Heparin-induced coagulopathy

WILLIAM R. BELL, NORMAN D. ANDERSON, AND ARTHUR O. ANDERSON

Baltimore and Frederick, Md.

Intravenous heparin, at doses of 3.0 U./gm. of body weight, produced an extravascular coagulopathy in rats which was manifested by intestinal tract hemorrhage, a reduction in plasma fibrinogen concentration, a rise in fibrinogen-fibrin degradation products, and the appearance of a rise in platelet count noted in the control animals. This coagulopathy could not be produced by conventional anticoagulant doses of heparin or the injection of large doses of heparin in the presence of protamine sulfate. Specific studies excluded hypoglycemia, metabolic acidosis, and endotoxemia as possible etiologic factors. The coagulation abnormalities observed in this study differ from those produced by injection of other polynuclear substances but their precise pathogenesis is still uncertain.

Thrombocytopenia has been described during the administration of heparin in experimental animals that have similar blood changes were rarely observed in man. While some reports attributed the apparent thrombocytopenia to counterarthritis, other studies have documented a fall in blood platelets during heparin therapy and attributed this to idiosyncratic reactions, perhaps with an immunologic basis. Recently, disseminated intravascular coagulation (DIC) has been reported in patients treated with heparin, in some cases the thrombocytopenia was accompanied by abrupt onset of hemor-

rhagic, prolongation of the whole blood clotting time, decreased fibrinogen concentration, and a rise in fibrinogen-fibrin degradation products (FDP). This finding prompted us to perform a clinical study which showed that thrombocytopenia occurred in 31 percent and DIC developed in 12 percent of patients receiving standard-dose heparin therapy. The present report demonstrates that similar changes can be produced by treating cats with large doses of heparin.

Materials and methods

Adults, male Wistar rats, weighing 250 to 250 grams (Microbiological Associates, Walkersville, Md.) were quarantined in air-conditioned quarters with laboratory chow and water available ad lib. for 3 weeks after delivery to insure absence of disease before studies were instituted. Heparin prepared from beef lung and porcine intestine mucosa in concentrations of 1,000 USP units per milliliter was supplied by the Upjohn Company, Kalamazoo. Methenamine, a bacteriostatic product of Malayan pit viper (Agathisrodion semifasciatus) venom, was obtained in a concentration of 10 units per

From the Department of Medicine, The Johns Hopkins University School of Medicine, Baltimore, Md., and the U. S. Army Medical Research Institute of Infectious Diseases, Frederick, Md.

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Reprint requests: Dr. William R. Bell, Department of Medicine, The Johns Hopkins University School of Medicine, Baltimore, Md. 21205.

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solutions from Tryp cards, London, England. Unkilled from human uvea was obtained from Stanford Workshop Company, Rensselaer, New York. Porcine sublimate solution containing 10 mg/mL was supplied by Eli Lilly Company, Indianapolis, Indiana.

Hematocrit values were determined on blood collected in microcapillary tubes coated with sodium and potassium oxalate. Platelet counts were performed on blood collected in glass tubes containing sodium citrate (1 part citrate : 9 parts whole blood) as the anticoagulant. Plasma was prepared by centrifuging whole blood at 1,050 × g for 15 minutes at 4°C. In control rats, hematocrit levels were determined according to the method of Randell and Memet and Allium's technique, whereas Arvin is used instead of thrombin to clot fibrinogen. Only the latter technique was employed in quantifying fibrinogen concentration in heparin-treated rats.

To be certain that the reduction in plasma fibrinogen concentration and the elevation of FDP levels seen in rats treated with heparin was not artificial, measurements were made on undiluted plasma samples after the in vitro addition of 600 U.S.P. units of heparin to provide a concentration greater than that achieved in the experimental animals. The standard procedure--epilation--anticoagulant--calcium chloride mixture and Arvin were compared in their capacity to remove fibrinogen from heparinized plasma. Similarly, FDP levels were determined before and after heparinization of plasma samples and after the addition of deionized water.

Fibrinogen degradation products were measured by the bound red cell hemagglutination inhibition immunoassay (THCL) as described by Memorial, Johnson, and Lakatos modified for rat FDP with the use of formalized and human type O-negative erythrocytes coated with purified rat fibrinogen. Results were expressed as the highest serum dilution which prevented agglutination of fibrinogen-coated red cells by anti-rat fibrinogen antibodies. Anti-rat fibrinogen antiserum was prepared by injecting 0.1 mg of purified rat fibrinogen emulsified in complete Freund's adjuvant into each of the footpads and two subcutaneous sites in New Zealand white rabbits at weekly intervals. Immune serum was collected 2 weeks after the last injection and absorbed three times with agar, normal rat serum at 25°C, over 30 hours. The absorbed antiserum produced a single precipitate band when reacted against rat fibrinogen in gel diffusion and a 1:10,000 dilution completely agglutinated erythrocytes coated with rat fibrinogen.

Anticoagulant Blood-Clotting Data for pH, Pco2, and PaO2 were obtained in a separate panel of rats, 5, 6, and 6 hours after the heparin injection. Anticoagulant blood was collected under the abdominal aorta and studied with a Beckman ILM-20 infrared absorbance spectrophotometer. Blood, heparinized with Dextran 40, was mixed with a standard grade plethysmograph (Nero Biometers, Houston, Texas). Each heparin preparation used in this study was tested for endotoxin by the Limulus lysate assay of Levin and Bredt. All blood samples obtained from rats before and 2 hours after heparin injection 3.0 units of heparin/kg were tested for endotoxin and cultured in nutrient broth and on blood agar plates at 37°C for 20 hours.

Slides from each organ were stained in methylene blue and Dry ice and sectioned at 4 x 10" in cryostat. After being dried in vacuo with calcium sulfide dust, they were observed for 30 minutes or room temperature. Sections were then observed to examine and were stained with fluorescent antirat fibrinogen (Cappel Laboratories, Downingtown, Pa.) for 1 hour at 20°C. After being washed and mounted in a 0.1 M NaHPO4-0.5 M NaH2PO4-0.85 gm% NaCl as sodium chloride-glycol solution, 0.1Mcro, the sections were examined with a Leitz fluorescence microscope. The activity and specificity of this antiserum were confirmed by double-diffusion method and specific staining of a 3 times washed (0.05 gm%106 m) of sodium-hirudin (preparations prepared from washed rat plasma. Additional controls, from the same organs, were fixed in buffered formalin, embedded in paraffin, and sectioned at 5 x 10" microns. Alternate sections were stained with hetroxaline and eosin. From Lowry's thio- barbituric, or periodic acid-Schiff reagents in an attempt to demonstrate irreversible thrombosis. Non-permeability, stainability of the mean, and comparative group analysis were computed by the method of M. D. and LAVAL, METHODS OF PROCEDURE. In these experiments the rats were restrained at 10-20 degrees, in facilitate collection of blood samples at sequential intervals without the use of anaesthetics. Heparin
Fig. 1. Ten rats (controls) were injected intravenously at time 0 with 1.0 ml of sodium chloride. Note the rise in platelet count observed beginning at 2 hours. Each point is the mean value and the lined line is the S.E.M.

was injected intravenously into separate groups of rats at doses of 0.3, 1.0, 2.0, and 3.0 U.S.P. U./gm. of body weight. Each dose was diluted in sterile 0.85% NaCl 100 ml of sodium chloride to a final volume of 1 ml which was injected over 1 to 2 minutes. Control rats were given 1 ml of 0.85% NaCl 100 ml of sterile sodium chloride intravenously. Blood samples were obtained immediately before heparin injection and serially at 1, 2, 4, and 6 hours thereafter by transcutaneously obtaining the blood from the tail and allowing the blood to flow directly into the containers described. These sequential studies were performed on a total of 2.5 to 3.0 ml of blood to overcome volume depletion artifacts and complete panels of animals were used when necessary. All rats were necropsied at the termination of the experiments. Histologic evidence of DIC was sought by fulfilling two rules from each experimental group at the sequential time intervals designated above and examining their lungs, liver, spleen, kidneys, and lymph nodes by light and fluorescence microscopy.

Results

Commercial preparations of beef lung and porcine mucosal heparin were evaluated separately in these studies. Since no differences were observed in their in vivo effects, data from both experimental groups were combined for presentation in subsequent sections of this report.

Control animals. Intravenous injection of 1.0 ml of sodium chloride solution into 10 rats did not alter the mean systemic blood pressure, arterial blood pH, Pco2, Pco3, clotting time, pressor activity, plasma fibrinogen concentration, or fibrinolytic activity. Beginning at 2 hours, the mean platelet count began to rise from a control value of 560,000/mm3 to a peak mean value at 4 hours of 1,000,000/mm3, which persisted for the duration of the study (Fig. 1). At the completion of the study, all animals appeared healthy and no significant abnormalities were found at necropsy.

Effect of 0.3 to 2.0 U. of heparin/gm. of body weight. The intravenous administration of 1.0 ml of solution containing heparin 0.3 U./gm. of body weight to separate groups of five rats per group did not alter the mean systemic blood pressure, the arterial blood pH, Pco2, Pco3, pressor activity, and plasma fibrinogen concentration. In the ac-
Table 1. Mean systolic blood pressure (mm. Hg)

<table>
<thead>
<tr>
<th>Dose of heparin (C/gm.)</th>
<th>Time after heparin injection</th>
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<tbody>
<tr>
<td></td>
<td>0</td>
</tr>
<tr>
<td>3.3</td>
<td>104</td>
</tr>
<tr>
<td>2.0</td>
<td>94</td>
</tr>
<tr>
<td>0.3</td>
<td>94</td>
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*N.R. = not recordable.

The mean systolic blood pressures are shown in three groups of animals (five rats per group), each receiving a different dose of heparin.

The mean systolic blood pressure was not different from the arterial blood pressure in 48 mm. Hg at 6 hours (Table I). This dose of heparin administered in 2, 4 and 6 hours but the arterial blood pH, Pco₂, Pao₂, hemocrit value, and plasma fibrinogen concentration were unaltered. A rise in platelet count, beginning at 2 hours and of the same magnitude as seen in the control animals, was noted in all groups of animals receiving 0.3, 1.0, and 2.0 U. of heparin/gm. of body weight. The clotting time was prolonged to 60 to 120 minutes in the group of animals receiving 0.3 U. of heparin/gm. and to more than 15 hours in all animals receiving 1.0 and 2.0 U. of body weight. At the end of the study, all animals appeared healthy. At necropsy the findings were unremarkable except in those receiving 2.0 U. of heparin/gm., where focal gastrointestinal hemorrhage was found.

Effect of 3.0 U. of heparin/gm. of body weight. The intravenous administration of 3.0 ml. of heparin containing 3.0 U./gm. of body weight caused a marked prolongation of the clotting time to greater than 24 hours. The mean systolic blood pressure fell within 10 minutes after injection and continued to decline to nonrecordable levels over the course of the experiment (Table I). The hematocrit value decreased moderately but progressively in these animals. The platelet count rise seen in all other treatment groups and the control group was not seen in these rats (Fig. 2). The plasma fibrinogen concentration fell to 40 to 50 percent of the pretreatment levels. Fibrinogen- fibrin degradation products increased to very high concentrations with peak values at 2 hours and remained elevated throughout the duration of the study (Fig. 2). The difference in the alterations in platelet count, plasma fibrinogen concentration, and fibrin degradation products were seen in this group of animals compared to all other groups was significant at the 0.0001 level. In this group of animals the arterial blood pH, Pco₂, and Pao₂ were normal throughout the duration of the experiment (Table II). All animals in this treatment group died 6 to 18 hours following the injection of heparin. At necropsy, fresh hemorrhage (necrotic tissue) was present in the alimentary tract in 13 of 15 animals, but there were no signs of potentially lethal hemorrhage at any other sites. Intraperitoneal and peritoneal accumulations of blood were not present. Necropsy studies in combination with blood pressure measurements of rats killed at sequential intervals indicated that the hypotension in this group commenced 2 to 3 hours before the first sign of hemorrhage in the alimentary tract.

Effect of 3.9 U. of heparin and 0.2 mg. of protamine sulfate/gm. of body weight. The intravenous administration of 3.9 ml. containing 3.9 U. of heparin/gm. of body weight immediately followed by 0.5 ml. of a solution containing 0.3 mg. of protamine sulfate/gm. of body weight in five rats did not alter the mean systolic blood pressure or the arterial blood pH, Pco₂ or Pao₂. The clotting time at 1 hour was normal and un-

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The document contains a table and text discussing the effects of heparin on blood pressure and clotting time, along with observations on platelet counts, fibrinogen levels, and other blood parameters. It also describes the effects of protamine sulfate on these parameters. The text is focused on the physiological responses to different doses of heparin and how these responses compare to control groups and to each other. The results highlight the dramatic effects of heparin on clotting time and blood pressure, with significant decreases observed in both parameters. The table provides specific data points for different doses of heparin, showing how these doses affect the blood pressure over time. The text also mentions the absence of hemorrhage at necropsy in some cases, indicating potential areas for future research.
Table II. Mean arterial blood changes with heparin, 0.5 U. per gram of body weight

<table>
<thead>
<tr>
<th>Time after heparin injection</th>
<th>0</th>
<th>10 min</th>
<th>1 hr</th>
<th>2 hr</th>
<th>6 hr</th>
</tr>
</thead>
<tbody>
<tr>
<td>pH</td>
<td>7.42</td>
<td>7.42</td>
<td>7.41</td>
<td>7.38</td>
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<td>76</td>
<td>76</td>
<td>76</td>
<td>76</td>
<td>76</td>
</tr>
</tbody>
</table>

*The mean arterial blood gas and pH changes before and following the intravenous injection of heparin are shown for a group of 15 rats changed from the preheparin clotting time. The hematocrit value and plasma fibrinogen concentration were unchanged from the preheparin studies. The rise in platelet count seen in the control animals and in those animals which received 0.3, 1.0, and 2.0 U. of heparin/gm. was also noted in these animals (Fig. 3). No PDP-III, as in the blood at the completion of the study period all animals were healthy and no necropsy of gross abnormalities were observed.

Effect of 0.3 mg. of protamine sulfate/gm. of body weight. The hematocrit determinations of 1.0 ml of sera of animals containing 0.3 mg/gm. of body weight of the rats gave results identical to those described in the control group. At necropsy no abnormalities were noted.

Histologic studies

Histologic examination with routine and special stains revealed neither fibrin nor platelet thrombmi in the vessels of any of the heparin-treated or control animals. Immunofluorescent studies did not demonstrate these deposits in these rats. Histologic abnormalities were seen only in the group of animals receiving 3.0 U. of heparin/gm. of body.
Fig. 1. A group of five rats were injected intravenously with 3.0 U. of heparin per gram of body weight immediately followed by 0.3 mg. of protamine sulfate per gram. The alterations noted in the rats which received 3.0 U. of heparin per gram alone are not observed in these animals. The rise in platelet count seen in the controls is also observed in this group. Each point is the mean value and the bracketed line is the S.E.M.

weight. In this group, vascular congestion and focal extravasation of blood were observed in the kidneys and lymph nodes in 13 of 15 animals.

In vitro determination of fibrinogen and FDP-fdp in the presence of heparin. The anticoagulant properties of heparin prevented the use of conventional methods of measuring fibrinogen as thrombin-clotable protein in this study. As indicated by the data in Table III, Arvin can be used to determine accurately the fibrinogen concentration in plasma specimens containing heparin. Despite the presence of high concentrations of heparin, Arvin can remove completely all clotable proteins without generating FDP-fdp or interfering with the immunologic determination of FDP-fdp. As shown in Table III, when Arvin, as contrasted with thrombin, was employed, the concentration of fibrinogen and the level of FDP-fdp were not altered by heparin.

Endotoxin studies

Endotoxin could not be detected in the heparin aseptically removed from the administration vials nor in the blood of the rats studied before and 2 hours after the intravenous administration of 3.0 U. of heparin per gram. No bacterial growth was observed from the blood of animals in the nutrient broth or on blood agar plates.

Discussion

The mechanism of heparin-induced thrombocytopenia has never been adequately explained. Heparin, attributing this phenomenon to direct toxic effects upon platelets or heparin-antibody-platelet interactions have not been substantiated. Although it has been suggested that some subjects developing thrombocytopenia during heparin administration may be experiencing an intravascular coagulopathy, most reports have not in-
<table>
<thead>
<tr>
<th></th>
<th>I Rat plasma</th>
<th>II Rat plasma, 650 U of heparin</th>
<th>III Rat FDP + of heparin (1:10240)</th>
<th>IV Rat plasma + FDP + (1:10240), 650 U of heparin + 10 U of thrombin</th>
</tr>
</thead>
<tbody>
<tr>
<td>Thrubin</td>
<td>155</td>
<td>157</td>
<td>153</td>
<td>82</td>
</tr>
<tr>
<td>Arter</td>
<td>No clot</td>
<td>No clot</td>
<td>No clot</td>
<td>No clot</td>
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</table>

*Comparison of fibrinogen concentration and FDP-tiler titer in plasma samples containing heparin in rats. Data are presented as the mean of four determinations. The comparison was made by the Student's t-test. The differences between the means were considered significant at p < 0.05.*

<table>
<thead>
<tr>
<th>Fibrinogen</th>
<th>FDP-tiler titer</th>
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<tbody>
<tr>
<td>(mg/100 mL)</td>
<td></td>
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<tr>
<td>0</td>
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duced studies required for documenting this process. Recognition of a DIC-like disorder in the anticoagulant patient is dependent upon demonstrating a fall in blood platelets and fibrinogen levels accompanied by a rise in FDP-tiler. In a recent study of 52 patients receiving standard doses of heparin, we observed thrombocytopenia (<100,000 platelets/mm³) in 16 subjects. Ten of these patients showed elevated levels of FDP-tiler during heparin therapy (no FDP-tiler were detectable prior to treatment) and in five subjects the plasma fibrinogen concentration fell to 25 to 50 percent of the pretreatment levels. These data suggest that such an intravascular coagulopathy may contribute to the significant incidence of bleeding seen with this anticoagulant.

The data in the present study demonstrate that the onset of bleeding, fall in blood pressure, reduction in plasma fibrinogen concentration and platelet count, and rise in FDP-tiler occurred in rats during the administration of heparin but only with amounts far exceeding those used in clinical medicine. The phenomena could not be produced in the presence of protamine sulfate, a heparin antagonist. Intravenous injection of large doses of heparin into rats caused prolonged clotting times, gastrointestinal hemorrhage, and hypotension which could be attributed to the anticoagulant effects of heparin. However, the demonstration of a relative thrombocytopenia (failure to exhibit the rise in platelet counts seen in other treatment groups and controls) was a fall in plasma fibrinogen levels and concomitant rise in FDP-tiler after injecting 3 U of heparin/mg. of body weight suggested that these animals were experiencing a DIC-like disorder. This thesis was supported by documenting that the reduction in plasma fibrinogen levels and rise in FDP-tiler were not artifacts caused by the antithrombin effects of heparin during in vitro assay. We were unable to detect intravascular fibrin deposits at platelet thrombi in tissues from these animals, but these data suggest that such deposits can be completely absent in some well-documented cases of DIC.

Several different mechanisms were considered as possible causes of the intravascular coagulopathy in these animals. Since all of the rats appeared clinically well at the start of these experiments and no evidence of significant disease was found at necropsy, it is unlikely that this coagulopathy was related to latent infection. The occurrence of the intravascular coagulopathy only in rats given 3 U of heparin/mg. of body weight suggested that this might be a dose-related, direct toxic effect. In vitro studies have shown that a single subcutaneous heparin may have adverse effects on platelets which are not pro-
duced by beef lung heparin. 15 In the present study both types of heparin were found to be equally potent in producing \textit{in vivo} intravascular coagulopathy. This indicated that the platelet-aggregating properties produced by intravenous heparin in vitro could not be correlated with the reduction \textit{in vivo} in platelet and fibrinogen levels. Such a contaminant may not be significant in routine doses of heparin but would be magnified at the doses employed in this study. However, sequential injections of heparin and protamine sulfate failed to induce coagula-

tion abnormalities in test animals. Endotoxin has been implicated as a cause of DIC 14 but we were unable to detect endotoxin in either the heparin or the blood of the animals before or after treatment with heparin. As heparin is strongly acidic, it was postulated that the concentrations employed in these studies (10 to 15 times the usual therapeutic dose) might cause a metabolic acidosis with secondary alterations in blood pressure and activa-
tion of the coagulation system. Hypothesis was also considered as a possible mechanism since DIC has been reported in this setting. 15, 16 These possibilities were excluded when consistently normal arterial pH and blood gases were found in heparin-treated rats. Hypotension is a possible cause and cannot be excluded. However, alterations in FDP, fibrinogen, and fibrin were noted at 1 hour when no striking fall in blood pressure occurred. Since previous investigators have described anticomplementary effects, 17-18 impaired phagocytosis, 19 and decreased lymph node-filtering capacity 20 in heparinized rats, we postulated that these changes could permit hematogenous dissemination of bacteria or bacterial products from the intestine and result in such a coagulopathy. Blood cultures were sterile and studies for endotoxin were negative in the present study, suggesting that this mechanism is unlikely.

While heparin is a highly charged polyanion capable of reacting with proteins and cell surfaces \textit{in vitro} and \textit{in vivo}, 21, 22 there is no convincing evidence that such interactions directly activate the coagulation system. Intravenous infusions with other polyanions (polysaccharide sulfonate) have been reported to activate the clotting system and cause renal cortical necrosis in dogs. 23 However, this appears to be a secondary event related to platelet aggregation and endothelial damage. 24-25 Since this form of intravascular clotting can be minimized by heparin therapy, 26 it appears to differ from the intravascular coagulopathy described in the present study. Other investigators have suggested that polyanions may activate the complement cascade which triggers intravascular coagulation. 27-28 This concept is based upon observations that preincubation of serum with heparin results in loss of complement components C1, C4, and C3-C9 activity, but it is still uncertain whether these changes reflect activation of the complement sequence or anticomplementary effects due to heparin-protein binding. The relevance of these in vitro findings to intravascular coagula-
tion must be questioned. Rent and associates 29 have shown that simultaneous addition of heparin and protamine enhances the loss of complement activity in serum samples. While we have no data on complement consumption in the present study, our results clearly indicate that sequential injections of heparin and protamine in vitro failed to induce clot-
ing abnormalities.

Arts was supplied through the courtesy of Prof. F. A. Robinson, London, England. The endo-
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