Failure of ticlopidine to inhibit deposition of indium-111-labeled platelets on Dacron prosthetic surfaces in humans

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ABSTRACT In a randomized double-blind trial we sought to determine whether short-term therapy with ticlopidine (250 mg bid for 14 days) inhibited platelet deposition on Dacron aortic bifurcation grafts that had been in place a year or longer. A total of 10 men, 42 to 69 years old, underwent indium-111 platelet imaging during both placebo and drug phases of the trial at 24, 48, and 72 hr after the injection of labeled platelets. Platelet accumulation was quantitated by a graft/blood ratio that compared background-corrected activity of indium-111-labeled platelets in the graft with whole-blood activity of indium-111-labeled platelets. Additionally, blinded qualitative visual analysis of the unprocessed images was used to compare graft area activity with activity in adjacent native arteries. Ticlopidine significantly prolonged the template bleeding time from 5.3 ± 0.5 to 17.1 ± 3.1 min (± SEM) (p = .003). However, by quantitative analysis there was no significant reduction in platelet deposition in the graft during ticlopidine therapy compared with placebo at 24 hr (graft/blood ratio 2.3 ± 0.4 vs 2.6 ± 0.3), 48 hr (3.1 ± 0.5 vs 3.2 ± 0.4), or 72 hr (3.9 ± 0.7 vs 4.0 ± 0.6) after injection of labeled platelets. By visual analysis, nine patients had positive results for abnormal platelet deposition when on placebo that were unchanged when on ticlopidine. The tenth patient had an equivocal result for abnormal platelet deposition when on placebo and a negative result for abnormal platelet deposition when on ticlopidine. We conclude that ticlopidine, in a dosage that significantly prolonged the bleeding time, did not inhibit platelet deposition on Dacron prosthetic surfaces in humans. The procedures described in this study may be useful for the evaluation in vivo of platelet accumulation on prosthetic materials and for the study of effects of antithrombotic drugs.


TICLOPIDINE hydrochloride, 5-(2-chlorobenzyl)-4,5,6,7-tetrahydrothieno(3,2-C)pyridine hydrochloride, is a promising investigational platelet inhibitor that significantly prolongs the bleeding time,1-6 inhibits platelet aggregation in vitro induced by adenosine diphosphate, collagen, and epinephrine,3-4,6-9 and decreases serotonin release to adenosine diphosphate and collagen5,6 in humans. In animals, ticlopidine has increased shortened platelet survival times in those with recently implanted Dacron grafts,10 decreased deposition of indium-111-labeled platelets on polytetrafluoroethylene grafts,11 and reduced thrombus formation in polyethylene arteriovenous shunts.12 Data from studies in humans have also suggested that ticlopidine decreased platelet responsiveness to artificial surfaces in patients undergoing cardiopulmonary bypass1 and in uremic patients with arteriovenous shunts.7 However, ticlopidine did not prolong shortened platelet survival times in a small number of patients with Dacron grafts.13 Thus, the effect of ticlopidine in altering the platelet response to prosthetic materials in humans remains largely undetermined.

It has previously been demonstrated that deposition of indium-111-labeled platelets on Dacron aortic bifurcation grafts implanted for more than 9 months is an ongoing process.14-18 Presumably, continuing platelet deposition is in part responsible for the significant thrombotic and embolic complications of Dacron grafts and other artificial materials inserted into the arterial circulation. Less reactive materials or more effective antithrombotic drugs are needed to reduce the thromboembolic complications of prosthetic materials, which are increasingly used in heart valves, vascular grafts, cerebrovascular shunts, artificial hearts, and vascular catheters. We have developed a reproducible and quantitative method of assessing accumulation of indium-111-labeled platelets on arterial graft surfaces,
so that serial changes in platelet deposition induced by drugs or graft maturation can be noninvasively evaluated in humans. On repeat testing over 2 to 16 weeks in patients who have had implanted grafts for over 9 months, indium-111 platelet imaging has been shown to be qualitatively and quantitatively reproducible.15

Using a placebo-controlled, randomized, double-blind design, we tested the hypothesis that ticlopidine in a dosage of 250 mg bid would cause a decrease in deposition of indium-111-labeled platelets on Dacron aortic bifurcation grafts in humans who had had the grafts a year or longer. Our results indicate that ticlopidine in this dosage is ineffective.

Methods

Subjects. Ten men (42 to 69 years old) were studied, each of whom had a knitted Dacron aortic bifurcation graft implanted more than 9 months previously (range 12 to 46). All were clinically stable and did not have recent surgery, advanced renal or hepatic disease, or uncontrolled hypertension. None of the patients was receiving any anticoagulants or platelet inhibitors (warfarin, heparin, aspirin, dipryridamole, sulfipyrazone, indomethacin, ibuprofen, or other nonsteroidal anti-inflammatory drugs). Before entry into the study, all subjects gave a medical history and underwent a physical examination, urinalysis, and tests for hemoglobin, white blood count, platelet count, total bilirubin, alkaline phosphatase, serum glutamic oxaloacetic transaminase, and serum creatinine levels. This study was approved by the University of Washington Human Subjects Review Committee, and all subjects gave informed consent.

Study design. The study was a randomized, double-blind, placebo-controlled, crossover trial consisting of three phases and lasting 35 days. During the first phase, each subject received drug or placebo for 14 days (days 1 to 14), followed by a 7 day washout phase (days 15 to 21), during which the subject received placebo; this was followed by 14 days of the alternate therapy (days 22 to 35). Four subjects received placebo first and six received ticlopidine. Platelet labeling was performed twice, once on day 10 or 11 and again on day 31 or 32. In each case, the patient had been on drug or placebo for at least 10 days. At the time of each platelet labeling, repeat laboratory screening was performed and a bleeding time was determined. The bleeding time was determined by a template method (Simplate II; Warner-Lambert, Morris Plains, NJ). The investigators were unaware of the bleeding times until the completion of data analysis.

Medication and placebo were supplied in tablets that appeared to be identical. The ticlopidine dosage was 250 mg bid. Patients’ compliance was encouraged by repeated phone calls and assessed by pill counts at the time of platelet labeling and imaging. Only one subject, who did not take his medication for 3 days during the placebo phase, did not comply. All patients completed the trial.

Platelet labeling, imaging, and analysis. Autologous platelets were labeled with indium-111 oxine by use of a closed blood bag modification of the technique of Thakur et al.20 as previously described.21 The initial and subsequent centrifugations have been changed to 350 g for 15 min and 1300 g for 15 min, respectively, from those initially described. The mean labeling efficiency was 44 ± 1% (± 1 SEM) for platelets from subjects on placebo and 41 ± 3% for those from subjects on ticlopidine (p = N5). The mean injected dose was not significantly different in the placebo (372 ± 7 μCi) or ticlopidine (377 ± 6 μCi) phase of the study. The total injected dose per patient for both studies ranged from 705 to 785 μCi. On the basis of prior studies of dosimetry, each 1000 μCi of indium-111-labeled platelets results in a total dose to the body of 0.3 to 0.9 rad, a dose to the liver of 0.6 to 2.5 rad, and a dose to the spleen of 25 to 34 rad.22-23 The mean percentage of indium-111 activity free in the plasma was not significantly different for the placebo and ticlopidine phase of the study at 24 hr (5 ± 1% vs 5 ± 1%), 48 hr (5 ± 1% vs 5 ± 1%), and 72 hr (6 ± 1% vs 7 ± 1%) after injection of labeled platelets.

Images of the graft were obtained by taking an anterior view of the lower abdomen that largely excluded the liver and spleen for 150,000 counts at 24, 48, and 72 hr after injection of labeled platelets.15 Both the 173 and 247 keV gamma photon peaks of indium-111 were collected with a 15% energy window. Images were recorded on Polaroid trielens film for visual assessment of the unprocessed data and on a computer disc system with a 128 × 128 matrix to allow quantitation of platelet deposition. Qualitative visual analysis of the unprocessed images from all 20 graft studies was performed by two observers who were unaware of any clinical data or of the study sequence. Images were defined as showing abnormal platelet deposition when there was an area(s) of activity present in the region of an aortic bifurcation graft that was clearly greater than the large vessel blood pool activity present above or below the area encompassed by a graft.15 Results of studies were graded as positive, negative, or equivocal for abnormal platelet deposition. There was observer agreement in 19 of the 20 studies; in one study a consensus was reached. Interobserver reproducibility was tested by having one blinded observer repeatedly read results for 20 studies 4 months apart. For 19 studies, the second reading was the same (positive in all 19); one study result was initially read as negative and was reread as equivocal. Interobserver reproducibility was tested by two observers interpreting 25 studies of platelet imaging from patients with Dacron bifurcation grafts not entered in this study. There was agreement in 24 of the 25 study results (22 positive, one equivocal, and one negative); in one case, observer 1 interpreted the results as positive and observer 2 considered the results equivocal. Thus, we considered the methods of visual analysis to be reproducible. In addition, we have previously documented that the visual and quantitative results are reproducible over 2 to 16 weeks by repeat study of patients who have had grafts for over 9 months.

Platelet accumulation in the graft was quantitated with a graft/blood ratio that compared gamma camera–derived background-corrected activity in the graft region with well-counted, whole blood, indium-111–labeled platelet activity.15 This ratio was determined for each patient at each imaging time. To obtain this ratio, each image from a given patient was first shifted with a computer program so that the aortofemoral blood pool and anatomic markers on each image were located at the same relative position in a 128 × 128 computer matrix. Regions of interest were then hand-drawn on the initial 48 hr image from each subject to encompass the entire extent of his aortofemoral graft as well as a region of background activity adjacent to the left limb of the graft. For drawing the graft region, the proximal and distal ends of the graft could be seen in most cases, since the native artery typically has considerably less activity than the graft. To ensure that the entire graft was included, the graft region was drawn generously in the few cases in which the anastomoses were poorly defined. For each subject, the single graft and background regions that were drawn were then sequentially applied to the 24, 48, and 72 hr images from both the placebo and drug studies to obtain graft and background counts at each imaging time. Thus, identical graft and background regions were used on all images for a given patient. Graft and
background counts at each imaging time were then normalized to an imaging time of 1000 sec. A background correction was performed to minimize the counts from adjacent underlying and overlying tissues and bone marrow, as well as to minimize the effects of the graft region of interest being somewhat larger than the graft itself. For the background correction, the average number of counts per pixel in the background region was subtracted from each pixel in the graft region. The graft/blood ratio was then determined by dividing the background-corrected graft counts by the indium-111–labeled platelet count activity present in 0.1 ml of whole blood counted for 200 sec in a well counter. Division of the background-corrected graft counts by whole blood counts serves to normalize for differences in injected indium dose, platelet recovery, and platelet survival times among patients and for differences in these variables from one study to the next in an individual patient. It also normalizes for the effects of isotope decay between the 24 hr images and later images. It does not correct for differences in isotope attenuation among patients, which are related to body surface area.

Intraobserver reproducibility for the determination of the graft/blood ratio was tested by having one blinded observer repeatedly analyze results of 10 studies 1 month apart. The correlation coefficient between the first and second analyses was \( r = .97 \) (p = .000). Additionally, two independent investigators analyzed results of 20 studies to determine interobserver variability. The correlation coefficient was similarly high (\( r = .95 \); p = .000). For purposes of comparison, the aortofemoral blood pool/whole blood ratios of young normal subjects without grafts are also presented; the data for normal subjects has been previously reported. Statistical analysis was by paired and unpaired t testing. All values are expressed as the mean ± 1 SEM.

Results

Results of platelet imaging. By quantitative analysis there was no significant difference in the graft/blood ratio of subjects in the placebo or ticlopidine phase of the study at 24 hr (2.6 ± 0.3 vs 2.3 ± 0.4), 48 hr (3.2 ± 0.4 vs 3.1 ± 0.5), or 72 hr (4.0 ± 0.6 vs 3.9 ± 0.7) after injection of labeled platelets (figure 1). During both the placebo and ticlopidine phases, the increase in the graft/blood ratio for subjects from 24 to 48 hr and from 24 to 72 hr was highly significant (all p < .01). For comparison, the mean aortofemoral blood pool/whole blood ratio for young normal subjects without grafts or ongoing platelet deposition was 2.0 ± 0.1 at 24 hr (p = .02 vs subjects on placebo and p = .22 vs subjects on ticlopidine), 1.8 ± 0.1 at 48 hr (p < .01 vs subjects on placebo and ticlopidine), and 1.7 ± 0.1 at 72 hr (p < .01 vs subjects on placebo and ticlopidine) after platelet injection. In normal subjects there was no significant change in this ratio from 24 to 48 or 72 hr, reflecting stability of these two measurements of platelet activity in the circulating blood pool.

By qualitative analysis, nine subjects had positive results indicating visually detectable platelet deposition when they were receiving placebo and when they were receiving ticlopidine (figure 2). For these patients, no difference could be seen in the magnitude or extent of platelet accumulation on images taken in the placebo and ticlopidine phases of the study. The tenth patient had a result that was equivocal when on placebo and negative when on ticlopidine.

Laboratory results and adverse effects. The mean bleeding time for our subjects increased significantly from 5.3 ± 0.5 min when receiving placebo to 17.0 ± 3.1 min when receiving ticlopidine (p = .003). The bleeding time increased at least 2.5 min for all but one patient. Serum creatinine levels were slightly higher when they were receiving ticlopidine (1.45 ± 0.13) than when they were receiving placebo (1.31 ± 0.11, p = .03). However, the serum creatinine levels obtained from subjects before entry into the study (1.39 ± 0.13) were not significantly different from the values obtained when they received ticlopidine. The significance of this change is unclear. The test results for hemoglobin, white blood count, platelet count, SGOT, total bilirubin, alkaline phosphatase levels, and urinalysis were unchanged by ticlopidine.

One subject noted transient nausea during placebo therapy and one during ticlopidine therapy. One patient had transient visual blurring during placebo therapy. One patient noted transient weakness associated with upper respiratory tract symptoms during ticlobi-
dine therapy. No bleeding complications occurred, and no subject failed to complete the trial because of adverse effects.

Discussion

We sought to determine whether ticlopidine reduces platelet accumulation on Dacron aortic bifurcation grafts in humans who have had the grafts a year or longer. In most patients with prosthetic grafts, deposition of indium-111–labeled platelets is readily detectable, even up to 10 years after graft implantation. In an earlier study, platelet accumulation was visually detectable in 13 of 15 (87%) patients with grafts in place longer than 9 months. In the present study, platelet deposition was visually detectable (i.e., positive) in nine of 10 (90%) patients, and the result was equivocal for one patient during the placebo phase. As previously described, the pattern of deposition changed over time in approximately one-half of patients, with new areas of accumulation appearing at later (48 or 72 hr) imaging times. In contrast, localized platelet deposition in the aortofemoral vessels has not been detectable in normal subjects without grafts.

Quantitative analysis with the graft/blood ratio further confirmed that platelet deposition in the graft occurred in the 10 patients in our study, even though all grafts were in place for at least 1 year. As shown in figure 1, at 48 and 72 hr after platelet injection, the graft/blood ratio was significantly greater than a similarly obtained aortofemoral blood pool/whole blood ratio for normal subjects without grafts. In addition, the change in this ratio from 24 to 72 hr clearly differentiates normal subjects, in whom the ratio is stable or declines, from patients with grafts, in whom the ratio increases. As previously noted, even patients with grafts who have equivocal or negative results by visual analysis show a characteristic increase in this quantitative measure of platelet accumulation over time. For example, the patient in our study who had an equivocal result by visual analysis of an image taken when he was receiving placebo had a 43% increase in the graft/blood ratio from 24 to 72 hr (1.4 to 2.0), in contrast to the insignificant (15%) decrease (2.0 to 1.7) among normal subjects. Thus, our study further supports the conclusion that platelet deposition is consistently present in Dacron arterial grafts in place for more than a year.

For the serial study of platelet deposition in individual patients and for the comparison of different patient groups, a quantitative approach is needed. The quanti-
tation should normalize for differences in injected indium-111 dose, platelet survival, and platelet recovery, since these factors may change from study to study and, thus, increase or decrease counts in the graft region. The quantitative measure used in this study, the graft/blood ratio, corrects for these differences by dividing gamma camera-derived graft counts by well-counted, whole blood activity. Any intrapatient or interpatient difference in graft region activity caused by variations in injected dose, platelet survival, and platelet recovery are cancelled out, since such differences equally affect both the numerator (background-corrected graft counts) and the denominator (simultaneous whole blood count activity) of the graft/blood ratio. This ratio does not correct for differences among patients in isotope attenuation; however, attenuation differences are not relevant to our study since each patient served as his own control. In a similar group of patients with grafts implanted for 9 months or more, we found that the results of platelet imaging were qualitatively reproducible and that the graft/blood ratio was quantitatively reproducible \((r = .88; p = .000)\) when patients were restudied over 2 to 16 weeks in the absence of therapy. In addition, the method of calculating the ratio with hand-drawn regions of interest has a high interobserver and intraobserver reproducibility \((r = .95\) and \(.97\), respectively), as described in Methods. Using this quantitative index, we have documented that platelet deposition decreases from 2 weeks to 6 months after graft implantation and then stabilizes between 6 months and 1 year. Because of the decrease in deposition not induced by drug in the first several months after implantation, we selected only patients with grafts that had been implanted for at least 9 months for our study.

Since platelet deposition in grafts implanted for over a year is consistently present, is stable over months, and is quantifiable and reproducible, platelet imaging should be useful for testing the effects in vivo of platelet inhibitors in humans. In addition, earlier studies have documented that fibrinogen consumption does not occur in patients with Dacron grafts implanted for over a year, suggesting that platelets have a predominant role in the thrombotic response to this type of artificial material. Since thrombosis and thromboembolism continue to be common complications of artificial materials inserted into the arterial or venous circulation, this particular model is also directly clinically relevant.

In humans ticlopidine, in dosages similar to those used in this study, inhibits platelet aggregation in vitro induced by a variety of stimuli. The duration of effect after discontinuing therapy was 4 to 8 days, and we thus included a washout phase in our trial. In addition, earlier studies have also shown a substantial increase in the bleeding time, which is similar to our observations. Aggregation in vitro was not measured in our study. The mechanism of action of ticlopidine is uncertain, but it does not appear to inhibit prostaglandin synthesis or phosphodiesterase activity.

In animals ticlopidine has reduced accumulation of indium-111–labeled platelets on recently implanted polytetrafluoroethylene arterial grafts and autogenous vein grafts. Additionally, ticlopidine (50 mg/kg/day) increased shortened platelet survival times and decreased postoperative platelet loss in dogs with recently implanted Dacron arterial grafts, although not as effectively as sulfinpyrazone. In rats with polyethylene arteriovenous shunts, ticlopidine at doses of 5 mg/kg or more decreased shunt thrombosis, while aspirin at doses of 10 to 300 mg/kg did not. In humans with implanted artificial surfaces, the effects of ticlopidine have been less clear. In a randomized trial of 100 patients on long-term hemodialysis who had arteriovenous external shunts or vascular grafts, ticlopidine (200 mg qd) reduced the incidence of thrombotic occlusion and the need for reconstructive surgery for vascular access. Additionally, in a randomized trial of 20 patients undergoing cardiopulmonary bypass, ticlopidine diminished the operative fall in platelet count without increasing intraoperative or postoperative blood loss.

In our study ticlopidine, in a dosage of 250 mg bid, failed to inhibit platelet deposition on Dacron aortic bifurcation grafts in place for over a year. A definite drug effect was present, as evidenced by a threefold increase in the bleeding time. The magnitude of change in the mean graft/blood ratio that would have been required (in our 10 patients) to be of statistical significance can be calculated. With 90% power (i.e., a 10% type II error), the graft/blood ratio would have been sensitive to a 31%, 31%, and 30% increase or decrease at 24, 48, and 72 hr, respectively, at the p = .05 level. Smaller reductions or increases would be missed unless larger numbers of patients were studied. As confirmed in this and earlier studies, we are clearly able to differentiate between patients with grafts and normal subjects by use of this technique, as well as between 24 hr images and later images taken in patients with grafts. In addition, we have detected statistically significant decreases in deposition between 2 weeks and 6 months after graft implantation. Thus, changes in deposition are detectable by the graft/blood.
ratio; whether changes in deposition beneath the resolution of the technique in our study would be clinically relevant is uncertain.

The negative results reported here are similar to those obtained in a nonrandomized trial of sulfipyrazone (200 mg qid) in a similar group of patients with Dacron grafts. During sulfipyrazone therapy, there was also no visual change in the magnitude or pattern of platelet deposition nor was there any significant change in the graft/blood ratio at any imaging time. Although we have not detected either a qualitative or quantitative decrease in platelet accumulation on Dacron grafts by sulfipyrazone or ticlopidine, we have detected reductions in platelet deposition by warfarin or platelet inhibitors in patients with left ventricular thrombosis or hemodialysis access sites. In contrast to our negative findings with sulfipyrazone and ticlopidine, Pumphrey et al., in a nonrandomized study, found an apparent decrease in deposition after aspirin (325 mg tid) and dipyridamole (75 mg tid) in eight patients with recently implanted Dacron aortobifemoral grafts compared with similar control patients not receiving therapy. However, their quantitative method did not correct for differences in platelet survival or recovery between the two groups. Since aspirin plus dipyridamole increases platelet survival times in patients with recently implanted Dacron grafts, this may have affected their results. Other potential causes of the difference in our findings may relate to multiple factors including graft age, the drug regimen used, and the patient population. Recently implanted grafts accumulate more platelets than older grafts, and, thus, a drug effect might be more pronounced in recently implanted grafts. Although ticlopidine, which inhibits both primary and secondary aggregation, appears to be a more potent platelet inhibitor in vitro than aspirin, ticlopidine has failed to normalize shortened platelet survival times in patients with Dacron grafts, while aspirin in combination with dipyridamole does prolong platelet survival in such patients. Additional studies are needed to establish whether platelet deposition or its inhibition with drugs can be used to predict ultimate graft function. The quantitative techniques outlined in this study may be useful for the further assessment of the effects in vivo of new platelet inhibitors or new prosthetic materials in man.

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