Platelet accumulation in experimental angioplasty: time course and relation to vascular injury

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ABSTRACT Since platelet accumulation may be an important determinant of restenosis after angioplasty, the time course of $^{51}$Cr-labeled platelet accumulation after experimental angioplasty was evaluated in a deendothelialized, hypercholesterolemic rabbit preparation of atherosclerosis. Marked platelet accumulation (39.5 ± 8.7 × 10^6 platelets/1 cm vessel length) was observed at 30 min and remained active until 4 hr after angioplasty. Total accumulation over 24 hr was 56.4 ± 4.7 × 10^6 platelets/1 cm length. Histologic dissection was directly related to the degree of platelet accumulation, with 64 ± 15 × 10^6 platelets/1 cm in the group with marked dissection and 8.7 ± 3.7 × 10^6 platelets/1 cm in the group with minimal dissection (p < .05). Increasing angiographic dissection also resulted in a trend toward increased platelet accumulation, and angiographic change in luminal diameter showed a significant correlation with platelet accumulation. It is concluded that marked platelet accumulation occurs early after transluminal angioplasty and is related to the extent of dissection. Restenosis may result from a complex interaction of platelet accumulation, vascular damage, and blood flow.


PERCUTANEOUS transluminal angioplasty has been used increasingly for the nonsurgical treatment of occlusive atherosclerotic arterial disease in man. With a primary success rate approaching 90%, the major biological obstacle to be overcome remains the problem of restenosis, which occurs in up to 30% of successfully dilated lesions.\(^1\) Endothelial denudation and platelet adhesion have been observed both by light and scanning electron microscopy after arterial intimal damage.\(^2\)\(^,\)\(^3\) Platelet adhesion may be responsible for thrombosis and vasospasm occurring early after angioplasty. In addition, platelet-derived growth factor has a proliferative effect on smooth muscle cells, which may be an important factor in restenosis. Earlier studies from this laboratory have shown a diminution in the degree of restenosis in rabbits treated with either aspirin-dipyridamole or sulfipyrazone, suggesting a role for platelets.\(^1\) If platelet accumulation is indeed important in the genesis of restenosis, then quantitation of this process and determination of its time course and relationship to vascular injury are critical to the development of protocols designed to maintain vessel patency after angioplasty.

Thus, the aims of this study were to use $^{51}$Cr-labeled platelets (1) to delineate the time during which active platelet accumulation occurs after experimental angioplasty, and (2) to relate the level of platelet accumulation after angioplasty to the degree of neointimal dissection determined both angiographically and histologically.

**Methods**

**Animal preparation.** Sixty-eight New Zealand white rabbits (weight 3 kg) were prepared with bilateral iliofemoral balloon deendothelialization followed by 6 weeks of a 2% cholesterol diet to create significant iliac atherosclerosis as previously described.\(^3\)

**Experimental design (figure 1).** After 6 weeks on the atherogenic diet all animals were anesthetized with pentobarbital and underwent aortoiliac angiography by right carotid arteriotomy with a No. 4F Swan-Ganz catheter advanced fluoroscopically to the aortic bifurcation. Only lesions causing 50% to 95% reduction in luminal diameter in either the left or right iliac artery or both were studied. A total of 51 animals were suitable for study after animals suffering anesthetic death during the initial procedure, bilateral total iliac occlusions, or less than 50% stenosis.
were excluded. After angiography, arteries with lesions were randomly assigned to undergo angioplasty or serve as control segments. After 16 vessels were accumulated as control segments, all subsequent lesions underwent angioplasty. Of the 51 animals, 14 had one site, 34 had two sites, two had three sites, and one had four sites dilated. Angioplasty was performed with a 2.5 mm intraoperative angioplasty catheter introduced via superficial femoral arteriography and advanced through the lesion under fluoroscopic control. The arteriomy site was in the superficial femoral artery and was at least 6 cm from the angioplasty site to ensure adequate runoff. The angioplasty balloon was inflated three times for 30 sec at 5 atmospheres, and successful dilation was confirmed by repeat angiography showing at least a 20% increase in luminal diameter. To determine vessel wall–platelet reactivity at specific times after angioplasty, 2 × 10⁵ homologous 51Cr-labeled platelets were injected 30 min before animals were killed. Groups of animals were killed at 30 min, 2, 4, 6, and 24 hr, and 1 and 4 weeks after angioplasty after 30 min exposure to radiolabeled platelets. In a separate group of animals, to determine total platelet accumulation over 24 hr, labeled platelets were injected and allowed to circulate in the recipient animal for a 24 hr period from angioplasty to death. At the time of death, blood samples were taken to determine specific platelet radioactivity. Animals were then killed with pentobarbital followed by rapid carotid pressure perfusion with a formalin-glutaraldehyde mixture at 100 mm Hg. The angioplasty site was identified by its relationship to other vessels on the cineangiograms and by its characteristic dilated appearance on gross inspection. One centimeter iliac arterial segments at the site of angioplasty were removed, cleaned of all adventitia, and prepared for gamma counting and histologic analysis. The number of platelets adherent to the 1 cm angioplasty site was derived by dividing the number of gamma counts at the site by the number of counts per blood platelet determined at time of death.

Platelet preparation. Rabbit platelets were prepared and labeled with 51Cr by the method of Adelman et al. Platelets were obtained from blood collected into acidified citrate dextrose anticoagulant through a 21 g scalp-vein needle placed in the ear artery of normal rabbit donors. The platelet pellet was resuspended in Ringer’s citrate dextrose solution and labeled with 100 Ci of Na251CrO4 (New England Nuclear, Boston) by incubation for 30 min at 37°C. Labelled platelets were then washed twice in platelet-poor plasma and resuspended in platelet-poor plasma for injection into the ear veins of experimental animals.

Analysis. Cineangiograms obtained before and after transmural angioplasty were viewed by two independent investigators without knowledge of the platelet accumulation data. Caliper measurements were taken of the smallest luminal diameter narrowing at the angioplasty site. True diameters were calculated with use of a 1 cm marker positioned at the level of the spine. Angiographic dissection was graded as 0 (no dissection), 1+ (intraluminal linear filling defect), or 2+ (extraluminal linear dye stain). Differences in either measurements or angiographic dissection grade were resolved by consensus. Histopathologic sections were obtained from serial sections of the 1 cm dilated segment of iliac artery. Sections were stained with hematoxylin-eosin and Verhoff–Van Gieson elastin stains. Histologic dissection was graded as minimal (no dissection or minimal intimal clefts) or marked (disruption through to the media). Sections were viewed by two investigators and grading differences were resolved by consensus.

Comparisons between group means were by the nonpaired Student’s t test. The relationship of platelet accumulation to increasing angiographic dissection was tested by one-way analysis of variance. The correlation between change in luminal diameter and platelet adhesion was assessed assuming a linear relation. In all tests a p value of <.05 was considered to indicate a significant difference.

Results

Time course of platelet accumulation. As shown in Table 1, platelet accumulation at the site of angioplasty
TABLE 1
Platelet accumulation at intervals after experimental angioplasty

<table>
<thead>
<tr>
<th>Group</th>
<th>n</th>
<th>Platelets (×10^6/1 cm vessel length)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nondilated controląż</td>
<td>16</td>
<td>2.3 ± 0.6</td>
</tr>
<tr>
<td>After angioplastyąż</td>
<td></td>
<td></td>
</tr>
<tr>
<td>30 min</td>
<td>32</td>
<td>39.5 ± 8.70</td>
</tr>
<tr>
<td>2 hr</td>
<td>8</td>
<td>51.6 ± 36.2D</td>
</tr>
<tr>
<td>4 hr</td>
<td>5</td>
<td>3.6 ± 0.9</td>
</tr>
<tr>
<td>6 hr</td>
<td>6</td>
<td>2.3 ± 0.6</td>
</tr>
<tr>
<td>24 hr</td>
<td>8</td>
<td>5.1 ± 1.5D</td>
</tr>
<tr>
<td>1 wk</td>
<td>5</td>
<td>0.9 ± 0.3</td>
</tr>
<tr>
<td>4 wk</td>
<td>8</td>
<td>0.8 ± 0.3</td>
</tr>
<tr>
<td>Accumulation over 24 hrż</td>
<td>5</td>
<td>56.4 ± 4.8D</td>
</tr>
</tbody>
</table>

*Mean ± SEM.
*ZLabeled platelets injected and allowed to circulate 30 min before animals were killed.
*ZLabeled platelets injected 30 min before angioplasty and allowed to circulate for 24 hr until animals were killed.
*ZP < .05 vs no angioplasty 30 min after injection of labeled platelets.

(mean ± SE) rose rapidly from a background level of 2.1 ± 0.6 × 10^6 platelets/1 cm length in nonangio-
plastied lesions to 39.5 ± 8.70 × 10^6 platelets/1 cm length at 30 min, and 51.6 ± 36.2 × 10^6 platelets/1 cm length at 2 hr. Platelet accumulation returned to background levels by 4 hr, with a small increment at 24 hr. Total platelet accumulation over 24 hr was 56.4 ± 4.8 × 10^6 platelets/1 cm length, supporting the conclusion that the vast majority of platelet adhesion occurs within the first 2 hr after angioplasty. Because of the wide variation in platelet accumulation in this preparation, and to identify predictors of heavy platelet accumulation after angioplasty, histologic and angiographic variables of vessel damage were examined.

Relationship to histologic dissection. The relationship between histologic dissection and platelet accumulation at 30 min was examined in 21 vessels. Shown in figure 2, A, is a cross section of a rabbit iliac artery taken 30 min after angioplasty. The fibrocellular nature of the atherosclerotic lesion is apparent, with stretching, minimal intimal disruption, and a thin carpet of platelets adherent to the damaged neointima. This figure illustrates an example of our minimal dissection grade. In contrast, figure 2, B, is an example of a marked neointimal fracture and dissection between the intima and media. Heavy platelet accumulation and frank intramural hemorrhage are present at the dissection site. In vessels with this degree of dissection, intramural hemorrhage was easily observed on gross examination.

The relation of histologic dissection grade to platelet accumulation 30 min after angioplasty is shown in table 2. The group with marked dissection had a significantly greater degree of platelet accumulation (64.1 ± 14.9 × 10^6 platelets/1 cm) than the minimal dissection group (8.7 ± 3.7 × 10^6 platelets/1 cm length).

Relationship to angiographic variables. Angiographic variables were also related to platelet adhesion in 16 vessels. Figure 3 shows examples of 0, 1+, and 2+ dissection grades. When lesions with no (17.7 ± 5.5 × 10^6 platelets/1 cm length), 1+ (61.9 ± 24.8 × 10^6 platelets/1 cm length), and 2+ (88.0 ± 30.2 × 10^6 platelets/1 cm length) angiographic dissection were compared (table 2), there was a trend toward increasing platelet accumulation with more severe dissection, but this relationship failed to reach statistical significance in a one-way analysis of variance due to scatter inherent in the chromium quantitation method. There was, however, a near-significant difference in platelet accumulation when the group with 2+ dissection was compared with the group without angiographic dissection. A significant correlation was seen between angiographic change in luminal diameter and platelet accumulation (r = .59, p < .05; figure 4).

Discussion

The mechanism of transluminal angioplasty appears to be an increase in luminal diameter brought about by fracture and stretching of the intima and media. This is seen in both human case reports6,7 and experimental preparations.5,8 Histologic analyses in experimental presentations and human6,10 studies late after angioplasty have shown dense fibrocellular lesions with partial to total obstruction of the lumen, often without evidence of the fissuring seen acutely. This suggests an active process of remodeling in the vessel wall at the lesion site involving the proliferation of smooth muscle cells.3

TABLE 2
Relation of histologic and angiographic dissection to platelet accumulation 30 min after dilation

<table>
<thead>
<tr>
<th>Dissection grade</th>
<th>n</th>
<th>Platelets (×10^6/1 cm vessel length)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Histologic</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Minimal</td>
<td>5</td>
<td>8.7 ± 3.7</td>
</tr>
<tr>
<td>Marked</td>
<td>16</td>
<td>64.1 ± 14.94</td>
</tr>
<tr>
<td>Angiographic</td>
<td></td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>4</td>
<td>17.7 ± 5.5</td>
</tr>
<tr>
<td>1+</td>
<td>6</td>
<td>61.9 ± 24.8</td>
</tr>
<tr>
<td>2+</td>
<td>6</td>
<td>88.0 ± 30.6</td>
</tr>
</tbody>
</table>

*p < .05 vs minimal; 6p = .06 vs 0 dissection.
FIGURE 2. Light micrographs of rabbit iliac angioplasty sites 30 min after dilation. A, An example of minimal dissection showing only a thin layer of platelets (black arrow). B, An example of marked neointimal dissection with frank hemorrhage (small black arrow) and significant platelet deposition (large white arrow).
Platelets have been shown to release a potent smooth muscle cell mitogen, platelet-derived growth factor (PDGF), which may be an important regulator of the extent of neointimal regrowth after angioplasty, and thereby an important determinant of restenosis. We thus sought to quantitate the extent and to delineate the time course of platelet accumulation in a rabbit preparation of angioplasty.

**Time course of platelet accumulation and relation to vascular injury.** In this experimental study, platelet accumulation at the angioplasty site was quantitated and shown to be most prevalent in the first few hours after the procedure. The platelet accumulation observed was tenfold greater than the $4 \times 10^6$ platelet/cm$^2$ previously described both by Groves and Adelman and their colleagues in models of mild arterial intimal damage in which only deendothelialization without fissuring is created by balloon withdrawal. In addition, the duration of active platelet accumulation at the angioplasty site appears to be somewhat longer than that measured by Groves et al., who found that platelet adhesion dropped to $0.1 \times 10^6$ platelet/cm$^2$ within 60 min after deendothelialization and fell further over the next 7 days. The contrast between the amount of platelet accumulation reported in mild arterial balloon deendothelialization and that seen after balloon angioplasty suggests that the degree of platelet accumulation and its duration may depend on the extent of fracture in the vessel wall with resultant exposure of highly thrombogenic medial collagen. In support of this hypothesis, a direct relation was demonstrated between the amount of platelet accumulation and the degree of neointimal damage in the present study.

**Animal preparation.** This study uses a well-described rabbit preparation of atherosclerosis induced by endothelial denudation and cholesterol feeding. The lesions thus produced may be subocclusive or may lead to total occlusion, and in that respect resemble human atherosclerosis; however, the lesions tend to be diffuse, and consist primarily of a foam cell proliferation rather than the fibrotic and often ulcerated lesions found in man. Nonetheless, this preparation has been used successfully to study the mechanisms of transluminal angioplasty and has proven an excellent preparation for the study of restenosis, which, although it occurs in only 30% of patients after angioplasty, is uniformly seen after angioplasty in rabbits. Another advantage of this preparation is that it reproduces the intimal tears and fibrocellular proliferation often seen in human angioplasty. In a recent article Steele et al. reported the use of nonatherosclerotic pig carotid arteries as an angioplasty preparation. A somewhat oversized dilating catheter (8 mm compared with the normal pig carotid diameter of 5 to 6 mm) was used to duplicate the intimal splitting seen in human angioplasty. Their results were quantitatively similar to those presented here ($44.7 \pm 20.7 \times 10^6$ platelets/cm$^2$ adherent to the site at 1 hr), but suggest persistence of a high degree of platelet accumulation up to 24 hr after angioplasty. In the present study, platelet accumulation was quantitated per centimeter length of lesion rather than per square centimeter surface area, as has been done in previous studies. This was done first to preserve histologic integrity of the dilated segment and second

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**FIGURE 3.** Aortoiliac angiograms taken 30 min after transluminal angioplasty. A. No angiographic dissection after bilateral iliac angioplasty. B. Dissection of grade 1+ with intimal flap (arrow) after right iliac angioplasty. The left iliac was totally occluded. C. Dissection of grade 2+ with frank extraluminal dye density after left iliac angioplasty. The right iliac was totally occluded.
because of the difficulty encountered in accurately measuring the surface area of a lesion that has been subjected to severe neointimal dissection and stretching. Given the surface area of a nondilated 1 cm segment of an atherosclerotic rabbit iliac artery (0.3 to 0.5 cm²), the platelet accumulation seen in this study is approximately two to three times that noted by Steele et al.⁴ in a nonatherosclerotic angioplasty preparation.

In this study the scatter inherent in chromium quantitation of platelet accumulation was similar to that seen by other investigators.² ³ ⁴ This may partially account for the lack of statistical relationship between angiographic dissection and platelet accumulation; however, the limitations of angiography in estimating vessel wall dissection must also be kept in mind. A dissection occurring in a plane parallel to the angiographic x-ray beam will likely be entirely missed while one lying perpendicular to the beam will be correctly gauged. Thus, angiography at best approximates the degree of intimal dissection as detected histologically.

**Relation of restenosis to platelet accumulation and arterial damage.** Marked platelet accumulation at the angioplasty site could lead to restenosis by two different mechanisms. The first is the induction of thrombosis and vasospasm that might be expected to occur early in angioplasty. Two groups have described both phenomena in the setting of the acute reclosure syndrome after initially successful angioplasty and have related this to the presence of intimal tear.¹⁵ ¹⁶ The second mechanism that may be of importance in restenosis occurring 3 to 6 months after angioplasty is the stimulation of smooth muscle cell regeneration at the dilated site by PDGF released at the time of angioplasty.⁷ ¹¹ This mechanism would be expected to have a larger impact on the regrowth of cellular lesions and may partially explain the higher restenosis rates reported for patients with recent onset of symptoms.¹

A unifying hypothesis could be advanced that the neointimal damage caused by angioplasty leads to marked platelet accumulation at the site with release of PDGF leading to smooth muscle cell proliferation and restenosis. Although theoretically appealing, there is some question regarding the application of this hypothesis to clinical angioplasty. Leimgruber et al.¹⁷ have shown a lower restenosis rate in patients with intimal tear who have had an initially successful angioplasty with a final gradient of less than 15 mm Hg. Others have found intimal dissection to have either no effect¹⁸ or to be protective against restenosis.¹⁹ A possible explanation for these disparate findings is that factors that may lead to a larger final lumen and greater blood flow are as important as platelet-mediated smooth muscle cell regrowth in determining the fate of the lesion after angioplasty. Such mechanical factors may be larger balloon size, higher pressures, and longer inflation times — all of which might be expected to yield a larger lumen while at the same time causing more prominent intimal dissection. Certainly indexes of smaller lumens and lower blood flow (high transsteno tic gradients and severestenoses before and after angioplasty) have been shown to be important predictors of restenosis.¹ The nature of the lesions themselves may also play a key role in restenosis.

It is clear that antiplatelet therapy will be an important part of the attempt to decrease restenosis. It has been disappointing so far that antiplatelet therapy has not diminished restenosis rates below 25% to 30%. Currently available platelet-inhibiting drugs may not
be sufficiently potent to prevent the platelet-mediated aspects of restenosis despite their demonstrated effect on maintaining patency of bypass grafts.

In conclusion, our study demonstrated marked platelet accumulation at the site of angioplasty primarily in the first 2 hr after the procedure. The degree of platelet accumulation was related to the extent of vessel wall damage. The biology of restenosis is complex and involves competing forces of thrombosis, spasm, and smooth muscle cell proliferation, which favor restenosis, and mechanical factors leading to larger luminal size and better blood flow that favor continued patency. Currently available antiplatelet therapy has failed to decrease restenosis rates below 25% to 30%. Further study of new more potent antiplatelet drugs may be worthwhile in the attempt to find a way to prevent restenosis.

References
Platelet accumulation in experimental angioplasty: time course and relation to vascular injury.
J R Wilentz, T A Sanborn, C C Haudenschild, C R Valeri, T J Ryan and D P Faxon

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