platelet agonists in vitro. Subsequently, Shattil et al. and Colman, demonstrated that normal platelets enriched with cholesterol become hyperaggregable and that purified platelets from hypercholesterolemic subjects contain increased membrane cholesterol compared to platelets from normolipemic controls. These observations have led to the hypothesis that alterations in platelet membrane lipid composition result in modified platelet function, thereby contributing to and possibly initiating atherosclerotic events.

Further studies by Harker et al. confirmed the finding of shortened platelet survival in hyperlipoproteinemic subjects but found normal platelet aggregation in vitro. When these authors subclassified their patients by lipoprotein typing, the type III and type IV patients had decreased platelet survivals. The eight type IIa heterozygotes had normal platelet survivals and only one of two type IIa homozygotes showed a modest shortening of platelet survival. These researchers observed no relationship between the degree of platelet lifespan reduction and lipid levels, extent of vascular disease, age, or sex of the patients. In the present study, we have expanded our effort to simultaneously measure platelet aggregation in a larger group of severely hypercholesterolemic patients whose extent of vascular disease is well characterized. This study was designed to determine whether severe hypercholesterolemia is associated with increased platelet reactivity and if this finding is correlated with reduced platelet survival.
Methods

Study Population

Patient and Control Selection

Twelve subjects with type II hyperlipoproteinemia (elevated low density lipoprotein cholesterol) were studied (table 1). Informed consent was obtained from all subjects according to National Institutes of Health guidelines. Patients abstained from all medications known to affect platelet function; medications to control lipid levels are indicated in table 1.

We studied 35 normal controls, aged 18 to 32 years, who showed no evidence of atherosclerotic disease, and who had normal fasting lipid levels and no history of platelet dysfunction. Of these controls, 10 also underwent $^{51}$Cr platelet survival measurement. Controls also avoided all medications known to affect platelet function during the study period.

Lipoprotein Type Classification

Lipoprotein phenotypes and genotypes were established by clinical appearance, fasting cholesterol and triglyceride levels, paper electrophoresis of plasma lipoproteins, family studies, low density lipoprotein receptor studies, and quantitative ultracentrifugation of plasma lipoproteins. Patients were classified as IIA if their low density lipoprotein (LDL) cholesterol was elevated and their very low density lipoprotein (VLDL) cholesterol was normal; they were classified as IIB if both their VLDL and LDL cholesterol levels were elevated.

Assessment of Atherosclerotic Disease

All patients underwent a routine physical examination, cardiac fluoroscopy and electrocardiography. A group of nine subjects (VC, VaC, TH, BC, HS, SA, RW, LR, and MB) had coronary artery angiography as part of their clinical evaluation. The extent of atherosclerotic involvement was estimated for each subject and is described in detail as follows and is summarized in table 1.

Patient Characteristics

Young patients. RN and DN are siblings who are asymptomatic, have normal electrocardiograms, and show no arterial bruits. VC and VaC are siblings who have been followed at the NIH Clinical Center for 6 years and have been treated with niacin and cholestyramine in the past. Both were found to be receptor-defective as determined by radiolabelled low density lipoprotein studies with skin fibroblasts. VC had nonspecific ST and T wave changes, mild angina, and bilateral carotid bruits. Catheterization demon-

Table 1. Clinical Characteristics, Lipid Levels, and Platelet Survival of Hypercholesterolemic Subjects

<table>
<thead>
<tr>
<th>Subject</th>
<th>Age (yrs)</th>
<th>Sex</th>
<th>Phenotype</th>
<th>Genotype</th>
<th>Plasma chol (mg/dl)</th>
<th>TGY (mg/dl)</th>
<th>VLDL</th>
<th>LDL</th>
<th>HDL</th>
<th>Medication</th>
<th>Atherosclerosis</th>
<th>Platelet half-life (hrs)</th>
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<tbody>
<tr>
<td>RN</td>
<td>11</td>
<td>M</td>
<td>IIA</td>
<td>FH het</td>
<td>366</td>
<td>91</td>
<td>13</td>
<td>347</td>
<td>36</td>
<td>none</td>
<td>none</td>
<td>118</td>
</tr>
<tr>
<td>DN</td>
<td>12</td>
<td>M</td>
<td>IIA</td>
<td>FH heter</td>
<td>328</td>
<td>71</td>
<td>4</td>
<td>279</td>
<td>45</td>
<td>none</td>
<td>none</td>
<td>150</td>
</tr>
<tr>
<td>VC</td>
<td>12</td>
<td>M</td>
<td>IIA</td>
<td>FH hom</td>
<td>830</td>
<td>96</td>
<td>24</td>
<td>734</td>
<td>23</td>
<td>none</td>
<td>A,LCA,C</td>
<td>120</td>
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<td>VaC</td>
<td>14</td>
<td>F</td>
<td>IIA</td>
<td>FH hom</td>
<td>834</td>
<td>151</td>
<td>70</td>
<td>731</td>
<td>33</td>
<td>none</td>
<td>minimal</td>
<td>120</td>
</tr>
<tr>
<td>TH</td>
<td>15</td>
<td>F</td>
<td>IIB</td>
<td>FH hom</td>
<td>942</td>
<td>291</td>
<td>132</td>
<td>796</td>
<td>14</td>
<td>none</td>
<td>A,LCA,RCA,C,F,Ang</td>
<td>103</td>
</tr>
<tr>
<td>LC</td>
<td>18</td>
<td>F</td>
<td>IIA</td>
<td>FH hom</td>
<td>436</td>
<td>65</td>
<td>19</td>
<td>365</td>
<td>52</td>
<td>Niacin</td>
<td>minimal</td>
<td>117</td>
</tr>
<tr>
<td>BC</td>
<td>41</td>
<td>F</td>
<td>IIA</td>
<td>FH hom</td>
<td>402</td>
<td>99</td>
<td>20</td>
<td>338</td>
<td>44</td>
<td>none</td>
<td>A,LCA,RCA,C,F,Ang</td>
<td>72†</td>
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<tr>
<td>HS</td>
<td>45</td>
<td>M</td>
<td>IIA</td>
<td>FH het</td>
<td>381</td>
<td>92</td>
<td>37</td>
<td>299</td>
<td>45</td>
<td>none</td>
<td>A,LCA,RCA,C,F,Ang,MI,CHF</td>
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<tr>
<td>SA</td>
<td>50</td>
<td>M</td>
<td>IIB</td>
<td>poly</td>
<td>411</td>
<td>210</td>
<td>65</td>
<td>305</td>
<td>41</td>
<td>none</td>
<td>A,LCA,RCA,Ang</td>
<td>105</td>
</tr>
<tr>
<td>RW</td>
<td>52</td>
<td>M</td>
<td>IIA</td>
<td>FH hom</td>
<td>350</td>
<td>107</td>
<td>27</td>
<td>295</td>
<td>28</td>
<td>none</td>
<td>A,LCA,RCA,C,F,Ang,MI</td>
<td>54</td>
</tr>
<tr>
<td>LR</td>
<td>54</td>
<td>F</td>
<td>IIB</td>
<td>poly</td>
<td>499</td>
<td>166</td>
<td>53</td>
<td>416</td>
<td>30</td>
<td>none</td>
<td>A,LCA,RCA,Ang</td>
<td>95</td>
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<tr>
<td>MB</td>
<td>69</td>
<td>F</td>
<td>IIB</td>
<td>FH het</td>
<td>284</td>
<td>234</td>
<td>53</td>
<td>174</td>
<td>57</td>
<td>none</td>
<td>A,LCA,RCA,C,F,Ang</td>
<td>78</td>
</tr>
</tbody>
</table>

Phenotype refers to lipoprotein phenotype as defined in reference 13; genotype is as defined in reference 12; see text for additional details.

Abbreviations: A = ascending aorta; LCA = left coronary artery; RCA = right coronary artery; C = carotid arteries; F = femoral arteries; Ang = angina; MI = status post myocardial infarction; CHF = congestive heart failure; VLDL = very low density lipoproteins; LDL = low density lipoproteins; HDL = high density lipoproteins; Chol = cholesterol; TGY = triglyceride; FH hom = homozygote for familial hypercholesterolemia (FH); FH het = heterozygote for familial hypercholesterolemia (FH); poly = polygenic.

*Deceased; postmortem examination performed.
†Lipid peroxidation technique, see text.
strated significant atherosclerosis in the ascending aorta and greater than 50% narrowing of the left main, left anterior descending, and circumflex coronary arteries. This patient later developed severe left main coronary artery disease, evidenced a high gradient (90 mm) across the aortic valve, and died during open heart surgery. His sister (VaC) had a normal electrocardiogram, was asymptomatic, and had no significant coronary artery disease on catheterization.

TH was receptor-negative, and had received no previous therapy. She had mild angina, non-specific ST and T wave changes, and bilateral carotid and femoral bruits. Cardiac catheterization revealed significant atherosclerosis in the ascending aorta and greater than 75% narrowing of the left main and right coronary arteries. LC was receptor-defective and had been treated with cholesterolamine, niacin, and intermittent d-thyroxine. She was receiving niacin during the study. Her electrocardiogram was normal; she was asymptomatic and had no vascular bruits.

Old patients. All subjects abstained from medication during the study. None had receptor binding studies. BC, HS, RW, and MB had family studies and were classified as heterozygous for familial hypercholesterolemia. SA and LR had less well-defined genetic studies and were classified as polygenic. All patients in the older age group had significant angina and greater than 50% narrowing of the left anterior descending and right coronary arteries. RW and MB also had evidence of atherosclerosis in the ascending aorta, with bilateral carotid and femoral bruits. HS and RW had sustained, documented myocardial infarctions; in HS this had caused a ventricular aneurysm and congestive heart failure. Both HS and SA subsequently died and at autopsy were found to have severe, diffuse atherosclerosis in their coronary arteries and ascending and descending aortas.

Platelet Survival Measurement

Autologous platelet life span was measured by the radiochromium technique of Abrahamsen.16 Both control and patient subjects had normal platelet counts. A blood sample taken before injection of labelled platelets determined platelet size and distribution and estimated red cell contamination.17 We determined the recovery of injected platelets after a 12-hour fast and inserted it into 0.08M trisodium citrate — 0.04M citric acid buffer (1 vol buffer to 6 vol blood) in 50 ml plastic tubes (#2074, Falcon Plastics, Oxnard, California). The tubes were capped with parafilm and gently mixed, and 8 ml aliquots were placed in smaller plastic tubes (#2059, Falcon Plastics, Oxnard, California) and centrifuged for 10 minutes at 150 g (22°C). The platelet-rich plasma (PRP-1) was removed and stored. The residual red cell-platelet fraction was centrifuged for 10 minutes at 350 g (22°C) to provide a second platelet-rich plasma (PRP-2). The remaining red cell pellet was centrifuged for 15 minutes at 3500 g (22°C) to yield platelet-poor plasma (PPP). The platelet count of PRP-1 and PRP-2 was determined17 and PRP-1 and PRP-2 were pooled in an appropriate ratio to yield a final PRP containing 300,000 platelets/µl. The pH of the final PRP was adjusted to 7.75 ± 0.05 by the addition of 1N NaOH and was periodically rechecked during the study.

Adenosine 5'-diphosphate (ADP) (Sigma, St. Louis, Missouri) was prepared as a 0.1M stock solution in 0.5M tris-HCl (pH 6.8) with 0.15M NaCl and was stored frozen at —80°C in 0.5 ml aliquots. Stock solutions were thawed once before use. L-epinephrine (Sigma, St. Louis, Missouri) was prepared as a stock solution of 0.01M in 0.025M HCl and stored at —80°C. Working solutions at 10⁻³M, 10⁻⁴M and 10⁻⁵M in 0.15M NaCl of ADP and epinephrine were prepared at the time of aggregation, used once, and discarded. Calf skin soluble collagen (7 mg/ml) (Worthington Biochemical, Freehold, New Jersey) was stored at 4°C and appropriate dilutions in 0.15M NaCl were prepared fresh at time of study.

Platelet aggregation was performed according to the technique of Born and Cross21 at 37°C using a Payton Dual Channel Aggregometer (Payton Instruments, Buffalo, New York). A normal control was run parallel to each patient study. The transmittance of PRP was set at 5% of full scale and that of PPP was set at 95% of full scale. The minimal concentration of each aggregating agent that produced maximal aggregation was measured in both control and patient subjects. This experimental design was adapted from the study by Carvalho et al.6 to facilitate comparison of our results with other studies in which platelet aggregation of severely hypercholesterolemic subjects was performed. Statistical comparisons were performed using a t test.
Results

The patients' clinical features are summarized in Table 1. Serum cholesterol concentrations (Table 2) for the different patient groups were significantly different from those of the controls ($p < 0.001$). Compared to heterozygotes and polygenics, homozygotes had a significant difference in serum cholesterol content ($p < 0.05$). The entire patient group also had a significantly higher serum triglyceride level (Table 2) than the group of control subjects ($p < 0.01$) but there was no difference in serum triglycerides between the two patient subgroups.

Maximum response to epinephrine-induced aggregation for controls (Figure 1) was $62.1\% \pm 22.0\%$ (so). The mean epinephrine dose for controls was $57.2 \pm 11.6 \mu M$ (SEM) compared to $6.7 \pm 3.8 \mu M$ (SEM) for homozygotes and $6.25 \mu M \pm 1.6$ (SEM) for heterozygotes plus polygenics. The difference between the entire patient group and the controls was significant ($p < 0.01$). There was no difference between the patient subgroups.

<table>
<thead>
<tr>
<th>Group</th>
<th>Cholesterol (mg/dl)</th>
<th>Triglyceride (mg/dl)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total patients</td>
<td>12</td>
<td>505 ± 66</td>
</tr>
<tr>
<td>Homozygotes</td>
<td>4</td>
<td>761 ± 11</td>
</tr>
<tr>
<td>Heterozygotes</td>
<td>8</td>
<td>378 ± 23</td>
</tr>
<tr>
<td>+ polygenics</td>
<td>35</td>
<td>165 ± 7</td>
</tr>
</tbody>
</table>

Values are means ± SEM.
Maximum response to ADP-induced platelet aggregation (figure 2) was 68.2% ± 13.1% (SD). The mean ADP dose for controls was 51.6 ± 10.6 μM (SEM), while that for the total patient group was: homozygotes, 43.1 ± 20.4 μM (SEM); heterozygotes, 6.9 ± 5.01 μM (SEM); and polygenics, 61.3 ± 28.9 μM (SEM). Only the homozygous group showed a minimal increase in ADP sensitivity, but the small sample size allowed only marginal statistical significance. Homozygous subjects were not significantly different from heterozygotes, although the mean dose was lower.

Maximum collagen-induced aggregation was 73.5% ± 21.8% (SD) for the control group. There were no differences in mean sensitivity of collagen aggregation for any patient group from the normal subjects; there was no trend toward increased collagen-mediated sensitivity observed in either patient group.

For 10 normal subjects the mean platelet half-life determined by the isotopic method was 119.5 ± 8.9 hours (SEM). Normal mean platelet T-50 measured by the nonisotopic technique in 10 control subjects was 114 hours. Five patients (table 1) had normal platelet survivals, two had moderate reductions in platelet T-50, while two showed severe reduction in platelet survival. Average postinfusion platelet recovery was 59% ± 4% SEM.

Platelet survival was normal in three of four homozygotes; these persons also had the most increase in platelet aggregability. Platelet survival was shortened in six of eight heterozygotes and polygenics who had the least change in aggregability. Only one of the five patients with normal platelet survival (LC, table 1) had a moderate amount of atherosclerotic disease. In contrast, all the patients with shortened platelet survival showed moderate to severe atherosclerotic vascular disease. Comparison of the probability of a shortened platelet survival between the patients with (n = 4) and without (n = 8) vascular disease by the Chi square test with Yates' correction showed a significantly greater incidence (0.01 < p < 0.05) of reduced platelet lifespan among patients with atherosclerotic vascular disease.

Discussion

Although we did observe an increase in platelet responsiveness, we did not see as large a change in our more severely hypercholesterolemic patients as would have been expected from earlier reports. Quantitative changes in platelet aggregability are difficult to measure and there are a limited number of severely affected type II patients available for study. Harker and Hazzard studied eight heterozygous type IIa patients and found normal platelet aggregation. Their study also included two homozygous type IIa subjects with normal platelet responses. Baker et al.22 also failed to find increased platelet aggregability in their study of hyperlipoproteinemic patients. These differences may be due in part to technical variation, to variations between individuals in platelet aggregation, or to differences in the patient populations studied.

Platelet survival measurements in patients with lipid abnormalities have also tended to be variable. Steele et al.3 documented slightly decreased average platelet lifespan in a population of males with coronary artery disease (CAD); 37 of these subjects had either type III or IV hyperlipoproteinemia. Average platelet survival was significantly shorter among hyperlipoproteinemia CAD subjects than among normolipemic CAD subjects. Platelet survival improved with the use of both clofibrate and sulfisoxazole, but the magnitude of lipid reduction did not correlate with the degree of platelet survival improvement. Another study by Steele and Rainwater again documented that subjects with CAD and lipid abnormalities were more likely to have shortened platelet survivals than normal lipidemic CAD patients. Studies by Harker and Hazzard showed that the effect of lipid abnormalities were variable and seemed to be dependent upon the type. As in the earlier studies, types III and IV had modestly decreased platelet survivals while type IIa heterozygotes were normal and only one of two type IIa homozygotes was abnormal.

Table 3. Lipid Levels and Platelet Survival of Hypercholesterolemic Subjects Divided According to Age

<table>
<thead>
<tr>
<th>Age</th>
<th>Patients</th>
<th>Plasma cholesterol (mg/dl)</th>
<th>Triglyceride (mg/dl)</th>
<th>Cholesterol (mg/dl)</th>
<th>Platelet survival (half-life hrs)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>VLDL</td>
<td>LDL</td>
<td>HDL</td>
<td>VLDL</td>
</tr>
<tr>
<td>Young</td>
<td>14 ± 1</td>
<td>6</td>
<td>623 ± 112</td>
<td>127 ± 35</td>
<td>44 ± 20</td>
</tr>
<tr>
<td>Old</td>
<td>52 ± 4</td>
<td>6</td>
<td>388 ± 29</td>
<td>151 ± 25</td>
<td>43 ± 7</td>
</tr>
<tr>
<td>Normal</td>
<td>18-32</td>
<td>10</td>
<td>165 ± 7</td>
<td>68 ± 10</td>
<td>87 ± 1</td>
</tr>
<tr>
<td>Normal adult</td>
<td>1088</td>
<td>1088</td>
<td>189 ± 1</td>
<td>87 ± 1</td>
<td>16 ± 1</td>
</tr>
</tbody>
</table>

*Statistically different (p < 0.01) from normal group or young-patient group (t test).

Values are means ± SEM.
Based upon other observations, we had anticipated that our patients with severe hypercholesterolemia would have increased platelet aggregability and markedly reduced platelet survival. Instead, the present study suggests that there is not a direct relationship between plasma cholesterol, in vitro platelet aggregability, and decreased platelet life span. The younger patients, the most severely hypercholesterolemic subjects, had the largest increase of platelet sensitivity, but had virtually normal platelet survivals. There was a strong trend among the older subjects, with lower cholesterol levels and less aggregation abnormalities, to exhibit the greatest reduction in platelet life span (table 3). When patients were divided according to the extent of vascular disease, we observed that subjects with increased atherosclerosis had the greatest reduction in platelet survival. Thus, it appears that the extent of vascular damage is a more important determinant of altered platelet survival and that hypercholesterolemia plays a greater role in affecting in vitro platelet aggregation than in vivo survival. Since the control group for platelet survival was not age-matched to the patient group, we cannot exclude the possibility that age alone could account for the decreased platelet survival.

Theoretically, patients with shortened survivals and normal platelet counts would have circulating platelets with a reduced mean age. Karpatkin has reported that young platelets exhibit an increased velocity of aggregation. When we compared the average sensitivity to epinephrine for patients with platelet half-lives of less than 100 hours to those with normal platelet survival, there is a difference (2.75 ± 0.95 vs 6.56 ± 2.26 μmoles/liter ± sem) but it is not significant (p = 0.1). The lack of significance may be due to the small sample size.

Ross and Harker have shown that the degree of shortened platelet survival in both homocystinuria- and hypercholesterolemia-induced vascular injury is proportional to the extent of vascular damage and that platelets from hypercholesterolemic animals have a normal survival when cross-transfused into animals without vascular disease. In a recent review, Genton has also pointed out the importance of preexisting vascular disease as a determinant of shortened platelet survival. Our findings are consistent with the hypotheses that the degree of platelet consumption is dependent upon the extent of vascular disease, and that hypercholesterolemia alone does not alter platelet life span.

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References

Index Terms: platelet survival • platelet aggregation • familial hypercholesterolemia
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