Plasma Heparin Activity and Antagonism During Cardiopulmonary Bypass with Hypothermia

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Plasma heparin activity in 11 patients undergoing open-heart surgery was measured by comparing thrombin time of patient plasma to thrombin time of plasma containing known heparin concentrations. Although all patients received 300 units/kg of heparin, their initial plasma heparin levels varied significantly, from 1.8 units/ml in lighter patients to 3 units/ml in heavier patients. During hypothermia (25°C), heparin decay was insignificant. At 37°C, heparin decayed at a rate between 0.37 and 2.01 units/ml/hr. This decay was significantly faster in those patients with higher initial posthypothermia plasma heparin levels.

Although there have been many methods of evaluating the effect of heparin on clotting, 1-12 a precise, practical method of evaluating circulating heparin levels during cardiopulmonary bypass in man has been lacking. As a result, over 30 protocols for heparin administration are in use in this country. 3 Among these, dosages of heparin, and of protamine used to reverse heparin, vary considerably, 3 with consequent excesses and deficits of both. Bull and coworkers 4 attempted to overcome this problem by using a dose-response curve in which clotting time is a function of heparin dose, but they still encountered marked variations in clotting time from patient to patient. We therefore undertook the following study to precisely assay plasma heparin levels in patients undergoing cardiopulmonary bypass, and to formulate an accurate method for reversing heparin with protamine.

MATERIALS AND METHODS

Plasma heparin activity was determined by comparing thrombin times of patient plasma samples to control thrombin times of pooled human plasma 5 containing known heparin concentrations. Before analysis, both control and patient plasma samples were

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were serially diluted 1:1 with veronal buffer, and then 1:1 with unheparinized pooled
human plasma, to minimize the influence of procoagulant variability. Then, 0.1 ml of
thrombin solution was added to 0.2 ml of diluted plasma which had been incubated
for 2 minutes at 37°C and clotting time determined mechanically. 6

Thrombin solutions were prepared by diluting 100 NIH units of lyophilized bovine
thrombin in 0.02 M CaCl₂, to 10 units/ml or 2.5 units/ml (for use in detecting low
heparin concentrations). Uniformity of these solutions was assured by adjusting the high
and low thrombin concentrations to obtain unheparinized control thrombin times of 7.2
to 7.6 seconds and 17.2 to 18.4 seconds, respectively.

The effect of hemodilution on the heparin assay was measured by comparing thrombin
time of heparinized plasma with that of the same plasma diluted 1:1 with control plasma,
heparinized pump-prime without blood, 6 Ringer’s solution, or distilled water.

Patients undergoing open-heart surgery were selected at random. Each patient
received 300 units of heparin/kg initially and 150 units/kg every 2 hours until the
termination of bypass. Plasma heparin determinations and blood-activated recalcification
time (BART) were performed frequently during each case and again after heparin was neutralized with protamine.

Heparin was reversed in 8 patients with a protamine dose based on the formula:

\[
mg = \frac{\text{mg} \times (\text{weight} \times 70 \text{ ml/kg}) \times \text{heparin units/ml plasma}}{(100 \text{ units/mg})}
\]

The precision of this method was evaluated by comparing predicted to actual residual
heparin levels produced by giving approximately 95 percent of the calculated prota-
mine dose. This was necessary because 100 percent antagonism could not be distin-
guished from protamine excess by the heparin assay. Subsequently, the balance of our conventional dose of protamine 6

**Fibrometer, Becton Dickinson Company, Cockys-
ville, Maryland.

6Pump-prime contains 1000 ml Normosol® 500 ml
dextran-40, 22.5 mg mannitol, and 2200 units
heparin.

165 ml in females.

Total protamine dose = (1 mg/kg) \(e^{-0.3165t}\) (t =
hours after the last heparin dose).

RESULTS

The thrombin time control curves (fig 1) demonstrate that in-vitro heparinization of
plasma prolonged thrombin time. The in-vitro assay is consistent within ±0.05 unit/ml
below heparin concentrations of 2 units/ml and ±0.1 unit/ml from 2 to 4 units/ml.
In-vitro dilution (1:1) of heparinized controls with pump-prime, plasma, or Ringer's
solution resulted in a proportional reduction in thrombin times, but in-vitro dilution
(1:1) with distilled water shortened control thrombin times by more than a factor of 6.

There was a consistent pattern of heparin activity during open-heart surgery. Figure
2 shows a typical patient’s record. Following a loading dose of heparin, plasma heparin
levels usually peaked out of the range of the assay, but fell within 30 minutes to be-
tween 3 and 5 units/ml. After instituting bypass, heparin levels fell further because
of hemodilution with pump-prime and then plateaued in association with hypothermia.
With each redose of heparin during hypo-
thermia, the plateau increased. Following
rewarming, heparin levels declined until heparin was reversed with protamine.

**Fig 1. Thrombin times at known heparin concen-
trations after in-vitro heparinization of control
plasma. The upper curve was generated using a
thrombin concentration of 2.5 units/ml; the lower
curve using thrombin 10 units/ml. (N equals the
number of determinations, if greater than 3.)
Although all patients received equal initial mg/kg doses of heparin, their plasma levels after initiating bypass varied from 1.8 units/ml in the lighter patients to 3 units/ml in the heavier patients. This relationship (fig 3, left) of level to weight is highly significant ($y = 0.022x + 0.92; r = 0.85, p<0.01$). The initial level was somewhat better correlated with total amount of heparin administered ($y = 0.08x + 0.34; r = 0.96, p<0.01$) than with weight (fig 3, right) because of the 2200 units of heparin contained in our pump-prime. Following a 2nd dose of heparin (6 patients), the plasma heparin activity increased to a new level which was proportional to the cumulative dose and patient weight ($p<0.01; r = 0.99$).

Heparin decay during hypothermia (fig 4) was insignificant (0.21 units/ml/hr ± 0.12 SD). After rewarming to 37°C, heparin levels declined linearly at a significant ($p<0.01$) rate between 0.37 and 2.01 units/ml/hr. These decay rates were directly proportional ($p<0.01; r = 0.93$) to the initial heparin levels following rewarming.

At the end of bypass, 8 patients received a protamine dose calculated to reverse approximately 95 percent of the assayed circulating heparin. The mean difference between the predicted and actual residual heparin level was 0.025 units/ml ± 0.09 SE. This represents a mean error in protamine dose of only 1.3 mg ± 2.7 SE/patient, or about a 2 percent error. The BART returned to within 4 percent of preoperative control values, and no heparin activity was detectable after administration of the remainder of our conventional protamine dose.
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Fig 3. Initial plasma heparin levels during bypass following a 300-unit/kg loading dose. Initial levels varied from 1.8 to 3 units/ml. Left: Initial heparin levels are proportional to weight: $y = 0.022x + 0.92; r = 0.85, p < 0.01$; Right: Initial heparin levels are somewhat better correlated with initial dose: $y = 0.08x + 0.34; r = 0.96, p < 0.01$.

![Graph showing initial plasma heparin levels during bypass](image)

Fig 4. Heparin activity during hypothermia (25°C) in 11 patients. Heparin activity during hypothermia declined insignificantly (0.21 units/ml/hr ± 0.12 SD) with time.

![Graph showing heparin activity during hypothermia](image)

**DISCUSSION**

Many methods of measuring heparin levels exist. Of these, only the microelectrophoresis method of Jaques measures heparin directly, but it requires 5 or more hours to complete. The BART, activated coagulation time, activated partial thromboplastin time, BaSon, and thromboelastogram are only semiquantitative methods. Other clotting-time tests measure heparin quantitatively. Of these, factor X analysis is a complex multiple-stage test, and the diluted activated partial thromboplastin time was significantly prolonged by incubation of controls for 30 minutes at 21°C.

Although all patients received equal mg/kg loading heparin doses, after initiating bypass their plasma levels were not equal but were instead proportional to weight. The 2 heaviest patients had over 66 percent higher levels than the 2 lightest patients. Heavier patients thus demonstrated a heparin space which was relatively smaller per kg body weight than that of the lighter patients. More uniform initial heparin levels might be achieved by administering loading doses calculated on the basis of lean body weight rather than by actual weight.

Heparin decay during hypothermia is relatively insignificant. Because decay is so profoundly reduced and heparin redoses only serve to raise the level of the plateau, redoses during hypothermia could be markedly reduced or eliminated entirely. Ideally, redoses should be based on monitored heparin levels.

Previous studies of heparin activity during normothermia revealed an exponential pattern of decay. Our patients had linear decay patterns after rewarming to 37°C, which may be the result of exposure to hypothermia. Alternatively, the linear segments may simply represent short portions of an exponential decay curve. This might explain why patients with higher heparin levels after rewarming had more rapid decay rates than those with low levels.

Neutralizing heparin with 1 mg protamine per mg of measured circulating heparin avoids the use of excess protamine. While excess protamine is known to act as
an anticoagulant in vitro, Ellison has disputed the possibility of this effect in vivo.\textsuperscript{13, 15} Ellison advocates a protamine dose of 1 mg/kg of heparin given during bypass to avoid heparin rebound, which he observed in 3/6 patients receiving half that dose.\textsuperscript{13, 14} The clinical significance of Ellison’s findings regarding heparin rebound and the benign nature of protamine is disputed by other workers. Guffin and coworkers,\textsuperscript{16} in a study of 60 patients, showed that postoperative bleeding was decreased by approximately 50 percent by reducing protamine from the dose recommended by Ellison to the lower dose with which Ellison observed rebound.

In a study of 92 patients whose heparin was not neutralized, Castaneda\textsuperscript{17} showed a return of Lee-White clotting time to normal within 4 hours after bypass. He observed no cases of unexpected postoperative bleeding. Other adverse effects of protamine include myocardial depression, 80 percent reduction in platelet count,\textsuperscript{15, 18, 19} and sluggish due to red cell aggregation with consequently increased vascular resistance,\textsuperscript{17} including an increase in pulmonary artery pressure of up to 121 percent and pulmonary venous pressure of 134 percent.\textsuperscript{18, 20}

While further studies of the relevance of heparin rebound and protamine excess would be useful, the weight of the evidence supports the use of the method of heparin neutralization presented in this study, in which no more protamine is given than is necessary to reverse the heparin.

CONCLUSIONS

The results of this study contain clinically important implications regarding the use of heparin for open-heart surgery. In order to achieve uniform initial plasma levels, loading doses of heparin should probably be based on lean body weight. Heparin decay during hypothermia is relatively insignificant. Therefore, redoses during this period may be reduced or omitted. Following rewarming, heparin decay becomes significant, but the decay rates are highly variable from patient to patient. Heparin can be accurately neutralized by 1 mg protamine per mg circulating heparin. Because of the influence of temperature and patient weight on heparin activity during bypass, accurate control and reversal of heparin requires ongoing analysis rather than any 1 dosage protocol.

REFERENCES


Guest Discussion

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I congratulate Dr. Cohen and his colleagues on a thoughtful and significant contribution to an area of medicine that has had much of its practice based on "visceral" data and anecdotal information. I would like to emphasize a few aspects of their paper and draw some comparisons with information presently available in the literature.

The most important point to emphasize is their finding of a variable patient response to fixed heparin doses by quantitation of the thrombin time. We all learned in basic pharmacology that any group of individuals has a "bell-shaped curve" response to drugs, but our clinical practice as physicians has not shown our understanding of this basic concept. As anesthesiologists we titrate drugs based on response probably more than most areas of medicine, but this demonstration by Dr. Cohen seems somehow overdue.

His information supports in a quantitative way recent data by Bull and his colleagues, who measured heparin activity by the less exact, but nonetheless clinically meaningful, technic of activated clotting time. The individual patient variation in that study was further accentuated by a variety of rigid protocols for heparin administration. The findings by Dr. Cohen that the obese patient has a relatively smaller heparin space emphasizes the importance of lean body mass for drug distribution and that this is important for heparin as well. There are, however, some areas in this report that are at odds with reports by previous investigators.

The use of the thrombin clotting time by Dr. Cohen showed a marked cessation of heparin metabolism at 26 to 28°C. Bull's group found such a small and unpredictable decline in metabolism of heparin that it was not considered significant at 30°C, using the activated clotting time. Wright and coworkers, in a rather gross estimation of heparin activity, did demonstrate marked cessation of metabolism in dogs and man with hypothermia, as have others, but Dr. Cohen has quantitated this plateau more accurately. Although temperatures were not exactly comparable, it would follow that metabolism of heparin, like other drugs, would be slowed, even though Bull's study did not show this.

The use of the calculated amount of heparin remaining after bypass to determine the protamine required to neutralize it yielded insignificant errors in actual residual heparin in the present study. This led to a considerably reduced protamine dose. Ellison and colleagues looked at the effects of excess protamine on the coagulation system and found that doses of 800 mg/70 kg produced minimal effects on coagulation. However, they did not measure a platelet count, which has been shown by Gans and Castaneda and others to be reduced following protamine administration.

Ellison used, among other tests, the protamine titration method of quantitating the remaining heparin for neutralization. All patients who received protamine (about 0.5 mg) for each 1 mg of heparin given (presumably a result of heparin decay) demonstrated anticoagulation (heparin rebound) by their criteria. In 3/6 with low-dose protamine, clinical bleeding required more protamine up to 5 hours following the initial reversal.

Patients in Dr. Cohen's report, however, received an even lower protamine/cumulative heparin dose (0.3 mg/1 mg), and none had postoperative bleeding complications. To add to the controversy, Guffin's group recently evaluated low-dose protamine based on heparin decay and found a marked reduction in chest drainage and operating time along with improved coagulation studies when compared with a 1 mg/1 mg protamine-to-heparin ratio. This information is in direct conflict with data from Ellison and others.

The question remains as to why such diverse results can be obtained with similar operations, drugs, and patient populations.