Reduction of indium-111 platelet deposition on Dacron vascular grafts in humans by aspirin plus dipyridamole

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ABSTRACT Aspirin plus dipyridamole reduces platelet accumulation on short-term Dacron vascular grafts in man. To determine whether drug inhibition of platelet deposition is sustained on older grafts, we studied 18 men aged 41 to 87 years who had Dacron aortic bifurcation grafts in place a mean of 43.4 months (range 9.8 to 121.0) before and during short-term therapy with aspirin (325 mg tid) plus dipyridamole (75 mg tid). During both the baseline and drug studies, indium-111 (111In) platelet deposition was quantitated by two techniques, standard planar imaging performed at 24, 48, and 72 hr after injection of platelets and single photon emission computed tomographic imaging performed at 24 and 72 hr after injection. All analyses were performed in a blinded fashion. On both the planar and tomographic images, platelet accumulation on the graft was quantitated by a graft/blood ratio that compared activity in the graft to simultaneously collected whole blood 111In platelet activity. Aspirin plus dipyridamole reduced the tomographic graft/blood ratio at 24 hr (20.6 ± 3.5 vs 17.3 ± 2.5) (± SEM) and at 72 hr (29.0 ± 4.8 vs 25.0 ± 4.1) after injection of platelets (p = .02). Similarly, the planar graft/blood ratio was reduced at 24 hr (2.7 ± 0.5 vs 2.4 ± 0.5), 48 hr (3.7 ± 0.9 vs 3.1 ± 0.7), and 72 hr (4.0 ± 0.9 vs 3.6 ± 0.8) (p = .04). We conclude that aspirin (325 mg tid) plus dipyridamole (75 mg tid) reduces platelet accumulation on long-term Dacron vascular grafts.


AFTER PROSTHETIC ARTERIAL GRAFT placement, occlusion is a common complication, particularly for grafts of smaller caliber. Although the mechanisms of occlusion are multifactorial,1 many studies in animals and in humans suggest that platelet accumulation may play a prominent role, either directly by the formation of occlusive thrombus or indirectly by stimulation of intimal hyperplasia. Studies of indium-111 (111In)–labeled platelets have documented that platelet deposition is greatest early after arterial graft placement.2,3 Although deposition decreases in the subsequent 6 months, platelet accumulation remains readily detectable for periods of up to 10 years.4,5 Aspirin plus dipyridamole, used early postoperatively when deposition is maximal, reduces 111In–labeled platelet uptake on Dacron or polytetrafluoroethylene arterial grafts in humans6,7 and has been associated with improved short-term patency compared with placebo therapy.6,8–10 Whether aspirin plus dipyridamole reduces platelet uptake on older grafts that have a lesser rate of platelet deposition, but a continued risk of occlusion, remains unclear. Prior studies have documented that other platelet inhibitory agents, including ticlopidine,11 sulcotidil,12 and sulfipyrazone13 do not reduce platelet accumulation on grafts greater than 9 months old. With two methods of platelet imaging, the hypotheses tested in this study was that aspirin (325 mg tid) plus dipyridamole (75 mg tid) would reduce 111In–labeled platelet deposition on Dacron grafts that had been in place for greater than 9 months.

Methods

Subjects. Eighteen men aged 41 to 87 were selected for study who had Dacron aortic bifurcation grafts that had been in place for greater than 9 months, had no contraindications to aspirin or dipyridamole therapy, were clinically stable, and were not receiving anticoagulants or platelet inhibitory drugs (heparin, warfarin, aspirin, dipyridamole, sulfipyrazone, indomethacin, ibuprofen, and other nonsteroidal antiinflammatory drugs). The grafts had been in place a mean of 43.4 months (range 9.8 to 121.0). The study was approved by the University of Washington.
ton Human Subjects Review Committee, and all subjects gave informed consent.

**Study protocol.** The study was a crossover trial consisting of two phases with blinded interpretation of the data. For 10 days before study entry and during the first platelet imaging, aspirin, dipyridamole, and other platelet inhibitory agents were stopped. Acetaminophen was given for analgesia. On study days 1 to 4, subjects underwent control 111In platelet labeling and imaging. They were then begun on aspirin (325 mg tid) plus dipyridamole (75 mg tid) on day 5 and had repeat 111In platelet imaging on days 15 to 18 while they continued to receive the drugs. At the time of the second platelet labeling, patients had received aspirin plus dipyridamole for 10 days. Drug compliance was encouraged by repeated phone calls. In the last nine patients entered, drug compliance was assessed by aspirin levels determined by a commercial assay (Stanbio Laboratory, Inc., San Antonio, TX) at the time of each platelet labeling. At the time of the initial baseline study, all nine subjects had levels between 0 and 2.0 mg/ml (the range noted in patients not ingesting aspirin). At the time of the drug study, eight subjects had a level of greater than 2.5 mg/ml and the ninth subject had a level of 1.6 mg/ml.

**Platelet labeling, imaging, and analysis.** 111In labeling of autologous platelets was performed with a closed blood-bag method as previously described. The mean labeling efficiency was 44 ± 2% (± SEM) during control testing and 43 ± 3% during the aspirin plus dipyridamole phase. There was no difference by paired t testing in the mean injected dose between control testing (338 ± 2 μCi) and aspirin plus dipyridamole testing (331 ± 11 μCi). The total injected dose for both studies ranged from 510 to 705 μCi. Prior dosimetric studies of 111In-labeled platelets in humans have estimated that each 1000 μCi results in a total body dose of 0.3 to 0.9 rad, a hepatic dose of 0.6 to 2.5 rad, and a splenic dose of 25 to 34 rad. The mean percentage of 111In activity present free in the plasma was not significantly different between the control or drug phases before injection (5 ± 1% vs 6 ± 1%) or at 24 hr (5 ± 1% vs 5 ± 1%), 48 hr (7 ± 1% vs 6 ± 1%), or 72 hr (6 ± 1% vs 6 ± 1%) after injection of platelets.

In platelet imaging was performed with two separate techniques, standard planar imaging and single photon emission computed tomographic imaging. Planar imaging was performed at 24, 48, and 72 hr after injection of labeled platelets in all patients by taking a 150,000 count anterior abdominal view that largely excluded the liver and spleen. The imaging time in seconds was recorded. Images were acquired with either an Ohio Nuclear Sigma 410 gamma camera equipped with a medium-energy, parallel-hole collimator (n = 15 patients) or a General Electric Maxicamera equipped with a medium energy parallel-hole collimator (n = 3 patients). Both gamma photon peaks of 111In (173 and 247 keV) were collected with a 15% energy window. Images were stored on a computer disk system in a 128 x 128 matrix. Because of a technical error, computer-stored planar images on one patient were lost; thus 17 patients had planar images available for analysis. All analyses were performed by a person who was blinded to both the time of the image (24, 48, or 72 hr) and to whether the image was from the control or drug phase. Platelet accumulation in the graft from the planar images was quantitated with a graft to blood ratio that compared background-corrected 111In activity in the graft to a simultaneously collected 0.1 ml whole blood sample counted in a well counter as previously described. For each image obtained on each patient, the same hand-drawn regions of interest for graft and background activities were applied to all of the planar images. The background-corrected graft activity was divided by well-counted whole blood activity to normalize for differences between studies in injected 111In dose, platelet recovery, platelet survival, and isotope decay. We have previously established that the test-retest reproducibility (r = .88), intraobserver reproducibility (r = .97), and interobserver reproducibility (r = .95) of this method of quantitating graft platelet deposition are high.

Single photon emission computed tomographic imaging was performed with a commercially available system (General Electric 400T) equipped with a medium-energy, parallel-hole collimator and a 20% energy window for the 173 keV photopeak and a 17.5% window for the 247 keV photopeak. Data were collected over the anterior 180 degrees of the patient with the camera acquiring counts for 32 sec at 64 discrete stops, separated by 2.81 angular degrees. Imaging was performed at 24 and 72 hr after injection of platelets in 15 patients and at 48 and 72 hr after injection of platelets in one patient. Because of scheduling or technical problems, two of the 18 patients did not have tomographic images obtained during both the control and drug phases. Transaxial tomographic reconstruction of the data was performed with filtered-back projection techniques as previously described, which yielded 0.6 cm thick transaxial slices of activity from approximately the xiphoid above to the inguinal area below. Before image quantitation, every three adjacent 0.6 cm thick slices were summed to form 1.8 cm thick slices (figure 1). In platelet activity was quantitated with a computer-generated circular region of interest that encompassed an area of 10.4 cm² (figure 2). This region of interest was...
sequentially applied to each aortic slice above the bifurcation and to the right and left limbs of the graft below the bifurcation. The proximal and distal ends of the graft were defined visually and by an increment in counts from the native vessel to the graft. For each patient, the same number of slices above and below the bifurcation were used to determine total graft counts on all serial images. Because of variations in graft length between patients, the number of 1.8 cm thick slices analyzed above the bifurcation ranged from two to six and the number of slices analyzed below the bifurcation from seven to 11. The graft activity in all slices so identified was summed to give the total graft activity. As for the planar data, a tomographically determined graft/blood ratio for each tomographic image on each patient was calculated by dividing total graft counts by simultaneously collected, well-counted whole blood counts. Unlike the planar imaging analysis, no empiric background correction was performed on the tomographic data. The intraobserver reproducibility of this technique was assessed by having one observer analyze 17 different studies 1 to 2 months apart. The correlation coefficient between the first and second analysis was r = .99.

Statistical analysis was performed using analysis of variance for repeated measures. Values are expressed as the mean ± 1 SEM.

Results

Aspirin plus dipyridamole reduced the tomographic graft/blood ratio at 24 hr (20.6 ± 3.5 vs 17.3 ± 2.5) and at 72 hr (29.0 ± 4.8 vs 25.0 ± 4.1) after injection of platelets (p = .02) (figure 3). Similarly, the planar graft/blood ratio was reduced at 24 hr (2.7 ± 0.5 vs 2.4 ± 0.5), 48 hr (3.7 ± 0.9 vs 3.1 ± 0.7), and 72 hr (4.0 ± 0.9 vs 3.6 ± 0.8) after injection (p = .04) (figure 4).

The percentage increase or decrease for each individual patient in the planar (24 + 48 + 72 hr values) and tomographic (24 + 72 hr values) graft/blood ratios on aspirin plus dipyridamole are shown in figure 5. The mean drug-increased decrease in the tomographic graft/blood ratio was 13 ± 4%. The mean drug-induced decrease in the planar graft/blood ratio was 12 ± 4%. Thus the two methods of imaging yielded concordant results. The decreases in both the planar and tomographic graft/blood ratios by aspirin plus dipyridamole were due entirely to a reduction in graft counts determined by the two imaging methods, since whole blood counts were slightly higher on aspirin plus dipyridamole compared with baseline at all times after injection of platelets.

Discussion

This study sought to determine whether aspirin plus dipyridamole reduces platelet deposition on Dacron vascular grafts that have been in place for longer than 9 months in humans. Prior studies have documented a reduction in platelet deposition by aspirin plus dipyridamole in synthetic vascular grafts in the first week after implantation of grafts,6,7 but whether such drug-induced reductions are sustained has not been determined. The current study demonstrated a modest decrease in two measurements of platelet deposition in patients with grafts in place a mean of 43.4 months.

FIGURE 3. The tomographic graft/blood ratio was significantly reduced by aspirin plus dipyridamole compared with control testing (p = .02). The reduction in the graft/blood ratio was due entirely to a decrease in graft counts, since whole blood counts were slightly increased.

FIGURE 2. Quantitation of tomographic platelet uptake. Platelet accumulation in each sequential 1.8 cm thick transaxial slice of the graft was determined by a circular region of interest (ROI). The tomographic graft/blood ratio was determined by summing the graft area activity from all slices and dividing by the count activity in 0.1 ml of whole blood counted in a well counter.
recently placed prosthetic materials was reduced. Other effective agents in animals include aspirin alone, \textsuperscript{27} dipyridamole alone, \textsuperscript{24, 26–28} ibuprofen, \textsuperscript{25, 27, 31–33} prostacyclin, \textsuperscript{25, 34} sulfipyrazone, \textsuperscript{24, 28} ticlopidine, \textsuperscript{27} nifedipine, \textsuperscript{26, 27} and verapamil.\textsuperscript{26, 27} In one recent study in baboons, although aspirin alone did not reduce platelet consumption caused by a polyurethane graft, aspirin potentiated the antithrombotic effects of dipyridamole.\textsuperscript{28} In several studies, agents that decreased platelet deposition on recently implanted grafts also improved short-term patency.\textsuperscript{23, 26, 27, 29–33, 35–38} These results underscore the importance of platelet mechanisms in arterial graft failure.

Trials in humans have also suggested an improvement in the short-term patency of lower extremity Dacron or polytetrafluoroethylene bypass grafts with platelet-inhibitor therapy.\textsuperscript{6, 8–10, 39} although one study failed to demonstrate a benefit.\textsuperscript{40} In addition, one study in humans demonstrated that lesser degrees of \textsuperscript{11}In–labeled platelet deposition early after surgery were associated with higher patency rates.\textsuperscript{41} Whether platelet-active agents will improve patency in older grafts has not been determined in either animal or human studies.

Prior imaging studies of other platelet inhibitory agents in patients with Dacron grafts that have been in place greater than 9 months have been disappointing. Using planar imaging techniques, we have noted that

The magnitude of the aspirin plus dipyridamole–induced reduction noted in these older grafts appears smaller than the approximate 50% reduction in counts per 100 pixels per injected microcurie noted by Pumphrey et al.,\textsuperscript{3} and the 34% reduction in a thrombogenicity index noted by Goldman et al.\textsuperscript{5} when patients were studied in the first week after implantation of grafts. Although the lesser reduction noted in the current study may be due to differences in imaging and quantitative techniques, a more likely explanation is that drug effects are more pronounced in recently implanted grafts, which accumulate more platelets than do older grafts.\textsuperscript{2, 3, 5}

The regimen and drug dosages chosen were based on the prior positive studies in patients with short-term grafts and on the capacity of the combination of aspirin plus dipyridamole to normalize shortened platelet survivals in patients with coronary artery disease\textsuperscript{21} and in patients with recently implanted Dacron grafts.\textsuperscript{22} Whether aspirin or dipyridamole alone, or aspirin in a lower dosage, would be effective in decreasing platelet uptake on prosthetic materials has not been determined in humans and merits further study. Results of short-term graft studies in animals in which aspirin or dipyridamole have been used alone have been conflicting.\textsuperscript{23–28} As can be seen in figure 5, not all patients responded to therapy. Suboptimal dosage is one possible explanation for the lack of drug effect noted in some patients.

The aspirin plus dipyridamole–induced decrease in platelet accumulation is consistent with observations in animals, in which platelet uptake or consumption on

FIGURE 4. Analysis of the planar images was independent from that of the tomographic images. Aspirin plus dipyridamole significantly reduced the planar graft/blood ratio compared with control testing ($p = .04$).

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FIGURE 5. Percentage change induced by aspirin plus dipyridamole for individual patients in the tomographic (left) or planar (right) graft/blood ratio compared with baseline testing.
sulfinpyrazone (200 mg qid), ticlopidine (250 mg bid), and sulodexid (200 mg tid) all failed to inhibit platelet uptake in patients with Dacron grafts of a similar age to those in the current study.11-13

The cause of prosthetic graft failure appears to change with time after placement.1 Failure within the first month is usually related to technical errors or to graft thrombosis caused by the thrombogenic stimulus of the prosthetic material; platelet-active agents might be effective during this period by reducing platelet thrombus formation. In contrast, late failure is more commonly caused by progression of atherosclerosis distal to the graft, resulting in poor runoff, or by progressive neointimal fibrous hyperplasia with intimal thickening and smooth muscle cell proliferation, which occurs predominantly at the downstream anastomotic site and can lead to localized graft stenosis.1,42-47 Platelet deposition on the flow surface, which continues for indefinite periods after graft implantation,4,5 might lead to continued release of platelet-derived growth factor(s) that would result in smooth muscle cell proliferation.48 Thus platelet active agents might be effective in reducing late graft failure by decreasing smooth muscle cell hyperplasia. In this regard, two studies in animals have demonstrated a reduction in neointimal hyperplasia at anastomotic sites in animals treated with aspirin plus dipyridamole compared with controls,29,38 although a third study was negative.49

There are several limitations to this investigation. The study was not randomized. However, we have previously demonstrated that platelet deposition on long-term Dacron grafts is stable and reproducible over a 2 to 16 week study period in the absence of therapy.5 In addition, all analyses were performed by blinded observers and, as noted, the two different imaging techniques produced concordant results. Another limitation is that aspirin levels to document protocol compliance were obtained on only the second nine subjects. However, it is likely that any noncompliance with the protocol, either by ingesting aspirin during the baseline period or by taking inadequate amounts of the agents during the drug phase, would tend to minimize the observed differences.

Two imaging techniques were used in this study, standard planar imaging, as has been used in all prior studies of 111In-labeled platelets, and single photon emission computed tomographic reconstruction. Tomographic imaging may improve the detection of localized platelet uptake because it largely removes activity overlapping or underlying the region of interest. For example, liver activity anterior to the aorta is clearly removed by transaxial tomographic imaging. For thallium myocardial imaging, tomographic imaging improved the detection of prior myocardial infarction compared with standard planar imaging.18 Another advantage of tomographic imaging may be improved image quantitation. The intraobserver reproducibility of the tomographic determination was slightly higher (r = .99) than had we been previously noted for the planar measurement (r = .90 and r = .97).4,11 In addition, the coefficient of variation, which is the standard deviation/100 divided by the mean, was 65% for the tomographic graft/blood ratio during the baseline study compared with 95% for the planar measurement, indicating less interpatient variability with the tomographic technique. In other studies of image quantitation using tomographic techniques, we have demonstrated that tomographic imaging can accurately size myocardial perfusion defects in dogs19 and can measure left ventricular volumes in man.20 The current study demonstrates the feasibility of tomographic quantitation of 111In data and shows that the technique has a somewhat better intraobserver reproducibility and measurement variability than do planar imaging techniques.

In contrast to prior studies of platelet inhibitory regimens in patients with long-term Dacron arterial grafts, the current study noted a modest reduction in platelet deposition induced by aspirin plus dipyridamole. The findings suggest that these agents should be tested to determine whether they will reduce the long-term complications of prosthetic arterial grafts in humans.

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