Indium-111-labeled Platelets: Effect of Heparin on Uptake by Venous Thrombi and Relationship to the Activated Partial Thromboplastin Time

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SUMMARY The goal of heparin therapy in deep vein thrombosis is to prevent thrombus extension. The relationship between thrombus extension and the results of coagulation tests used to monitor heparin therapy is unclear. To explore this relationship, we studied the effect of several heparin regimens on the accretion of indium-111-labeled platelets on fresh venous thrombi, as detected by gamma imaging, and monitored the activated partial thromboplastin time (APTT). Six dogs were treated with a 300-U/kg bolus of heparin followed by a 90-U/kg/hour heparin infusion, a dose of heparin sufficient to increase the APTT to levels greater than eight times baseline (APTT ratio); platelet accretion (thrombus imaging) occurred only after the heparin effect was reversed with protamine sulfate. Nineteen dogs were treated with a 150-U/kg bolus of heparin followed by a 4-hour, 45-U/kg/hour heparin infusion; a thrombus was demonstrated only after protamine injection in 12 (mean APTT ratio 1.3 ± 0.19) and before protamine injection in seven. In thirteen of these 19 dogs, 30 minutes separated the platelet injection from heparin therapy, while in six this duration was less than 30 minutes. In four of these six dogs, thrombi were demonstrated before protamine therapy and at APTT ratios greater than 3.0. Finally, 10 dogs were treated with a 100-U/kg bolus followed by a 3-hour, 50-U/kg/hour heparin infusion, after which the APTT was allowed to return to baseline values spontaneously. In all 10 dogs, a thrombus was demonstrated only after cessation of the heparin infusion, and at a mean APTT ratio of 1.4 ± 0.15 times baseline. These results suggest that, except with very early platelet injection, platelet accretion by thrombi is consistently inhibited by heparin at APTT ratios greater than 2.5.

Platelet accretion by venous thrombi occurs within narrow limits of heparin effect as reflected by the APTT.

ALTHOUGH heparin is considered the drug of choice for preventing the extension of acute deep vein thrombosis, there is no consensus on the most effective dose regimen to achieve this objective. Recommendations regarding heparin regimens for the treatment of deep vein thrombosis range from empiric dose selection1 to careful periodic monitoring with specific coagulation tests;2 continuous intravenous,3 intermittent intravenous,4 and subcutaneous dosing5 have also been recommended. Such diversity arises because there has been no sensitive method for relating the biologic effect desired in vivo — inhibition of thrombus growth — to specific dose regimens or to the “therapeutic range” designated by a given coagulation test. Thus, the relationship between heparin’s antithrombotic effect (cessation of thrombus growth) and anticoagulant effect (prolongation of coagulation assays) remains unclear.

Thrombus growth occurs by accretion of platelets and fibrinogen. Failure of such accretion to occur during heparin therapy would appear, therefore, to be a signal that an effective dose of heparin is being provided. Noninvasive in vivo monitoring of platelet/fibrinogen accretion to venous thrombi would provide a means to assess different heparin regimens and to define the relationship between such regimens and the behavior of selected coagulation assays.

In the present study, we explored this thesis by intravenously injecting indium-111-labeled platelets in dogs after formation of venous thrombi. Platelet accretion on thrombi was continuously monitored by gamma camera. We sought to determine (1) what i.v. dosage regimens of heparin would consistently inhibit platelet accretion to these venous thrombi; (2) at what point after reversal or cessation of heparin a thrombus could be demonstrated; and (3) the relationship between the heparin effect, as monitored by the activated partial thromboplastin time (APTT), and thrombus growth, as reflected by accretion of labeled platelets onto venous thrombi.

Methods
Preparation of Indium-111 Oxine
The indium-111 oxine complex was formed by a modification of the method of Thakur et al.6 One to 3 mCi of $^{111}$In Cl$_3$ solution in a volume of 1.5 ml were added to 1.5 ml of distilled water. The pH was adjusted to 5.5 by addition of 0.2 ml of 0.3 M sodium acetate; 0.2 ml of an oxine-ethanol solution was added and the solution was mixed. After 15 minutes, the solution was removed and added to 3 ml of dichloromethane. After a 1-minute vortex mix, the dichloromethane-oxine layer was removed and evaporated to dryness. The complex was resuspended in 0.1 ml of DMSO, and 0.1 ml of 0.9% NaCl was added just before use.

Platelet Preparation and Labeling
Forty-three milliliters of venous blood were drawn into a clean, dry syringe that contained 7 ml of ACD solution. The blood was centrifuged for 15 minutes at
200 g and the platelet-rich plasma removed. This was then spun at 1400 g for 10 minutes and the platelet-poor plasma removed. The resulting platelet button was resuspended in 10 ml of 0.9% saline solution by gentle suctioning and then incubated for 30 minutes with the indium-111 oxide complex.

Production of Experimental Venous Thrombi

Mongrel dogs that weighed 16–24 kg were anesthetized with sodium pentobarbital, 25 mg/kg. Venous thrombi were induced without intimal damage as previously described. A modified Swan Ganz catheter was introduced through a venous cutdown just above the paw and the tip advanced to the femoral triangle. When in position, the balloon was inflated with 1 ml of air. Two minutes later, 10 U of topical thrombin were injected into the newly created proximal orifice and flushed with 2 ml of normal saline. Thirty minutes later, the balloon was deflated, but the catheter left in place. Thus, all induced thrombi were 30 minutes old when reexposed to the venous blood flow. Using contrast venography, we have shown that balloon deflation is associated with restoration of flow over the thrombus, but failure of the thrombus to embolize; the same period of balloon occlusion without thrombin injection is not associated with platelet uptake on the venous intima or catheter.

Coagulation Assay

APTTs were determined in duplicate using a photoelectric optical density device (Lancer Coagulizer Jr. II). Nine parts of freshly collected blood were mixed with one part of 3.8% sodium citrate and centrifuged at 2000 g for 10 minutes; 0.1 ml of plasma was added to 0.1 ml of the partial thromboplastin reagent (General Diagnostics, Automated APTT), and allowed to incubate for 5 minutes. The solution was then recalculated with 0.1 ml of CaCl2, and the APTT recorded. The entire procedure was repeated if the two samples differed by more than 10%.

Nuclear Imaging

The scintillation cameras were equipped with a medium-energy, parallel-hole collimator, and the pulse-height analyzer was set to include both the 173-keV and 247-keV photopeaks of indium-111. A total of 3–6 × 106 autologous platelets were labeled with 1–3 mCi of indium-111. In all protocols, continuous anterior static images were acquired for 200,000 counts, and Polaroid images over the venous thrombosis were obtained every 5 minutes to determine the onset and course of platelet accretion. In addition to these images, data were acquired simultaneously on a nuclear medicine dedicated computer system for later analysis. Regions of interest over the thrombus, the contralateral femoral vein, and a background area were outlined with a light pen and subjected to computer analysis to construct time-activity curves. The time at which a thrombus was demonstrated was determined for statistical purposes from scintiphotos independently evaluated by three observers and was compared, after the study, with the computer-generated time-activity curves.

Experimental Protocols

Protocol 1 involved the formation of venous thrombi in six dogs followed by a 300-U/kg heparin bolus and a 4-hour infusion of heparin, 90 U/kg/hour, by a constant infusion pump. Fifteen minutes after the heparin bolus, the indium-111-labeled platelets were injected. Immediately after the heparin infusion ended, protamine sulfate, 1 mg per 100 U of heparin, was administered to return the APTT to baseline values. The APTT was determined before the heparin infusion and hourly thereafter.

Protocol 2 involved the formation of venous thrombi in 19 dogs followed by a 150-U/kg bolus and a 4-hour infusion of heparin in a dose of 45 U/kg/hour. In six dogs, indium-111-labeled platelets were injected less than 30 minutes after heparin infusion had begun. In the remaining 13 dogs, heparin was injected 30 minutes after the platelet injection. APTTs were obtained every 30 minutes. At the end of the infusion, protamine sulfate was administered, and an APTT was obtained at the moment the thrombus was demonstrated.

Protocol 3 involved the formation of venous thrombi in 10 dogs followed by a 100-U/kg bolus and a 3-hour infusion of heparin in a dose of 50 U/kg/hour. In all dogs, indium-111-labeled platelets were injected 30 minutes after the heparin infusion began. APTTs were obtained every 30 minutes. At the end of the infusion, the APTT was allowed to return to baseline values spontaneously. During this period, an APTT was determined every 15 minutes until the thrombus was demonstrated.

In all three protocols, porcine intestinal mucosa heparin was used (Liquaemin Sodium, 1000 U/ml, Organon Laboratories). At the conclusion of each protocol, a blood sample was obtained, the dog was killed, the thrombi were removed and the ratio of thrombus to blood radioactivity was determined in a well-counter.

Results

Protocol 1

In the six dogs that received a 300-U/kg bolus followed by a 4-hour, 90-U/kg/hour infusion, the APTT remained greater than eight times control throughout the infusion period. No thrombus was demonstrated until after the protamine injection. In all six dogs, a thrombus was demonstrated within 30 minutes after protamine injection, and the APTT returned to control levels within 1 hour.

Protocol 2

In the 19 dogs that received a 150-U/kg heparin bolus followed by a 45 U/kg/hour infusion, the APTT varied substantially in individual dogs during the infusion. Some APTT values fell below 2.5 times control despite constant infusion (fig. 1). The mean APTT at the end of the infusion was 4.5 ± 3.1 times baseline, and ranged from one to more than 10 times baseline.
minutes after the heparin bolus, and the APTT was less than 2.5 times control when a thrombus was demonstrated (table 1).

In the 13 dogs in which platelets were injected 30 minutes after heparin therapy, the mean APTT when a thrombus was demonstrated was 1.4 ± 0.47 (table 2). In the seven dogs in which platelets were injected earlier, the APTT at the time of thrombus demonstration was highly variable.

**Protocol 3**

In the 10 dogs treated with a 100-U/kg bolus followed by a 3-hour heparin infusion of 50 U/kg/hour, the APTT again varied substantially during the infusion. The mean APTT at the end of the infusion was 3.3 ± 1.7 times baseline (range 1.5–6.8 times baseline) (fig. 2). In no dog was a thrombus demonstrated during the heparin infusion. Thrombi were demonstrated at various times after the heparin infusion was discontinued, but within a relatively narrow APTT range (mean 1.4 ± 0.15; range 1.2–1.6 times control) (table 3). The APTT at the time of thrombus demonstration did not differ significantly from that in the protocol 2 dogs.

The computer-generated time-activity curves showed that at the time of thrombus demonstration, platelet accretion occurred rapidly and at a well-defined point (fig. 3). Visual determination of platelet uptake from Polaroid images correlated well with the point at which the computer-generated time-activity curve suddenly changed (fig. 4).

In all three protocols, the ratio of thrombus to blood radioactivity ranged from 7 to 22. No bleeding complications occurred in any of the 35 dogs.

### Discussion

The level of heparin effect necessary to prevent the extension of venous thrombosis and the best means for measuring that effect are controversial. Current recommendations are diverse and are based primarily upon trials in patients with deep vein thrombosis, in which the incidence of thromboembolic recurrence was used as the index of heparin efficacy. However, recurrence may be an insensitive index of the inhibitory effects of the heparin on platelet function.

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**TABLE 1. Activated Partial Thromboplastin Time Ratio at Demonstration of Thrombus: Protocol 2**

<table>
<thead>
<tr>
<th>Thrombus demonstration</th>
<th>n</th>
<th>Time from heparin to platelet injection (min)</th>
<th>APTT ratio</th>
</tr>
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<tbody>
<tr>
<td>After protamine</td>
<td>10</td>
<td>30</td>
<td>1.3 ± 0.19*</td>
</tr>
<tr>
<td></td>
<td>1</td>
<td>3</td>
<td>1.3 ± 0.19*</td>
</tr>
<tr>
<td></td>
<td>1</td>
<td>16</td>
<td></td>
</tr>
<tr>
<td>Before protamine</td>
<td>1</td>
<td>3</td>
<td>8.2</td>
</tr>
<tr>
<td></td>
<td>1</td>
<td>13</td>
<td>7.4</td>
</tr>
<tr>
<td></td>
<td>1</td>
<td>13</td>
<td>10</td>
</tr>
<tr>
<td></td>
<td>1</td>
<td>25</td>
<td>3.3</td>
</tr>
<tr>
<td></td>
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<td>2.4</td>
</tr>
<tr>
<td></td>
<td>1</td>
<td>30</td>
<td>2.4</td>
</tr>
</tbody>
</table>

*Mean ± SD.

Abbreviation: APTT = activated partial thromboplastin time.

**TABLE 2. Activated Partial Thromboplastin Time Ratio at Demonstration of Thrombus: Protocol 2**

<table>
<thead>
<tr>
<th>Time from heparin to platelet injection (min)</th>
<th>n</th>
<th>APTT ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>30</td>
<td>13</td>
<td>1.4 ± 0.47*</td>
</tr>
<tr>
<td>3</td>
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<tr>
<td>13</td>
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<td>7.4†</td>
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<td>16</td>
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<td>1.5</td>
</tr>
<tr>
<td>25</td>
<td>1</td>
<td>3.3†</td>
</tr>
</tbody>
</table>

*Mean ± SD.

†Thrombus demonstrated during heparin infusion (before protamine).

Abbreviation: APTT = activated partial thromboplastin time.
tion of thrombus growth, particularly if recurrence is detected clinically.

Factors that confound the translation of the results of human trials to dose recommendations include demonstrations that heparin clearance rates vary considerably

**TABLE 3. Activated Partial Thromboplastin Time Ratio at Demonstration of Thrombus: Protocol 3**

<table>
<thead>
<tr>
<th>Dog</th>
<th>Time from heparin to platelet injection (min)</th>
<th>APTT ratio at 180 minutes</th>
<th>APTT ratio at demonstration of thrombus</th>
</tr>
</thead>
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<tr>
<td>1</td>
<td>30</td>
<td>2.2</td>
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</tr>
<tr>
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<td>30</td>
<td>4.3</td>
<td>1.6</td>
</tr>
<tr>
<td>Mean ± SD</td>
<td></td>
<td>3.3 ± 1.7</td>
<td>1.4 ± 0.15</td>
</tr>
</tbody>
</table>

A thrombus was not demonstrated during the heparin infusion in any dog.

Abbreviation: APTT = activated partial thromboplastin time.

even among normal subjects, that significant interindividual variation exists in response to coagulation tests to similar heparin regimens, and that heparin requirements have been reported as both unchanged and increased in the presence of venous thrombosis. Additional interpretive difficulties are posed by the many coagulation assays recommended for monitoring, the timing of such assays, and whether safety, efficacy or both have been the monitoring objectives.

Our data suggest that gamma imaging of autologous indium-111-labeled platelets may provide a noninvasive method for continuous in vivo assessment of the inhibition of thrombus growth. Our results also suggest that a minimal level of heparin effect must be maintained to inhibit thrombus growth and that this effect is reflected by the APTT. Platelet accretion was conditioned by two independent variables: the APTT and the time between the initiation of heparin treatment and the injection of radiolabeled platelets. In no instance did platelet accretion occur at an APTT ratio of greater than 2.5 when 30 minutes of heparin therapy preceded platelet injection. When the heparin effect was allowed to resolve spontaneously, the level of effect necessary to inhibit thrombus extension was narrow, an APTT of 1.4 ± 0.15. Similarly, when protamine was administered to reverse the heparin effect, the mean APTT value at the time of thrombus demonstration was 1.3 ± 0.19.

The time between the onset of heparin therapy and platelet injection also appeared to condition platelet accretion by venous thrombi. In four of six dogs in which this interval was less than 30 minutes, a thrombus was demonstrated at APTT ratios substantially above 3.0. The reason for this must remain speculative. This phenomenon was not observed when 30 minutes separated the two events, nor when much larg-
other heparin doses were given (protocol 1). This observation may support the claim that larger amounts of heparin are initially needed to prevent extension of fresh thromboemboli.12

The factors that condition platelet accretion to venous thrombi in vivo are not known. The availability of free thrombin at the thrombus surface, however, is an obvious candidate. Thrombin activates platelet aggregation, which is inhibited by heparin.13 Perhaps as the heparin level and the APTT decrease, this inhibitory effect diminishes, free thrombin becomes available on the thrombus surface, and platelet accretion occurs. Whatever the specific events, our time-activity curves indicate that platelet accretion occurs rapidly and at a well-defined point in time as the APTT returns toward baseline values.

Other investigators have used indium-111-labeled platelets to diagnose deep vein thrombosis. Goodwin et al.14 imaged platelet uptake in three patients in whom venograms were abnormal in corresponding areas. One patient was receiving heparin and one cou-

Figure 4. Representative scintiphotos of femoral veins taken before (A) and after (B) platelet uptake. Thrombus is readily apparent in the proximal right femoral vein.

madin at the time of scanning, although the level of anticoagulation was not defined. Knight et al.15 comparing indium-111-labeled platelets to iodine-125 fibrinogen in a canine model, found higher thrombus-to-blood ratios with indium-111 platelets than with fibrinogen in thrombi less than 24 hours old. We previously showed, using a model that does not produce intimal damage, that venous thrombi are consistently imaged when formed after or up to 8 hours before injection of indium-111-labeled platelets.16 We also have shown that thrombolysis is more rapid in the dog than in man, making studies of aged thrombi difficult to design and interpret.17 The less active fibrinolytic system of man may allow imaging of older venous thrombi. This likelihood is supported by the results of Davis et al.18 They obtained positive indium-111 platelet images in 12 patients with clinically suspected deep vein thrombosis. In only six patients, however, was the diagnosis confirmed by venography or impedance plethysmography. Ten of the 12 patients with positive images were receiving therapeutic doses of i.v. heparin, prolonging their partial thromboplastin times to 2.0–2.5 times control. Davis et al.18 concluded that heparin did not appear to block localization of labeled platelets in venous thrombi. Since sequential APTTs were not reported, we can only speculate that during heparin therapy the APTT fell below the critical level we have defined, allowing platelet accretion by the thrombus. As we have documented, platelet uptake occurs rapidly, and very little time below this critical level is necessary to produce scan positivity. Preliminary data in our laboratory indicate that scan positivity cannot be reversed by heparin administration once platelet accretion has occurred. Alternatively, the platelet-thrombus-heparin interaction in the dog may differ substantially from that in man. We are unaware of data to support this concept.

Translating the results of animal experiments to human disease must always be done with caution. Species differences exist both in platelet function and in heparin response. Compared with human platelets, canine platelets irregularly undergo a biphasic aggregation response to ADP and adrenaline, contain more serotonin, and have different ADP/ATP ratios.19 Species differences also exist in the APTT response to heparin and in heparin clearance rates.20 Nonetheless, our results indicate that injection of indium-111 platelets may offer a means of studying the relationship between heparin effect and thrombus growth in man.

Indium-111 labeled platelets may prove valuable in diagnosing human venous thrombosis and in studying platelet-thrombus kinetics, the natural history of deep vein thrombosis and response to therapeutic intervention. The half life (2.8 days) and emission characteristics of indium-111 are well suited to detection by a gamma camera, and provide advantages over iodine-125-labeled fibrinogen, which has proved its value as a diagnostic and investigative agent.21–23 The higher thrombus-to-background ratio with indium-111 platelets may make it more suitable for detecting pelvic and proximal thigh vein thrombosis.
The details of platelet labeling technique have been extensively explored in different species.\textsuperscript{5, 14, 15, 18, 24–29} Because normal platelet function over a limited time was more important than prolonged survival in this study, incubation of indium-111 oxine platelets in saline was adequate. Recent work by Heaton et al.\textsuperscript{27} suggests that platelet suspension and incubation in a modified ACD solution, with an additional plasma wash, leads to high labeling efficiency, no loss of platelet function, and nearly normal platelet survival. This method appears to be particularly suited to studies in man. The preparative sequence, from blood withdrawal to reinjection of labeled platelets, can be accomplished within 90 minutes.

The demonstration that platelet accretion has been inhibited provides only part of the evidence needed to confirm that thrombus growth has been halted. Fibrinogen plays a major role in such growth; the relationship between fibrinogen deposition and the APTT may or may not parallel that for platelets. Other workers have used fibrinogen as an indicator of heparin efficacy.\textsuperscript{30–32} Despite significant methodologic differences, the results suggest that fibrinogen deposition also relates to the results of coagulation assays during heparin administration. We are now using radiolabeled fibrinogen and platelets to study the relationship between thrombus accretion of these two agents.

If fibrinogen and platelets both participate in thrombus growth below a clearly defined range of APTT (or any other coagulation test) in this animal model, extension of these observations to patients with documented acute venous thrombosis may be possible. The ability to monitor thrombosis should provide a means for defining the optimal heparin dose and the coagulation assays that most accurately reflect that dose.

References

Indium-111-labeled platelets: effects of heparin on uptake by venous thrombi and relationship to the activated partial thromboplastin time.
P F Fedullo, K M Moser, K S Moser, R Konopka and M T Hartman

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