Functional and Biochemical Alterations of Platelets in Atherosclerosis and Thromboembolism

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In atherosclerosis, platelets appear to circulate in an activated state. Our findings are based on results obtained from 240 patients. These platelets demonstrated an increased degree of surface activation and an increased amount of aggregation. Platelet aggregation was also demonstrated by a heightened sensitivity to adrenalin and ADP. Greater than normal secretion of ADP, PF-4, platelet fibrinogen, and platelet antiplasmin was noted. Platelet antiplasmin was increased, whereas platelet antithrombin III was decreased. Membrane transport of glucose was found to be defective in atherosclerosis. There appears to be no alteration in the phospholipid distribution, although the amount of phospholipids increases. The order of secretion of phospholipids in ADP and collagen-induced secretion was found to differ in platelets from atherosclerotic patients when compared to normal platelet populations. The availability of phospholipids rich in arachidonic acid was enhanced. Also, in atherosclerosis, we found a decrease in platelet c-AMP and an increase in c-GMP. The balance between TXA₂ formation in platelets and PGI₂ formation by the vessel wall is important in determining whether thrombus formation is encouraged or prevented.

Our findings are based on results obtained from 240 patients seen over the last decade (5). Lipids were typed on 115 patients, and 92 were found to have type II hyperlipoproteinemia. The hyperfunction of platelets that we observed parallels lipid alterations (14) and agrees with data previously reported by Colman (15).

**Surface Response**

Platelet adhesion is increased in patients with atherosclerosis. Preliminary findings in this area were first reported in patients with ischemic heart disease (16) and were confirmed later (10,17-19). We observed the same phenomenon in our laboratory using the methods of Salzman and Hellem.

Such platelet hyperadhesion parallels elevations in fibrinogen and von Willebrand factor (Table I) observed in these same patients (2,10,20-21). In atherosclerosis, the elevation of fibrinogen and von Willebrand factor levels stimulates platelet adhesion and interaction with the subendothelium. This correlation has been confirmed by Foster and Bowie in experiments with pigs having the homozygote form of von Willebrand's disease. They found that atherosclerosis was not produced (22) because the impaired platelet subendothelial interaction present prevented the formation of atheromatous plaques.

Rebuck and co-workers (23-24) devised a method that utilized transmission electron microscopy to measure the
TABLE I

<table>
<thead>
<tr>
<th>Test Procedures</th>
<th>Control</th>
<th>Atherosclerosis</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fibrinogen (mg%)</td>
<td>248.48 ± 38.24</td>
<td>375.68 ± 73.26</td>
<td>p&lt;0.001</td>
</tr>
<tr>
<td>(N:25)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>FVIII vWF (u%)</td>
<td>87.33 ± 12.3</td>
<td>135.4 ± 15.8</td>
<td>p=0.001</td>
</tr>
<tr>
<td>(N:18)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hellem II glass retension (%)</td>
<td>42.8 ± 8.6</td>
<td>76.5 ± 11.6</td>
<td>p&lt;0.001</td>
</tr>
<tr>
<td>(N:15)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ADP max. amp. mm 0.1 / ml</td>
<td>26.48 ± 8.636</td>
<td>55.88 ± 28.16</td>
<td>p&lt;0.001</td>
</tr>
<tr>
<td>(N:25)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Adrenalin max. amp. mm 0.25 ml</td>
<td>42.69 ± 16.834</td>
<td>73.4 ± 29.96</td>
<td>p&lt;0.001</td>
</tr>
<tr>
<td>(N:25)</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

TABLE II

<table>
<thead>
<tr>
<th>Platelet Types (%)</th>
<th>Control</th>
<th>Atherosclerosis</th>
<th>Aspirin</th>
<th>Dipyridamole</th>
<th>Indobufen</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(N:10)</td>
<td>(N:16)</td>
<td>(N:4)</td>
<td>(N:4)</td>
<td>(N:6)</td>
</tr>
<tr>
<td>Round (%)</td>
<td>4.5±2.6</td>
<td>2.6±2.4</td>
<td>32.4</td>
<td>17.7</td>
<td>27.6</td>
</tr>
<tr>
<td>Dendritic (%)</td>
<td>63.6±8</td>
<td>20.8±6.2</td>
<td>38.4</td>
<td>47.6</td>
<td>41.4</td>
</tr>
<tr>
<td>Intermediate (%)</td>
<td>21.4±4.2</td>
<td>27.7±6.4</td>
<td>11.5</td>
<td>14.9</td>
<td>13.8</td>
</tr>
<tr>
<td>Spread (%)</td>
<td>10.5±5.6</td>
<td>48.9±11.6</td>
<td>17.7</td>
<td>19.8</td>
<td>17.2</td>
</tr>
<tr>
<td>Aggregates per 100 single platelets</td>
<td>6.4±4.2</td>
<td>36.0±14.6</td>
<td>9.4</td>
<td>14.2</td>
<td>10.8</td>
</tr>
</tbody>
</table>

*Selected antiplatelet agents were evaluated in the abnormal platelet group.

degree of surface activation and amount of aggregation exhibited by platelets when they were exposed to a standardized surface (Formvar film) under controlled conditions. Ulutin, et al (25-27) and Walsh, et al (28) independently used this procedure to monitor platelet populations from patients with transient ischemia, cardiovascular disease, and atherosclerosis. Platelet differential counts from patients with atherosclerosis differed from those of normal subjects (Table II). Both the percentage of activated platelets (spread forms) and the number of aggregates were elevated. Increases in platelet adhesiveness and the number of activated forms were more pronounced in the prethrombotic stage and during documented transient ischemic attacks. Antiplatelet drugs, such as aspirin, dipyridamole and Indobufen, normalized the abnormal surface activation, as reflected by a decrease in the percentage of spread forms and a concomitant increase in the number of round forms.

Increased platelet aggregability is also seen in patients with atherosclerosis and thromboembolic phenomenon when platelet aggregometry is used. In patients with diabetes and atherosclerosis, lower than normal concentrations of aggregating agents are required to induce both aggregation and secretion (10,17,20,29-30). Under in vivo conditions, this excess sensitivity undoubtedly facilitates platelet aggregate formation and may potentiate the formation of thrombi and the occurrence of transient ischemic attacks.

**Secretion**

An initial injury that disrupts the endothelial layer of the vessel wall will cause platelets to stick and release a variety of factors. We found that PF-3 (lipoprotein platelet thromboplastin, a procoagulant activity) was increased in patients with atherosclerosis (21), while the osmotic fragility of these platelets remained normal (31). PF-3 activity is also reportedly increased in patients with transient cerebral ischemia and hyperbetalipoproteinemia (32).
In 1968, we reported that PF-4 was a releasable factor (33), and in the same year, Farbiszewski, et al (34) confirmed this finding. They reported that PF-4 was secreted from platelets during intravascular coagulation and thrombosis. Akman showed (35,36) that the same inducer released more PF-4 from platelets of patients with atherosclerosis than from controls. Later, the same enhanced release phenomenon was observed (2,10,19,20,29,37) for the secretion of ADP and platelet fibrinogen (Table III).

Similar changes have been noted in the preceding stage of chronic diffuse intravascular coagulation (DIC). In DIC, a release reaction by the circulating platelets creates an acquired storage pool deficiency, which can be demonstrated by biochemical and ultrastructural studies (10, 38-40). Secreted PF-4 is demonstrated extracellularly, and the Mayo Clinic investigators interpret its rise as indirect evidence of sequestration and consumption of platelets in the blood clotting process (41,42). In atherosclerosis and thromboembolism, the presence of PF-4 and betathromboglobulin (p-TG) have diagnostic value (35,36,43,44). Since we have observed great similarity between atherosclerosis and the preceding as well as the early compensated stages of chronic DIC, it is possible that continuous intravascular coagulation occurs in some cases of atherosclerosis (Table IV).

Another releasable platelet substance promotes the proliferation of smooth muscle cells. This close relationship between platelets and the early sign of atherosclerosis (i.e., smooth muscle cell proliferation) is important because in multiple vessel injury platelets seem to play a role not only in thrombus formation but also in the intimal thickening and ultimately the pathogenesis of atherosclerosis (52,53).
TABLE IV
Stages of Disseminated Intravascular Coagulation and Averaged Laboratory Findings in Ten Cases

<table>
<thead>
<tr>
<th>Stage</th>
<th>Preceding</th>
<th>Compensated</th>
<th>Decompensated</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fibrinogen</td>
<td>Increased</td>
<td>Normal</td>
<td>Decreased</td>
</tr>
<tr>
<td>Prothrombin</td>
<td>Normal</td>
<td>Slightly decreased</td>
<td>Decreased</td>
</tr>
<tr>
<td>Factor VIII/vWF</td>
<td>Increased</td>
<td>Increased or normal</td>
<td>Decreased</td>
</tr>
<tr>
<td>Platelet count</td>
<td>Normal or elevated</td>
<td>Normal or slightly decreased</td>
<td>Decreased</td>
</tr>
<tr>
<td>PF-4</td>
<td>Normal</td>
<td>Decreased</td>
<td>Decreased</td>
</tr>
<tr>
<td>ADP-release</td>
<td>Increased</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>PF-4 - release</td>
<td>Increased</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>ADP content of platelets</td>
<td>Normal</td>
<td>Decreased</td>
<td>Decreased</td>
</tr>
<tr>
<td>Thrombin time</td>
<td>Shortened</td>
<td>Normal or prolonged</td>
<td>Prolonged</td>
</tr>
<tr>
<td>ELT</td>
<td>Prolonged</td>
<td>Normal or shortened</td>
<td>Shortened</td>
</tr>
<tr>
<td>PDP</td>
<td>Normal</td>
<td>Increased</td>
<td>Increased</td>
</tr>
<tr>
<td>Fibrinogen (mg %)</td>
<td>540 ± 80</td>
<td>310 ± 95</td>
<td>92 ± 45</td>
</tr>
<tr>
<td>Prothrombin (u/ml)</td>
<td>240 ± 30</td>
<td>160 ± 40</td>
<td>95 ± 38</td>
</tr>
<tr>
<td>Total PF-4 (U/hr/10^11 platelets)</td>
<td>0.04-0.06</td>
<td>0.2-0.02</td>
<td>0.0</td>
</tr>
<tr>
<td>ADP release %</td>
<td>82 ± 16</td>
<td>15 ± 7</td>
<td>—</td>
</tr>
<tr>
<td>ADP (mg/3x10^10 platelets)</td>
<td>1.950 ± 0.350</td>
<td>0.310 ± 0.110</td>
<td>0.240 ± 0.07</td>
</tr>
<tr>
<td>FDP mg %</td>
<td>6 ± 3</td>
<td>22 ± 8</td>
<td>52 ± 24</td>
</tr>
</tbody>
</table>

TABLE V
Comparison of Platelet Antiplasmin and Platelet Antithrombin III Levels in Patients with Atherosclerosis and Normal Subjects

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>Atherosclerosis</th>
</tr>
</thead>
<tbody>
<tr>
<td>Platelet antiplasmin u/cumm</td>
<td>2.4 ± 0.8</td>
<td>10 ± 1.9</td>
</tr>
<tr>
<td>Inhibition (%)</td>
<td>3.5</td>
<td>12</td>
</tr>
<tr>
<td>p &lt; 0.001</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Platelet antithrombin III Immunologic method ( \mu g/10^9 \text{plt.} )</td>
<td>14.74 ± 1.9</td>
<td>8.84 ± 1.59</td>
</tr>
<tr>
<td>Rivanol method Unit/Thrombin</td>
<td>1.20 ± 0.04</td>
<td>1.04 ± 0.06</td>
</tr>
<tr>
<td>Chromogenic Substrate 2239 % Ath. III.</td>
<td>1.6 ± 6.08</td>
<td>0.9 ± 0.2</td>
</tr>
</tbody>
</table>

Another factor released by platelets also causes vasoconstriction of the vessel wall. However, because the prostacyclin (PGI₂) formed in the vessel is a vasodilator, it diminishes the action of this material.

More specifically, studies of prostacyclin (PGI₂) have shown that it is formed in the endothelial cells of the vessel wall in the arachidonic acid-endoperoxide pathway by a phospholipid prostacyclin synthetase enzyme. Furthermore, it is now known that prostacyclin is the strongest inhibitor of platelet aggregation and secretion and that the balance between TXA₂ and prostacyclin partially dictates either the formation or prevention of thrombosis (20,29,54,55) (Figs. 1,2). It is thus significant that experimental evidence indicates increased thrombus formation at the site of injured or disrupted endothelium where PGI₂ formation is either decreased or absent (55). In addition, it has been shown in humans that PGI₂-like activity is absent in atheromatous plaques.

In experiments with rabbits fed on atherogenic diets, a decrease in spontaneous and arachidonic acid-induced prostacycline formation was noted in the mesenteric artery and aorta. At this stage, the platelets are presumably normal. Only hyperaggregability to ADP can be shown, due to a decreased level of c-AMP, which in turn relates to a probable deficiency of PGI₂. After some time, however, arachidonic acid metabolism accelerates. As a result, platelet TXA₂ generation increases and PGI₂ formation decreases, both of which increase the risk for thrombus formation (56). Using isolated platelets from atherosclerotic patients, Yardimici and Emekli from our laboratory have shown that the increase in c-AMP and the decrease in c-
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Platelets

Platelet stimulation

Phospholipase A

Free ARACHIDONIC Acid

Cyclo-oxygenase

PGG
, PGH

Thromboxane Synthetase

TXA


ATP → C-AMP → AMP

Endothelial cells

VWF

Arachidonic acid

PGG
, PGH

Prostacyclin Synthetase

Fibrinolytic System Activators

Fig. 1

Prostaglandin pathways in the vessel wall and platelets. The balance between TXA and PGI.

GMP were significant when compared to normal platelets (57). These findings lend further support to the concept that activated platelets circulate in atherosclerotic patients.

These observations reveal the importance of TXA as an inducer of platelet aggregation produced by the platelets, the formation of PGI (an inhibitor of platelet aggregation produced by the vessel wall), and the balance between these two materials. We believe that thrombus formation is facilitated by decreased PGI synthesis from arachidonic acid in the endothelial cells or by an increased formation of TXA again from arachidonic acid, but in the platelets. If we decrease TXA formation in platelets or inhibit its release, thrombus formation is hampered.

Phospholipid Distribution and Quantitation

We also investigated both the distribution order of phospholipids in the platelet membrane and their sequence of secretion during the release phenomenon for platelets isolated from normal subjects and patients with atherosclerosis. When either ADP or collagen (20,29,58-61) was the inducer, no differences between the order of phospholipid distribution occurred. The order was as follows: phosphotidyl choline (PC) > phosphotidyl ethanolamine (PE) > sphingomyelin (SPH) > phosphotidyl serine (PS) >, and phosphotidyl inositol (PI). However, in atherosclerotic patients, the quantities of phospholipids were increased.

Depending on the inducer, the secretion of phospholipids (and therefore their availability) differed when normal platelets were compared with atherosclerotic platelets. This dependency was observed in both groups (58,61). In normal subjects, the sequence of secretion after induction with ADP was SPH > PI > PC > PS > PE (Fig. 3); whereas with collagen, it was PI > PS > SPH > PC > PE (Fig. 4). Using atherosclerotic platelets, the sequence of secretion with ADP was altered to PI > SPH > PS > PC > PE; and with collagen it was also changed: PS > PC > PE > PI > SPH.
atherosclerotic patients, PS and PI (Fig. 5) showed an increased amount of release ($P > 0.001$), whereas the release of SPH and PC decreased significantly and PE remained changed. In collagen-induced release, a greater release of PS, PE and PC (Fig. 6) was observed in atherosclerotic patients. On the other hand, there was a significant decrease in PI and SPH release.

In atherosclerosis, the phospholipids secreted early in the sequence are those rich in arachidonic acid. These observations gained importance when it was found that membrane phospholipids, arachidonic acid, PGE$_2$, PGF$_2\alpha$ and TXA$_2$, play key roles in platelet aggregation, secretion, and thrombus formation (20,29). Among the other findings that support this view are the increase in platelets of phospholipids rich in arachidonic acid (20,29) and the increase in thromboxane synthetase activity in patients with atherosclerosis as well as those with diabetes mellitus (30,62). Recently, Gursoy (Aktulga) demonstrated that when the platelets of atherosclerotic patients were induced with collagen (63), their PGF$_2\alpha$ formation was significantly higher than that of normal subjects (63).

Membrane Transport

Yardimci from our group (34) has shown a defect in the membrane transport of glucose in atherosclerosis. In patients with atherosclerosis, diabetic or nondiabetic, there was a significant decrease in platelet glucose transport when compared to normal subjects (Figs. 5-8). Thus, we can conclude that these patients have a defect in carbohydrate metabolism at the cellular level.
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Fig. 3
ADP-induced platelet phospholipid release in normal subjects (N) and in atherosclerotic patients (P).

Fig. 4
Collagen-induced platelet phospholipid release in normal subjects (N) and in atherosclerotic patients (P).

Fig. 5
Time course accumulation of glucose by human platelets isolated from normal subjects and from atherosclerotic patients. The final $^{14}C$-glucose concentration in the incubation medium was $1 \times 10^{-5}$M and the assay temperature was 37°C. Each point represents mean ± SE. Normal subjects, N:21; atherosclerotic patients, N:26 (54).

Fig. 6
Kinetics of glucose transport by human platelets isolated from normal subjects and from atherosclerotic patients. The assay is performed at 37°C. Normal subjects, N:6; atherosclerotic patients, N:7 (54).
Fig. 7
Double reciprocal plot of concentration dependence curve of glucose transport by human platelets isolated from normal subjects. Each point is mean ± SE for N:6 (54).

Fig. 8
The effect of metabolic inhibitors and non-metabolizable glucose analogues on glucose transport by control human platelets and those from atherosclerotic patients. Final glucose concentration was 1x10^-5M. Assay temperature was 37°C; the amount of glucose accumulation is measured at 10 min of incubation times. Bars represent mean ± SE, N:7 for normals and N:14 for atherosclerotics. The accumulation at 4°, with KCN, with DNP, with DOG, and with 30MG compared to the amount accumulated at 37° is all highly statistically significant; with ouabain it is significant for normals. For atherosclerotics the differences are not significant (54).

Summary
In atherosclerosis, platelets undergo functional changes including increases in adhesion, surface activation, and aggregation. These changes would appear to facilitate thrombus formation. The balance between TXA2 formation in platelets and the PGI2 formation by the vessel wall is also important in thrombus formation. We believe that the most important observation in atherosclerosis about platelets is that they circulate in an activated state.
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References


