Heparin Induced Platelet Aggregation: 
*In Vitro* Confirmation of Thrombotic Complications 
Associated with Heparin Therapy

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**ABSTRACT**

Eleven patients who developed thromboembolic complications while receiving heparin were studied for a possible adverse reaction to heparin as the cause of their progressive thrombosis. Fifteen additional patients who were receiving heparin for recurrent thromboembolism, but who did not develop signs of thrombotic complications, were studied as patient controls. The most significant finding was an abnormal *in vitro* aggregation response to heparin alone in all of the patients who developed complications who were tested for it (64 percent). None of the patient controls demonstrated this abnormality. In addition, thrombocytopenia was noted in all of the former but in only one of the latter. Results of prothrombin times, fibrinogens and fibrin split products eliminated disseminated intravascular coagulation as the cause of the thrombocytopenia in the majority of cases. Finally, an approach to the early detection of the abnormal heparin response is presented and guidelines for its therapeutic management are recommended.

**Introduction**

Heparin has been used for the prophylaxis and therapeutic management of thromboembolism for over 40 years. The *in vivo* response to this drug has varied markedly from therapeutic anticoagulation without visible side effect to increased morbidity, amputation and even death.1,2,4,5,11,12,13,14,15,17,18,19 Whereas thrombocytopenia was noted to be a possible side effect of heparin therapy as early as 1942,4 it is now recognized that heparin induced thrombocytopenia does exist. Indeed, its incidence is being reported with increasing frequency.1,2,7,8,11,13,14,17,18 Recent studies have further identified an anti-heparin-like antibody in some of the...
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patients exhibiting this phenomenon.\textsuperscript{17, 18} However, the development of new thrombosis in these patients during the course of heparin therapy has received only occasional mention.\textsuperscript{13, 14, 15, 19}

Thus, our experience is reviewed with patients who develop severe complications while on heparin, all of whom improved when heparin was discontinued.

Materials and Methods

Patients were selected for study because they developed thrombocytopenia, new thrombi or extension of pre-existing thrombi during the course of heparin therapy. A second group of patients who were receiving heparin for recurrent thromboembolic episodes was evaluated as control subjects. Normal controls were obtained from healthy hospital personnel. Informed consent was obtained from all individuals prior to study.

Blood specimens for platelet aggregation studies were collected by atraumatic venipuncture using disposable polypropylene syringes and disposable 20 gauge hypodermic needles. Nine ml of freshly drawn whole blood were anticoagulated with one ml of 3.8 percent sodium citrate in a 17 x 100 mm polypropylene test tube.

Platelet rich plasma (PRP) was obtained by centrifuging the fresh citrated blood at 75 \( \times \) g for 10 minutes. Platelet counts were performed on the PRP.*

Platelet poor plasma (PPP) was obtained by centrifuging the blood at 1450 \( \times \) g for 15 minutes.

ADP (adenosine diphosphate)\textsuperscript{†} was prepared by dissolving 200 mg in 100 ml of distilled water. This stock solution was stored in aliquots at -20°C. The working solution was prepared immediately before use by making a 1:20 dilution of the thawed stock reagent with Owren's buffer, pH 7.35.

Heparin (bovine lung or intestinal mucosal)\textsuperscript{‡} was prepared for use by diluting it to 25 units per ml in Owren's buffer, pH 7.35. The diluted solution was stored at 2 to 4°C.

Platelet aggregation studies were performed with a platelet aggregometer§ and recorder.\textsuperscript{‖} At 37°C, 2.4 ml of PRP were added to a siliconized cuvette containing a siliconized metal stir bar followed by 0.1 ml of the aggregating agent.

The aggregating agents used were ADP alone (final concentration 1 \( \times 10^{-6} \) M), heparin alone (final concentration one unit per ml of PRP) and ADP and heparin in combination. When the latter was used, heparin was added to the cuvette first, followed by the PRP and then the ADP. Owren's buffer was run as a control to identify the presence of spontaneous aggregation.

The final platelet count for all studies was 250,000 \( \pm \) 50,000 per mm\(^3\). If the platelet count on the PRP of the patient was too low to qualify for use, the patient PPP was mixed with normal PRP in a 1:1 ratio.

Fibrin split products were measured with a modification of the tanned red cell hemagglutination inhibition immunoassay (TRCHII) as described by Mertens et al.\textsuperscript{10} The specimen consisted of 2 ml of whole blood which was clotted in the presence of 12.5 mg of Amicar (e-aminocaproic acid) and incubated for 30 minutes at 37°C. The resultant serum was checked for residual fibrinogen with thrombin (approximately 50 NIH units) before assay.

Circulating platelet counts were performed\textsuperscript{*} on EDTA whole blood.

The PPP as prepared for use in platelet aggregation studies was also used for fibrinogen determinations. Clottable fibrinogen was determined according to Clauss.\textsuperscript{3}

\begin{itemize}
  \item * A Coulter ThromboCounter was used.
  \item † Sigma Chemical Co.
  \item ‡ Upjohn Co.
  \item § Chrono-Log Corporation.
  \item ‖ VOM Bausch and Lomb Co.
  \item † Baltimore Biological Laboratories.
\end{itemize}
Results

The normal and abnormal responses of PRP to ADP, heparin and ADP and heparin are shown in figure 1. A normal response to ADP (final concentration $1 \times 10^{-6}$ M) was characterized by a single (primary) wave of aggregation followed by deaggregation. In the presence of heparin, a similar curve was obtained with ADP on PRP from normal subjects, although at times the extent of the aggregation was greater than with ADP alone. Heparin alone produced a gradual but slight increase in light transmission through normal PRP, but this change was not comparable to a true wave of aggregation as seen with ADP. Buffer alone produced no change in light transmission other than what might be expected by its dilutional effect.

The abnormal response to ADP alone was characterized by a double wave of aggregation which was irreversible. The secondary wave represented the additional aggregation superimposed on the primary owing to the release of endogenous ADP (released reaction). An abnormal response to heparin alone consisted of irreversible aggregation following an initial lag period. The abnormal response to ADP with heparin demonstrated a somewhat more intense secondary wave. Finally, an abnormal response to buffer alone exhibited spontaneous irreversible aggregation.

Aggregation studies were performed on 22 normal controls selected at random from healthy hospital personnel and on 26 patients who were receiving heparin (table I). Normal aggregation curves were obtained in 50 sets of studies on the 22 normal controls.

Fifteen of the 26 patients served as additional controls. They were chosen for study because they were being treated with heparin for recurrent thromboembolism, i.e., thrombophlebitis and/or pulmonary embolism as evidenced by venography and/or arteriography. Clinically, however, these patients showed no signs of progressive thrombosis once heparin therapy had been initiated. The outstanding laboratory finding was the absence of abnormal aggregation with heparin alone as well as any spontaneous aggregation in the presence of buffer.

The remaining 11 patients developed thromboembolic complications while receiving heparin. An aggregation response to heparin alone could be demonstrated in seven of these patients, one of whom also showed spontaneous aggregation with buffer. The other four patients were studied before the evaluation of this parameter was started.
TABLE I
Platelet Aggregation Studies on 26 Patients

<table>
<thead>
<tr>
<th>Heparin</th>
<th>+ ADP</th>
<th>ADP</th>
<th>Control</th>
<th>New Thrombosis</th>
</tr>
</thead>
<tbody>
<tr>
<td>N</td>
<td>N</td>
<td>N</td>
<td>6</td>
<td>0</td>
</tr>
<tr>
<td>N</td>
<td>A</td>
<td>N</td>
<td>5</td>
<td>0</td>
</tr>
<tr>
<td>N</td>
<td>A</td>
<td>A</td>
<td>4</td>
<td>0</td>
</tr>
<tr>
<td>A</td>
<td>A</td>
<td>N</td>
<td>0</td>
<td>5</td>
</tr>
<tr>
<td>A</td>
<td>A</td>
<td>A</td>
<td>0</td>
<td>2</td>
</tr>
<tr>
<td>Not done</td>
<td>A</td>
<td>N</td>
<td>0</td>
<td>4</td>
</tr>
</tbody>
</table>

Aggregation responses are classified as either normal (N) or abnormal (A). None of the patient controls demonstrated aggregation with heparin alone whereas this abnormality was demonstrated in all of the patients who were tested for it and who developed new thrombi while receiving heparin. Concentrations in the reaction mixture of adenosine diphosphate (ADP) and heparin were $1 \times 10^{-6}$M and one unit per ml of platelet rich plasma, respectively.

Platelet counts were also determined. Thrombocytopenia was noted in one of the patient controls. Although progressive thrombotic complications did not develop, heparin was discontinued in this patient and the platelet count returned to normal. On the other hand, the progressive thromboembolic syndrome noted in the other 11 patients was always accompanied by thrombocytopenia.

In order to rule out disseminated intravascular coagulation (DIC) in these patients, the prothrombin time, fibrinogen and fibrin split product level (FSP's) were checked (table II). One patient (SP) fulfilled the criteria for DIC with thrombocytopenia, prolonged prothrombin time, fibrinogen deficiency and FSP's of 160 $\mu$g per ml. However, all of these parameters, plus clinical status, improved when heparin was discontinued and dextran was administered. Another patient (WH) had a marked increase in FSP's without other evidence of DIC.

Clinically, those patients who developed the abnormal in vitro aggregation response to heparin demonstrated thrombotic complications. Involvement of the venous system was most common. Five of the patients with venous involvement required amputation and two of these died. Two other patients also died.

In the absence of the aggregation response to heparin, one patient developed an isolated thrombocytopenia which, as previously mentioned, resolved when heparin was discontinued. Arterial embolism developed in another patient control as a result of mural thrombus formation secondary to myocardial infarction.

In table III are shown the effects of continuing heparin therapy after the development of clinical signs of thrombotic complications. The frequency with which complications resulted in amputation and/or death under these circumstances is striking. When heparin therapy was not discontinued, the antiaggregating/anticoagulant effects of neither dextran (Rheomacrodex), aspirin (acetylsalicylic acid), dipyridamole (Persantin), nor Coumadin were able to alter significantly the morbid and usually fatal course of these patients. However, prompt discontinuation of heparin coupled with administration of one or more antiaggregants successfully reversed the progression of thrombosis.

Discussion

Although the possibility of an association between heparin and thrombocyto-
penia has been considered for many years, heparin was not recognized as the cause of a progressive thrombotic syndrome with thrombocytopenia until 1973. That year, Rhodes et al\textsuperscript{13} described two patients who developed thrombocytopenia while being treated with heparin for myocardial infarction. Heparin therapy was continued in these patients because the appropriate management had not been clearly defined. Progressive thrombosis occurred in one of the patients. The thrombocytopenia in both did not resolve until heparin was discontinued.

In 1976, Bell et al\textsuperscript{2} conducted a prospective study of patients being treated with heparin in which 16 of 52 developed thrombocytopenia with platelet counts of less than 100,000 per mm\(^3\). Although 10 of these 16 also had increased FSP's and five had decreased fibrinogens, which would be compatible with DIC, the platelet counts returned to normal after heparin was discontinued. However, these authors did not mention the occurrence of any "heparin induced" thrombotic complications in their patients.

Most recently, immune mechanisms have been described to explain the development of heparin related thrombocytopenia.\textsuperscript{8,13} Trowbridge et al\textsuperscript{17} demonstrated the presence of a heparin-dependent IgG platelet aggregating antibody, whereas Wahl et al\textsuperscript{18} described an antibody that is completely absorbable with anti-human IgM and partially absorbed with anti-human IgG.

The primary tool for detection of the abnormal response to heparin \textit{in vitro} is platelet aggregometry. Enhancement of both ADP and epinephrine induced aggregation by heparin\textsuperscript{16,20} as well as aggregation by heparin alone\textsuperscript{6,11} have been studied. Since most patients who are receiving heparin demonstrate enhanced ADP induced aggregation regardless of whether or not thrombocytopenia develops, our experience with heparin alone has shown it to be the most clinically sig-

<table>
<thead>
<tr>
<th>Patient</th>
<th>Duration (Days) of Therapy*</th>
<th>Permanent Effects</th>
<th>Antiaggregating Agents and/or Anticoagulants§</th>
</tr>
</thead>
<tbody>
<tr>
<td>JJ</td>
<td>6</td>
<td>Death</td>
<td>None</td>
</tr>
<tr>
<td>WH</td>
<td>5</td>
<td>Death</td>
<td>Dextran</td>
</tr>
<tr>
<td>CD</td>
<td>11</td>
<td>Amputation + death</td>
<td>None</td>
</tr>
<tr>
<td>LN</td>
<td>8</td>
<td>Amputation + death</td>
<td>Dipyridamole</td>
</tr>
<tr>
<td>VJ</td>
<td>3</td>
<td>Amputation</td>
<td>Dextran, aspirin</td>
</tr>
<tr>
<td>EE</td>
<td>6</td>
<td>Amputation</td>
<td>Dextran, coumadin</td>
</tr>
<tr>
<td>MN</td>
<td>9</td>
<td>Amputation</td>
<td>Dextran</td>
</tr>
<tr>
<td>LJ</td>
<td>9†</td>
<td>None</td>
<td>Dextran, aspirin, dipyridamole</td>
</tr>
<tr>
<td>MS</td>
<td>0†</td>
<td>None</td>
<td>Dextran</td>
</tr>
<tr>
<td>OG</td>
<td>0†</td>
<td>None</td>
<td>Dextran, aspirin</td>
</tr>
<tr>
<td>SP</td>
<td>0†</td>
<td>None</td>
<td>Dextran</td>
</tr>
</tbody>
</table>

\*After onset of complications.
†Any time period of less than 24 hours.
§Administered where indicated.

nificant indicator of the disposition to thrombosis with heparin therapy.

Based upon the results obtained with platelet aggregometry, two types of abnormal responses to heparin were identified in our patients. The first type of abnormal response appeared to involve a plasma factor. In this response, the addition of heparin caused irreversible aggregation in patient PRP and also of normal platelets suspended in patient plasma. Clinically these patients resembled those described by Rhodes et al,\textsuperscript{14} Roberts et al\textsuperscript{15} and Weismann and Tobin\textsuperscript{19} in that they developed thrombosis of the venous or arterial systems.

Abnormal aggregation with heparin as well as spontaneous aggregation characterized the second type of response. Here, the abnormal response was only demonstrable with patient PRP, whereas a normal response was obtained when control platelets were mixed with patient plasma in the presence of heparin, i.e., no plasma factor could be demonstrated. This response occurred in only one of our cases which suggests the following pos-
sibilities: the plasma factor was present in an undetectable concentration; the plasma factor was totally adsorbed to the patient's platelets; or another mechanism is involved.

A third type of abnormal response to heparin, an isolated thrombocytopenia, was detected in one of our control patients. Although Bell et al.2 consider this to be the most commonly occurring abnormality, the relative frequency with which it occurs in the absence of abnormal aggregation merits further investigation. Green et al.8 were able to demonstrate an abnormal aggregation response along with thrombocytopenia. And indeed, our results tend to favor the occurrence of thrombocytopenia in association with an abnormal aggregation response rather than as an isolated phenomenon.

In reviewing the results of prothrombin times, fibrinogens, FSP's and platelet counts obtained on those patients who demonstrated the abnormal response to heparin (table II), the possibility of DIC cannot always be readily dismissed. Indeed, our present knowledge of platelet mechanisms and their interrelationships with various aspects of intrinsic fibrin formation make a tempting prospect of the idea of intravascular coagulation precipitated by widespread platelet agglutination. The point in fact, however, is that all of our patients who were continued on heparin despite the development of thrombotic complications required amputation and/or died. Those in whom heparin was discontinued and who were subsequently treated with antiaggregants and/or anticoagulants improved markedly.

In our experience, the development of thrombocytopenia correlated in time with the appearance of clinical signs of thrombosis. On this basis we recommend daily physical evaluation of the patient for evidence of further thrombosis during the entire course of heparin therapy, with platelet counts at the discretion of the attending staff. Since in vitro aggregation with heparin alone in the absence of thrombocytopenia could not be demonstrated in our patients, aggregation studies without any other evidence of the syndrome do not appear warranted on a routine basis.

However, evidence of new thrombosis or a decrease in platelet count to less than 100,000 per mm³ should, under ideal circumstances, be further investigated with aggregation studies to demonstrate the abnormal response to heparin.

The clinical immediacy surrounding this abnormality cannot be over-emphasized. Once its presence has been identified, heparin must be promptly discontinued. Failure to do so promotes further intravascular thrombosis which will immediately produce significant complications such as gangrene of the extremities, myocardial infarction, pulmonary embolism and stroke. Our experience also showed that patients with thrombocytopenia and evidence of new thrombus formation had a more rapid reversal of the adverse effects of heparin when antiaggregating agents were administered. In a patient who is unable to accept medication by mouth, dextran (Rheomacrodex) can be administered intravenously at the rate of 25 cc per hour. If the patient can accept medication orally, the attending physician may prefer treatment with aspirin or Coumadin with or without Persantin as an alternative.

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References


HEPARIN INDUCED PLATELET AGGREGATION


