Platelet Aggregation in Aortic and Coronary Venous Blood in Patients With and Without Coronary Disease

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SUMMARY  We studied 16 patients with coronary artery disease (CAD) to evaluate platelet aggregation in blood samples withdrawn simultaneously from the aorta and coronary sinus. At rest, mean platelet aggregation in coronary venous blood was significantly lower than that in aortic blood. Platelet counts in coronary venous blood were also lower than in the aortic blood in each of the six CAD patients in whom counts were done. Platelet aggregation was lower in seven patients who were taking propranolol than in the remaining nine who were not taking propranolol. During tachycardia stress, platelet aggregation increased in all patients, but the magnitude of increase was greater in patients not taking propranolol. In four other patients without CAD, platelet aggregation and counts were also studied in the same fashion and were similar in both the aortic and coronary venous blood. These data suggest that in certain CAD patients, platelet consumption or destruction within atherosclerotic vasculature may occur. Propranolol may reduce platelet aggregation at rest and modify excessive aggregation during tachycardia stress in certain CAD patients.

ABNORMAL PLATELET FUNCTION has been reported in several ischemic disease states, such as coronary heart disease1-8 and cerebrovascular disease.9,10 Abnormal platelet function has also been found in association with hyperlipidemia,11 diabetes mellitus12-14 and hypertension,15 conditions that are "risk factors" in the development of ischemic syndromes. Animal studies suggest that extension of myocardial infarction may relate to excessive platelet aggregation16 and that induction of platelet aggregation may result in focal myocardial necrosis.17-19 Recently, increased formation of platelet aggregates in the coronary circulation of dogs with narrowed coronary arteries has been suggested as additional evidence implicating platelets in myocardial ischemia and necrosis.20

The purpose of this study was to evaluate possible changes occurring in platelet aggregation as platelets traverse the coronary circulation in patients with coronary artery disease. Platelet aggregation studies were performed in aortic and coronary sinus blood samples from subjects with and without coronary disease. The influence of tachycardia stress on platelet aggregation was also assessed.

Materials and Methods

Patient Selection

Twenty patients, ages 46-64 years, were included in this study. Sixteen patients had angiographically documented coronary artery disease (CAD) defined as > 50% diameter narrowing. All of these CAD patients had typical stable angina pectoris ranging from one month to five years. Each had evidence for transient ischemia (electrocardiographic ST changes) on exercise by generally accepted criteria. These patients
were undergoing catheterization studies to further evaluate chest pain. The other four subjects had angiographically normal coronary arteries. The clinical indications for catheterization studies in these latter patients included further evaluation of chest pain (two patients) and symptoms related to mitral stenosis in one and mitral insufficiency in another. No patient had congestive heart failure. None of the subjects included had taken aspirin or related agents known to alter platelet function during the two week period before the study. Only antianginal agents consisting of nitrates with or without propranolol were continued.

**Catheterization Technique**

Studies were performed after obtaining informed consent. Without premedication, a #8 Sones catheter (woven dacron), 100 cm long, was advanced to the ascending aorta via an antecubital cutdown. A #7 Zucker catheter (woven dacron with pacing electrodes), 100 cm long, was positioned in the mid-coronary sinus under fluoroscopic control. All hematologic studies were performed before angiography. Samples of blood were obtained simultaneously from the aorta and coronary sinus at rest, during atrial pacing-induced tachycardia, and 15-30 minutes after cessation of tachycardia. The pacing rates varied between 90-150 beats/min (approximately 80% more than resting heart rate).

**Blood Samples**

Blood (9 ml) was collected and immediately transferred into polypropylene tubes containing 1 ml of 3.9% sodium citrate and 0.2 ml of hydroxymethyl (TRIS) aminomethane. In our laboratory this buffer controls the pH of platelet-rich plasma (PRP) so that no change is detected at 2 hours. From these samples PRP was obtained by centrifugation at 225 g for 15 minutes at room temperature and adjusted for platelet count. The remaining blood was centrifuged at 5,000 g for 30 minutes at room temperature to yield platelet-poor plasma (PPP).

Platelet aggregation studies were performed in duplicate within 2 hours of collection. The pH was repeatedly checked and found to be stable during this period. Samples of 0.45 ml PRP were prewarmed to 37°C for 1 minute by constant stirring in siliconized cuvettes in the Biodata Aggregation Profiler® before addition of aggregation stimulating agents. These agents included epinephrine 1:10,000 (55 uM), adenosine diphosphate (ADP) 1 uM and ADP 2 uM. ADP 20 uM stock solution was kept frozen and was diluted at the time of testing. Percentage aggregation was read as the percentage increase of light transmission observed at 5 minutes from the graphic record after the aggregating agent was added. The PPP was used as 100% light transmission. Whole blood platelet counts were performed in duplicate on counting chambers using 3% procaine hydrochloride under phase microscopy.

Platelet aggregation studies were also done to evaluate the effect of drawing blood through the catheters used in these studies. In five additional volunteers, blood was simultaneously withdrawn from an antecubital vein through a #20 metallic needle and through the woven dacron catheters used in the study. These blood samples were handled and analyzed as described above.

To assess the effects of platinum electrodes at the tip of the Zucker catheter, five experiments were done. Right atrial blood was withdrawn simultaneously through the Zucker catheter and another nonpacing dacron catheter for platelet studies. Blood was also withdrawn during intermittent electrical stimulation of the Zucker catheter with the same energy and rate as used in the tachycardia studies.

**Data Analysis**

Average platelet aggregation with each aggregating stimulating agent was determined for each patient. When values from the duplicate studies differed by more than 20%, multiple determinations were made to compute the average value. Mean value and standard error were determined for the percentage aggregation with each stimulating agent. The t test for paired and unpaired data was used to determine the significance of the difference between mean values. A P value < 0.05 was considered statistically significant.

**Results**

**Effect of Withdrawing Blood Through the Cardiac Catheters on Platelet Aggregation**

No difference in platelet aggregation was observed in blood samples obtained through catheters compared to the metallic needle. Mean platelet aggregation was 84 ± 6% for the five samples withdrawn through the catheters and 87 ± 6% in samples obtained through the metallic needle with epinephrine. Similarly, with ADP 1 uM and ADP 2 uM, no difference in platelet aggregation was observed. No differences in platelet aggregation and counts were observed, comparing blood samples drawn through the Zucker catheter with those drawn from the nonpacing dacron catheter. Electrical stimulation likewise did not significantly affect the platelet studies.

**Coronary Artery Disease Patients**

Data are summarized in table 1.

**Platelet Aggregation in Aortic and Coronary Venous Blood**

Platelet aggregation was lower in the coronary sinus blood than in aortic blood at rest. Mean aggregation in all 16 patients with epinephrine was 75 ± 7% in aortic blood, compared to 62 ± 9% in coronary sinus blood (P < 0.05). With the addition of ADP 1 uM and 2 uM, a similar difference in platelet aggregation between aortic and coronary sinus blood samples was seen (table 1).
Platelet aggregation data were widely variable among these CAD patients. Analysis of the aggregation data with regard to the clinical severity showed no apparent association. Clinically, the patients represented a relatively homogenous group (all New York Heart Association functional class II or class III). Analysis of these data with respect to whether or not the patients were taking propranolol revealed two groups. Group A included nine patients not taking propranolol. Group B included seven patients currently treated with propranolol (40–80 mg every 6 hours). The last dose of propranolol in each case had been taken between 4–6 hours before catheterization. Analysis of aggregation data relative to the angiographically determined extent of CAD revealed no apparent relationship as all the patients had extensive disease. Four patients in group A and three in group B had three vessel disease. Five patients in group A and four patients in group B had two vessel disease.

**Effect of Propranolol on Platelet Aggregation**

Platelet aggregation was significantly lower in group B patients both in aortic and coronary sinus blood samples compared to group A patients (table 1). In aortic blood, epinephrine produced 87 ± 5% aggregation in group A compared to 60 ± 12% (P < 0.05) in group B patients. With ADP 1 μM, aggregation in the aortic blood in group A was 71 ± 9% compared to 33 ± 10% (P < 0.05) in group B. Using ADP 2 μM, 87 ± 3% aggregation occurred in group A compared to 49 ± 13% (P < 0.01) in group B. In the coronary sinus blood, epinephrine resulted in 86 ± 6% aggregation in group A patients and 32 ± 10% in group B (P < 0.01). With ADP 1 μM, platelet aggregation was 42 ± 6% in group A compared to 17 ± 4% in group B (P < 0.01). ADP 2 μM resulted in 73 ± 5% and 20 ± 7% aggregation in groups A and B, respectively (P < 0.01).

**Effect of Tachycardia on Platelet Aggregation**

Data are summarized in table 2. Group A. An increase in platelet aggregation occurred in coronary venous blood during tachycardia. Using ADP 1 μM, aggregation increased from 42 ± 6% to 69 ± 9% (P < 0.01). An increase in aggregation was also apparent in the coronary sinus samples using ADP 2 μM. Aggregation returned toward resting values following tachycardia. Platelet aggregation in the aortic blood was unchanged during tachycardia with either epinephrine or ADP.

Group B. During tachycardia, aggregation increased significantly in the coronary sinus blood with ADP 1 μM and 2 μM (table 2). Mean platelet aggregation did not reach the same magnitude as in group A. Platelet aggregation was more than 50% with ADP 1 μM during tachycardia in only one of the seven patients on propranolol, whereas all but one of the nine patients not taking propranolol had aggregation more than 50%. A decrease in aggregation to resting values was observed in the post tachycardia coronary sinus samples with each aggregating agent. Again, in aortic blood, no change in aggregation with tachycardia was observed (table 2).

**Platelet Counts**

Platelet counts were lower in the coronary sinus than in the aortic blood samples in each of the six CAD patients in whom whole blood platelet counts were done. Four of these patients were not taking propranolol and two were on propranolol. The platelet count in the aortic blood was 233,167 ± 30,545/mm³.
Table 2. Effect of Tachycardia Stress on Platelet Aggregation (%)

<table>
<thead>
<tr>
<th></th>
<th>Epinephrine</th>
<th></th>
<th>ADP 1 μM</th>
<th></th>
<th>ADP 2 μM</th>
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<tr>
<td></td>
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<td></td>
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<td>92</td>
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<tr>
<td></td>
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<td>6</td>
<td>4</td>
<td>9</td>
<td>6</td>
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<td></td>
<td>P value</td>
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<td>&lt;0.01</td>
<td>NS</td>
<td>&lt;0.05</td>
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<td>Coro. sinus blood</td>
<td>Mean</td>
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<td>92</td>
<td>84</td>
<td>71</td>
<td>65</td>
</tr>
<tr>
<td></td>
<td>± SEM</td>
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<td>5</td>
<td>7</td>
<td>9</td>
<td>9</td>
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<tr>
<td></td>
<td>P value</td>
<td>NS</td>
<td>NS</td>
<td>&lt;0.05</td>
<td>NS</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>Aortic blood</td>
<td>Mean</td>
<td>233,750</td>
<td>37,150</td>
<td>233,750</td>
<td>37,150</td>
<td>233,750</td>
</tr>
<tr>
<td></td>
<td>± SEM</td>
<td>233,750</td>
<td>37,150</td>
<td>233,750</td>
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<td></td>
<td>P value</td>
<td>NS</td>
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</tr>
</tbody>
</table>

All P values expressed in comparison to resting platelet aggregation (%). Abbreviations: R = rest; T = during tachycardia; A = after tachycardia.

(mean) and in the coronary sinus blood 173,667 ± 15,583/mm³ (mean) (P < 0.05).

Patients with Normal Coronary Arteries
Data are summarized in Table 3.

Platelet Aggregation
There was no significant difference in aggregation between aortic and coronary sinus blood samples in each of the four patients with normal coronary arteries. Tachycardia in these patients did not significantly affect platelet aggregation in either the aortic or coronary sinus blood.

Platelet Counts
Platelet counts were similar in all four patients in both the aortic and coronary sinus blood samples, mean 233,750 ± 37,150/mm³ and 233,750 ± 39,389/mm³, respectively (P = NS).

Discussion
Our results show that a significant difference occurs in platelet aggregation as platelets traverse the atherosclerotic coronary vasculature in certain patients. Lower platelet counts are present in the myocardial effluent blood. Platelet aggregation appears to increase during tachycardia stress and this increase in aggregation promptly resolves following cessation of tachycardia.

Decreased platelet aggregation in the coronary venous blood at rest may be a manifestation of platelet adhesion to atherosclerotic vascular subendothelium. Lower platelet counts observed in the coronary venous blood support this hypothesis. Contact of platelets with the subendothelium, particularly collagen, will cause platelets to form thromboxane A₂ by releasing membrane arachidonic acid. Thromboxane A₂ is a potent stimulus for both platelet aggregation and vasoconstriction. It is possible that such a mechanism plays a role in platelet consumption or sequestration in CAD patients. The slightly lower pH in the ischemic area could decrease platelet aggregation in the coronary venous blood. The minor pH changes that may be present at rest in the absence of symptoms and other evidence for ischemia are unlikely to account for such a marked gradient in platelet aggregation across the heart. Decreased platelet aggregation in coronary venous blood could also result from a refractory state due to previous contact with ADP.

The question of whether withdrawing the samples through catheters could influence aggregation was considered. This possibility is unlikely, since the catheters used to withdraw blood from the aorta and coronary sinus were similar. The platinum electrodes on the coronary sinus catheter did not appear to affect platelet aggregation or counts. Moreover, similar catheter techniques were used in all the subjects whether or not they had coronary disease. No gradients in platelet counts and aggregation studies were observed across the myocardial vascular bed in subjects with angiographically normal coronary arteries.
arteries. Thus, it appears that lower platelet aggregation values and counts in myocardial effluent than in affluent blood are related to the presence of CAD.

Previous studies in CAD patients suggest a greater sensitivity of platelets to aggregation stimulating agents. These observations, however, were made using peripheral venous blood. Assuming that platelet sensitivity in peripheral venous blood is comparable to that in the aortic blood, our findings of similar platelet aggregation in subjects with normal coronary arteries and those with CAD (not taking propranolol) in the aortic blood are at variance with their observations.

It is known that stress states can lead to myocardial ischemia as well as increased platelet aggregation. The marked increase in platelet aggregation observed in coronary venous blood but not in aortic blood in this study appears related to changes occurring in the myocardial vascular bed during a state of increased oxygen demand. Whether increased platelet aggregation itself is a secondary or primary event in transient myocardial ischemia is not clear from our studies. Release of catecholamines and other metabolic products during ischemia induced by tachycardia stress could increase platelet aggregation. A tachycardia-related increase in coronary flow could reduce turbulent flow in the narrowed vessels and possibly increase platelet aggregation. A recent study by Vik-Mo suggesting increased formation of platelet aggregates in dog coronary vascular beds during ischemia supports our interpretation of an increase in platelet aggregation during ischemia induced by tachycardia.

In our CAD patients treated with propranolol, platelet aggregation was markedly diminished in both the aortic and coronary venous blood. The magnitude of the aggregation increase observed during tachycardia stress in these patients was also reduced. This observation of lower aggregation is consistent with previous reports that propranolol may inhibit platelet aggregation in certain patients with hypersensitive platelets by direct actions on platelet membrane.

Alternatively, the platelet aggregation effects observed could be mediated indirectly by effects of propranolol on β-adrenergic receptors, the oxyhemoglobin dissociation curve, myocardial metabolism and oxygen demands. However, since those patients on propranolol had reduced aggregation in both the aortic and coronary sinus blood at rest and the aggregation gradient across the myocardium persisted, we believe our findings favor a more direct action. The blunted increase in platelet aggregation in response to tachycardia may be due to actions of propranolol on coronary blood flow, myocardial metabolism and overall oxygen demands during stress. These observations may relate to the beneficial response seen in patients with ischemic heart disease treated with propranolol.

Our observations suggest that altered platelet function may be intimately related to coronary artery disease. Findings of lower platelet counts and aggregation in the myocardial effluent blood might indicate platelet interaction with a damaged vessel wall. These observations may have important implications in the pathogenesis of myocardial ischemia and warrant additional studies.

### Table 3. Platelet Aggregation (%) in Subjects with Normal Coronary Arteries

<table>
<thead>
<tr>
<th></th>
<th>Epinephrine</th>
<th>ADP 1 μM</th>
<th>ADP 2 μM</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>Aorta</td>
<td>Coronary sinus</td>
<td>Aorta</td>
</tr>
<tr>
<td>Rest</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean</td>
<td>96</td>
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<td>81</td>
</tr>
<tr>
<td>≈ SEM</td>
<td>2</td>
<td>5</td>
<td>6</td>
</tr>
<tr>
<td>P value</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td>Tachycardia</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean</td>
<td>94</td>
<td>95</td>
<td>61</td>
</tr>
<tr>
<td>≈ SEM</td>
<td>5</td>
<td>4</td>
<td>9</td>
</tr>
<tr>
<td>P value</td>
<td>NS</td>
<td>NS</td>
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</tr>
</tbody>
</table>

*P value at rest refers to comparison between aortic and coronary sinus blood; P value during tachycardia refers to comparison with resting values.*

**Abbreviations:** ADP = adenosine diphosphate.

### References

Platelet aggregation in aortic and coronary venous blood in patients with and without coronary disease. 3. Role of tachycardia stress and propranolol.
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