Iliac Artery Mural Thrombus Formation

Effect of Antiplatelet Therapy on $^{111}$In-Platelet Deposition in Baboons

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To measure the rate, extent, and time course of arterial mural thrombus formation in vivo and to assess the effects of antiplatelet therapy in that setting, we have studied autologous $^{111}$In-platelet deposition induced by experimental iliac artery aneurysms in baboons. Scintillation camera imaging analyses were performed at 1, 24, 48, and 72 hours after implantation of the device. Correction for tissue attenuation was determined by using a small, comparably located $^{111}$In source implanted at the time of surgery. In five animals, $^{111}$In-platelet activity accumulated progressively after device implantation, reaching a maximum after the third day. Repeat image analysis carried out 2 weeks after the surgical procedure also showed progressive accumulation of $^{111}$In-platelets over 3 days but at markedly reduced amounts as compared with the initial study. In five additional animals, treatment with a combination of aspirin and dipyridamole begun 1 hour after surgical implantation reduced $^{111}$In-platelet deposition to negligible levels by the third day. Although platelet survival time was shortened and platelet turnover was reciprocally increased in all operated animals, platelet survival and turnover were not affected by antiplatelet therapy. We conclude that, in contrast to platelet survival and turnover measurements, $^{111}$In-platelet imaging is a reliable and sensitive method for localizing and quantifying focal arterial thrombi and for assessing the effects of antiplatelet therapy.


Models of experimental arterial thrombosis have generally involved morphologic observations at fixed timepoints after an initiating vascular insult. Direct measurements of the rate, extent, and time course of thrombus formation in vivo have recently become feasible with the availability of isotopes suitable for platelet and protein labeling and subsequent dynamic imaging.1–3 Thrombosis models using exteriorized arteriovenous cannulae to access blood and to induce thrombus formation have been useful in evaluating quantitatively mechanisms of thrombus formation and its presentation under well-controlled conditions of blood flow.4–6 While blood passing through the arteriovenous shunt in these models is subject to physiologic filtration, dilution, and inactivation mechanisms, the actual site of thrombus formation is remote from normally adjacent vascular tissues that have the potential to modify the thrombotic response by mechanisms such as increased release of prostacyclin7 or tissue plasminogen activator.8

Although in vivo imaging has provided considerable information regarding thrombus localization,2,9–17 absolute quantitation of deposited blood elements has been limited, in part, by the variable tissue attenuation of emitted activities.2 To quantify continuously the overall rate of acute arterial thrombus formation in vivo, we have used a primate model of $^{111}$In-platelet deposition induced by an experimental iliac artery aneurysm. In this model, tissue attenuation of isotope emissions was determined by implanting a small, known source of $^{111}$In in a corresponding location adjacent to the contralateral iliac artery. By imaging the source activity both before and after surgical placement, tissue attenuation was measured directly. We have used this experimental design to assess quantitatively the effects of antithrombotic therapy in a setting that includes possible modulating factors arising from adjacent vascular structures.

Methods

Animal Studies

Ten normal male baboons (Papio anubis) weighing 8 to 14 kg were studied. All animals were dewormed and observed to be disease-free for at least 6 weeks prior to use. Circulating platelet concentrations averaged 446,000 ±
60,000 platelets/µl (± 1 sd), and the mean blood white cell count was 8300 ± 4,100/µl. All procedures were approved by the institutional Animal Care and Use Committee in accordance with the 1985 Federal Guide for the Care and Use of Laboratory Animals.

In all animals, a 1.5 cm long segment of 5 mm internal diameter (i.d.) knitted Dacron vascular graft (De Bakey Vascularour, U.S. Catheter, Incorporated, Billerica, Massachusetts) with a closed distal end was surgically anastomosed to the open end of the graft to the vascular margins of an arteriotomy in the right common iliac artery as shown in Figure 1 A. The surgical procedure was performed according to the following description. After induction with ketamine hydrochloride (10 mg/kg intramuscularly), the baboon was intubated and anesthetized with halothane (1%). Electrocardiographic leads were connected to a cardiac monitor for evaluation of cardiac function throughout the procedure. The abdomen was prepared and draped in sterile fashion. A midline incision was created from the supraumbilical region to the pubic symphesis. This incision was carried through the midline fascia, and the peritoneal cavity was entered. With use of sharp dissection, the retroperitoneal tissues were dissected, and the aorta and iliac vessels were identified. Vessel loops were placed proximally and distally on the common iliac artery to obtain vascular control. The internal and external iliac arteries were also controlled with vessel loops. Before restriction of blood flow, the baboon was anticoagulated with 1000 U of heparin. An arteriotomy was then created on the anterior surface of the right common iliac artery. This incision was extended proximally and distally to approximately 1.0 cm in length. The knitted Dacron vascular graft segment was precollotted with autologous, nonradioactive whole blood before heparin anticoagulation. The vascular graft segment was anastomosed to the common iliac artery in an end-to-side fashion using 6-0 prolene suture. The distal portion of the knitted Dacron vascular graft was ligated using 3-0 silk suture. The vessel loops were then removed, and circulation was restored. Surgical hemostasis was achieved locally at the anastomotic site. After forming this anastomosis, the presence of a distal pulse was confirmed.

For purposes of calibration, a small 111In-radioisotope source (approximately 5 µCi) was sealed at the end of 0.6 mm i.d. polyethylene tubing (PE-50, Clay Adams Incorporated, New York, New York) and placed adjacent to the contralateral common iliac artery in a comparable location and plane as the graft. The midline fascia was reapprorximately by use of a simple running suture, and the skin was closed. The animals tolerated the procedure well. The estimate of blood loss was approximately 25 to 50 ml.

Laboratory Procedures

Whole blood was drawn into 4 mg/ml ethylenedinitri- tetaacetic acid (EDTA) tubes, and platelet counting and sizing were then performed with a Model 810 whole blood counter (J.T. Baker Instruments, Piscataway, New Jersey). Autologous baboon platelets were labeled with 111In-oxine according to the following protocol. Whole blood (100 ml) was collected directly into plastic bags (TA-3, Fenwal Labs, Deerfield, Illinois) containing 20 ml acid-citrate-dextrose anticoagulant (NIH formula A). The blood was centrifuged in the bag at 300 g for 10 minutes. The supernatant platelet-rich plasma (PRP) was transferred to a second bag, and the pH was adjusted to 6.5 by the addition of 0.15 M citric acid (0.1 ml/10 ml PRP). The red blood cell fraction was returned to the donor animal. The platelets were formed into a pellet by centrifugation of the PRP at 1300 g for 15 minutes. The supernatant platelet-poor plasma (PPP) was completely decanted and discarded. To remove residual plasma proteins, the bag containing the platelet pellet was carefully washed once by overlaying with 30 ml of Ringer’s citrate dextrose (RCD, pH 6.5), which was then decanted and discarded. The pellet was gently resuspended in 5.0 ml RCD, and incubated for 30 minutes with 500 to 700 µCi 111In-oxine (Amersham Corporation, Arlington Heights, Illinois). Contaminating red cells were removed by a final slow centrifugation at 200 g for 5 minutes. Labeling efficiency was determined by diluting 200 µl of the labeled-platelet concentrate with 5.0 ml of RCD and by comparing the activity in 0.5 ml of the diluted platelet suspension with the activity in 0.5 ml of cell-free supernatant after centrifugation at 3000 g for 30 minutes. Labeling efficiency was better than 95%. A measured volume of labeled platelet suspension containing less than 5% of nonplatelet bound isotope was then injected directly into the recipient animals. In all studies with the scintillation camera, blood pool activities from all sources (platelet and nonplatelet) were a small fraction of total deposited platelet activity and were subtracted by using appropriate computer-assisted image analysis routines as described below.

Circulating platelet 111In-activity was determined from 4 ml blood samples drawn before and after graft placement and collected in 2 mg/ml EDTA. Then 1.0 ml of each sample was used for platelet counting and hematocrit determinations, and 1.0 ml was counted for whole blood 111In-activity. The remaining 2 ml were centrifuged at 3000 g for 30 minutes, and 1.0 ml of the supernatant (PPP) was counted for plasma 111In-activity. All blood and plasma samples were counted with a gamma spectrometer (Nuclear Chicago, Chicago, Illinois).

The mean platelet survival times were calculated by fitting the disappearance curve of platelet radioactivity to y functions, as described by Murphy et al. The proportion of labeled platelets remaining within the systemic circulation (i.e., recovery) was calculated from the initial platelet activity per milliliter of whole blood, multiplied by the estimated blood volume (65 ml/kg), and divided by the platelet 111In-activity injected.

Analytical Methods

Scintillation camera imaging of the 172 kev γ photon peak of 111In (with a 5% energy window) was performed in the present studies with good resolution by using a high sensitivity 99Tc collimator. Images of the Dacron aneurysmal implant, associated large arteries, and implanted 111In-oxine standard were acquired with a Picker DC 4/11 Dyna scintillation camera (Picker Corporation, Northford, Connecticut) and stored on and analyzed by a Medical Data
Figure 1 A. Experimental aneurysmal Dacron graft device. A segment of knitted Dacron vascular graft, sutured blind end and preclotted with autologous blood, was anastomosed end-to-side by arteriotomy to the right common iliac artery (see Methods). 111In-platelets were injected approximately 10 minutes after completion of the graft implantation.

Figure 1 B. Image analysis of 111In-platelet deposition in graft mural thrombus. 111In-platelet activity over the implanted graft aneurysm in the right common iliac artery was compared to a comparable location over the contralateral left common iliac artery and independent areas of vascular background by using regions of interest defined by γ scintillation camera imaging routines (see Methods).

Figure 1 C. Thrombus formed by implanted graft device. The implanted aneurysmal device exposed a preformed clot to circulating blood; flow was from right to left. After 4 days, the iliac vessels were pressure perfusion-fixed in situ with 2.5% glutaraldehyde in 0.1 M phosphate-buffered saline and the luminal surface of the artery was examined morphologically. A central "red" thrombus overlies the thrombus-filled graft aneurysmal device with a central cavity extending into the graft thrombus. A propagated "white" thrombus forms a tail extending into the lumen oriented in the direction of flow.
Results

Control Studies with $^{111}$In-Platelets

Platelet survival curves assessed following $^{111}$In-platelet labeling in six normal animals displayed a predominantly linear disappearance pattern (Figure 2). Platelet survival times in normal control animals were determined from the computer-fitting procedure and averaged 5.59 ± 0.10 days. Platelet recoveries, determined by extrapolating the best-fit survival curves to time zero, averaged 79.8% ± 2.9%. Platelet counts were 439,000 ± 35,000/μl, and platelet turnover averaged 99,000 ± 8000 platelets/μl/day. These values for platelet survival, recovery, and turnover were in agreement with those previously obtained in baboons with a $^{51}$Cr platelet label.4, 5, 20

$^{111}$In-Deposition in Mural Thrombus

In five baboons, autologous $^{111}$In-platelets were injected approximately 10 minutes after blood flow was restored after implantation of the graft aneurysmal device. $^{111}$In-platelet deposition was assessed in the mural thrombus induced by the implanted graft devices placed 1 hour previously and again at 24, 48, and 72 hours after injection of the labeled cells. All images were for 1 hour. Measurements of $^{111}$In-platelet deposition in five animals are shown in Figure 3. At 1 hour, deposited graft radioactivity was only 10% to 20% greater than the circulating whole blood radioactivity within the contralateral iliac artery. $^{111}$In-platelet deposition increased progressively thereafter, reaching a peak ratio with whole blood of about 10 by 3 days.
PLATELET DEPOSITION IN EXPERIMENTAL ANEURYSM Hanson et al. SIS

Figure 3. Time course of $^{111}$In-platelet deposition on graft mural thrombus. Platelets labeled with $^{111}$In were injected 10 minutes after implantation of the experimental aneurysm. Images were accumulated for 1 hour beginning at 1, 24, 48, and 72 hours after the injection of $^{111}$In-platelets (○). When autologous platelets had been relabeled with $^{111}$In in two animals 2 weeks after implantation of the graft device and imaging was performed at 1, 24, 48, and 72 hours after injection (○), the deposition of $^{111}$In-platelets increased progressively but was substantially reduced compared with the initial results.

In these five animals implanted with the graft aneurysm device, whole blood platelet counts averaged 449,000 ± 27,000/μl. Following device implantation, platelet survival time was significantly shortened to 3.48 ± 0.69 days (compared with 5.59 ± 0.10 days in normal animals; p < 0.01) (Figure 2). Platelet turnover was reciprocally increased to 160,000 ± 36,000 platelets/μl/day. Gross morphologic evaluation of the luminal surface of one graft-bearing vessel 4 days after implantation demonstrated mural thrombus filling the cavity of the graft segment with a propagated "white" thrombus projecting into the vascular lumen (Figure 1 C). Examination by scanning electron microscopy of the luminal surface of the mural thrombus at this same time revealed masses of platelets with red cells trapped in a fibrin mesh (Figure 4).

When $^{111}$In-platelet imaging studies were carried out in two animals 2 weeks after implantation of the graft device, deposition was also progressive over the 3 days of study (Figure 3). However, overall $^{111}$In-platelet deposition was substantially decreased compared with the initial study.

Effect of Antiplatelet Therapy

Dipyridamole (2 mg/kg) was injected intravenously 1 hour after surgical placement of the graft device in five additional animals. Oral dipyridamole and aspirin (15 mg/kg/day and 30 mg/kg/day, respectively) were given orally in combination as two divided doses daily for the subsequent 5 days.

Figure 4. Luminal thrombotic surface. Scanning electron microscopy of the luminal aspect of the "red" thrombus overlying the graft device demonstrates masses of platelets and fibrin with entrapped red cells. Bar = 10 μm.
Platelet turnover also remained increased in these treated animals (5.59 ± 0.10 days; p < 0.01) but not different from untreated graft-bearing operated animals (3.48 ± 0.69; p > 0.5). Platelet turnover also remained increased in these treated animals (148,000 ± 29,000 plat/μl/day) (p < 0.01 compared with unoperated normal controls and p > 0.5 compared with untreated operated animals).

### Discussion

The present study was designed to assess directly, by using a nonhuman primate model, the deposition of platelets acutely and over time following exposure to aneurysmal mural thrombus under arterial flow conditions, and to evaluate the effects of antiplatelet therapy on the rate and extent of platelet deposition. We used a preclootted Dacron vascular graft device as the thrombogenic stimulus to simulate the mural thrombus found in clinical aortic iliac aneurysms in man. We selected the baboon because this species appears to be hemostatically and anatomically similar to humans. The results document that quantitative \(^{111}\)In-platelet imaging provides sensitive detection and localization of forming thrombus and reliably measures both the changes over time and the effects of platelet-modifying therapy.

These in vivo studies were performed by using platelets labeled with \(^{111}\)In-oxine in vitro. We have previously documented\(^5\) that recovery of platelets labeled by this method is normal within 10 minutes after infusion into the circulation, and that platelet survival curves are predominantly linear, similar to the results obtained with a \(^{51}\)Cr-platelet label.\(^4\) In addition, we have previously shown\(^6\) that platelets labeled by this technique are representative of the parent platelet population with respect to density distribution and rate of incorporation into forming thrombus when evaluated 1 hour after reinjection. These results suggest that the labeled and unlabeled platelet populations were equivalent by the criteria relevant to this study.

To achieve direct quantitation of \(^{111}\)In-platelet deposition in this study, a small \(^{111}\)In-oxine source was implanted at a comparable location adjacent to the contralateral common iliac artery at the time of surgery. Consequently, tissue attenuation for the initial imaging study was measured directly, and this correction factor was used for subsequent imaging studies in that particular animal. In previous work\(^6\) acute platelet deposition has been expressed as total deposited platelets (i.e., labeled plus unlabeled). However, in the present study involving observations of longer duration, dilution of the circulating \(^{111}\)In-platelet pool by newly formed platelets together with unquantifiable losses of \(^{111}\)In-platelets from the mural thrombus by disaggregation, microembolization, or lysis, precluded the expression of accumulated \(^{111}\)In activity as total platelets deposited. Thus, meaningful comparisons of deposited \(^{111}\)In-platelet activity over time required that the results be expressed as a ratio of \(^{111}\)In-thrombus activity over \(^{111}\)In-platelet activity in circulating whole blood.

Following implantation of the graft device, \(^{111}\)In-platelet activity accumulated progressively in the mural thrombus throughout 3 days and perhaps longer since equilibrium was not yet established by the third day. In this context, it is of interest to note that \(^{111}\)In-platelet activity also increased progressively over 3 days when imaging was performed 2 weeks later. At this time, it could be assumed that the size of the thrombus was relatively steady state with respect to the 3-day period of observation. This pattern of progressive accumulation of \(^{111}\)In-platelet activity into steady state mural thrombus has been reported by others in patients with aortic aneurysms.\(^15\) 16 In vivo studies were performed by using platelets labeled with \(^{111}\)In-oxine in vitro. We have previously documented that platelets labeled by this technique are representative of the parent platelet population with respect to density distribution and rate of incorporation into forming thrombus when evaluated 1 hour after reinjection. These results suggest that the labeled and unlabeled platelet populations were equivalent by the criteria relevant to this study.

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The shortening of platelet survival time by about one-third and the reciprocal increase in platelet turnover in the...
animals after surgical placement of the graft devices presumably reflected both the effects of surgery and the formation of mural thrombus (Figure 3). Since the decrease in platelet survival time in these operated animals was comparable to the reduction in platelet survival observed in patients undergoing abdominal surgical procedures, and since the exposed area of the mural thrombus was relatively small, i.e., less than 1 cm² (Figure 1 C), most of the increased platelet consumption was attributed to utilization in the surgical wound. The conclusion that platelet consumption is largely surgery-induced was further supported by the fact that antiplatelet therapy did not significantly prolong platelet survival time (Figure 2), despite the marked effect of therapy on decreasing the 111In-platelet deposition on the mural thrombus.

Additional support for the conclusion that most of the platelet consumption was due to surgical trauma involved calculations of platelet utilization associated with surgery vs graft mural thrombus. The overall rate of platelet utilization, induced directly or indirectly by both the surgical procedure and the graft mural thrombus, was estimated to be about 100,000 plat/µl/day or about 3 x 10⁹ plat/hr for 10 kg animals, derived by estimating the number of platelets removed by random extrinsic mechanisms (vs senescence) as described previously. The proportion of overall utilization attributable to graft-associated thrombus was calculated from the initial imaging data (initial slope in Figure 3) as the rate at which platelets were deposited (i.e., 0.1 x 10⁹ plat/hr). This estimate assumes that microembolization from the mural thrombus was negligible, an assumption that may be valid in the acute period following graft placement. However, if microembolization were significant in our experiments, platelet destruction produced by the graft thrombus would be underestimated in those calculations. Nevertheless, the calculated rate of platelet consumption by the mural thrombus is only about 1/30 of the total utilization produced by both surgery and the graft thrombus together. Thus, even a large underestimation of mural thrombus-dependent platelet utilization would not change the general conclusion that increased platelet destruction was largely related to the hemostatic and wound-healing demands of surgery.

The marked reduction in mural thrombus 111In-platelet deposition produced by the platelet-modifying drugs aspirin and dipyridamole (Figure 5) was similar to the anti-thrombotic effects of this regimen previously reported with the model of arterial thromboembolism induced in arterio-venous (A-V) cannulaus. Thus, the present study establishes that the anti-thrombotic efficacy of this regimen is not limited to thrombus produced by thrombogenic prosthetic A-V shunts. However, the relative importance of aspirin vs dipyridamole vs the combination in achieving the anti-thrombotic effect has not been directly established in the present report. It is also noteworthy that this regimen fails to reduce platelet utilization in the surgical wound (i.e., whereas 111In-platelet deposition on graft mural thrombus is markedly reduced, platelet survival and turnover are unaltered by the drug treatment). Presumably, this differential effect of therapy is explained by the severe thrombogenic stimulation caused by tissue injury compared with mural thrombus formation.

It is interesting to speculate on the cause of the progressive inhibitory pattern of 111In-platelet deposition induced by antiplatelet therapy in this study. Probably the most important factor is the delay of drug administration, which was begun 1 hour postoperatively. This timing for therapy was chosen to avoid the potentially confounding effects of excessive bleeding and possible hematoma formation in the imaging field with 111In-platelet-containing blood and also to insure that the nature of the thrombus initially formed was similar in both the control and the treatment groups. Although postoperative therapy simulates the usual clinical treatment setting in which antithrombotic therapy would be initiated after thrombus has already become established, preoperative antiplatelet therapy has been shown clinically to be successful. An additional factor that may contribute to the delayed effect of antiplatelet drug therapy may be the period of time required for the drugs to be fully active. Although no precise data are available to specify the time before the effect of these drugs is fully manifest, indirect information suggests that in vivo effects on platelets increase over at least the first day. Other theoretically possible explanations for a delayed effect of these drugs include drug-mediated enhancement of 1) platelet disaggregation, 2) microthromboembolization, or 3) endogenous thrombolysis.

In summary 111In-platelet imaging reliably and sensitively detects and localizes ongoing focal mural arterial thrombosis. Moreover, this method has the capacity to demonstrate the beneficial effects of antiplatelet therapy when the extent of platelet deposition is far less than that required to modify platelet survival times significantly.

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References


Index Terms: mural thrombosis in vivo • 111In-platelet deposition • antiplatelet therapy • experimental baboon thrombosis model
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