

Original Article

The Effect of Hypothermia on Heparin Anticoagulation as Measured by Activated Clotting Time and Factor Xa Inhibition

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ABSTRACT

The effect of hypothermia on heparin anticoagulation was studied using (siliceous earth) activated clotting time (ACT) measured by the modified Hattersley technique and anti-factor Xa assay measured by the modified Teien technique during cardiopulmonary bypass (CPB). Seventeen adult patients with normal blood coagulation function undergoing their first time coronary artery bypass grafting surgery or valve replacement surgery were randomly selected. Blood samples were collected 3-5 minutes after CPB initiation, before cooling at a mean temperature of 32.9°C, and then again after cooling to a mean temperature of 28.4°C. No additional heparin, citrate or packed red blood cells were added to the CPB circuit between sampling points.

The mean heparin concentration measured by the anti-factor Xa assay was 4.1 ± 0.5 units/ml before cooling and 4.1 ± 0.4 units/ml after cooling. The mean ACT was 465 ± 75 seconds prior to cooling and 499 ± 69 seconds at the cold temperature ($p < 0.05$). This suggests that the cooling process itself must induce changes in whole blood coagulation resulting in a prolongation in the ACT. The change in ACT with cooling is not due to a change in heparin concentration. The presented data supports previous research on the inadequacy of ACT to trend heparin concentration when steady state conditions are altered.

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INTRODUCTION

Heparin has been used as an anticoagulant for cardiopulmonary bypass (CPB) since its inception in 1953. Activated clotting time (ACT) is affected by the heparin concentration and is easily used at the bedside to indicate blood anticoagulation status. However, the relationship between heparin concentration and ACT is not linear, and may be *convex*, thus making extrapolation of ACT to heparin concentration difficult (1). Historically, when the ACT is above 400 to 480 seconds, it is considered safe to initiate CPB (2,3), but several studies have reported ACT values below 400 seconds at CPB initiation without complications (4). A number of factors other than the heparin, including hypothermia, hemodilution, and platelet function can affect ACT during CPB (5-11). Paul et al reported that surface cooling to 20°C for 60 minutes in anesthetized dogs without cardiopulmonary bypass provoked a significant decrease in the platelet count and the constant release of a heparin-like factor which reacted identically to heparin as a specific inhibitor of factor Xa (12). This heparin-like factor has not been further characterized. The mean maximum heparin-like factor release in Paul's study was equivalent to the anticoagulant effect of 0.5 unit heparin/ml.

In the present study we investigated heparin concentration and ACT during the moderate hypothermic CPB. We compared ACT and factor Xa inhibition (a heparin and heparin-like factor specific assay) during cooling on CPB. We postulated that the increase in ACT seen with hypothermia does not reflect an increase in heparin or heparin-like factor.

METHODS AND MATERIALS

Adult patients undergoing their first time coronary artery bypass grafting surgery (CABG) or valve replacement surgery were randomly selected. Patients were excluded if they had received intravenous heparin, subcutaneous heparin, aspirin, or nonsteroidal anti-inflammatory containing medications, within one day or warfarin within seven days prior to surgery. Patients were eliminated if they received aminocaproic acid, desmopressin acetate, or aprotinin prior to, or during the study period.

CPB procedures employed the use of a roller head pump^a, a hollow fiber oxygenator (Maxima)^b, 3/8 inch and 1/2 inch PVC tubing, arterial filter (H-640)^c, venous reservoir bag (MVR1600)^b, cardiotomy^d and a 2:1 blood cardioplegia delivery set^e. The circuit was primed with a solution containing 2 liters Plasma Lyte-A solution^f, 10000 units of heparin, 22 mEq of sodium bicarbonate, 25 g of mannitol and 50 g of albumin. A hemoconcentrator if present was not used between sample collection. No more than 200 ml of a crystalloid solution or any blood product was added to the CPB circuit between sample collection. According to the clinical protocol, anticoagulation was achieved with a heparin^g loading dose of 300-350 units/kg

body weight, to achieve a pre CPB ACT of 480 seconds or greater^h prior to aortic cannulation.

Two blood samples were obtained for research analysis. Six ml aliquots of whole blood were collected from the sample port of the venous line just proximal to the venous reservoir for ACT and factor Xa inhibition assay. The first sample was collected 3 to 5 minutes after CPB initiation when the temperature of the venous return blood was 31-33°C. Patient cooling was then begun.

The second sample was collected when the temperature of venous return blood decreased to 27-29°C but before cardioplegia containing citrate was administered. No additional heparin was administered between the first and second blood sample collection.

Pre-study trials showed that automated kaolin ACT results determined by two identical machines varied considerably and that there was less variability using a modified Hattersley technique (unpublished results). Due to the variability noted in our routine clinical automated kaolin ACT's, the ACT test was performed using a modified Hattersley technique (13,14). Two separate 2 ml aliquots of venous blood were inserted into two prewarmed, 37°C vacutainer tubes containing six to ten milligrams of siliceous earth. The tubes were then inverted five times to mix and placed in a heat blockⁱ set at 37°C. At one minute and at ten second intervals thereafter, the tube was withdrawn from the heat block and tilted to spread the blood along its length. Timed to the nearest ten seconds, the appearance of the first unmistakable clot (one to three millimeters in diameter) determined the end point for the ACT. All the ACT assays were performed by the same investigator (LT). If the two ACT readings differed more than 6%, the data was discarded. The mean of the two ACT readings was used for analysis.

An additional 2 ml aliquot of whole blood for factor Xa inhibition assay was transferred to a 3 ml test tube containing 0.2 ml of 0.105 molar buffered citrate solution^j, mixed, then placed in an ice container and transported to the hematology laboratory. The sample was frozen, then later thawed for batch processing of the factor Xa inhibition assay.

This is a two-stage assay for heparin or heparin-like factor based on the acceleration of inhibition of factor Xa by antithrombin III (15,16). Antithrombin III forms a complex

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- a Sorin Biomedical, Inc., Irvine, CA 92714
 - b Medtronic Cardiopulmonary, Anaheim, CA 92807
 - c Bard Cardiopulmonary Division, Tewsbury, MA 01876
 - d Baxter Healthcare, Bentley Lab., Inc., Irvine, CA 92714
 - e Sarns 3M Healthcare, Ann Arbor, MI 48103
 - f Baxter Healthcare Corp., Deerfield, IL 60015
 - g Elkins-Sinn, Inc., Cherry Hill, NJ 08003
 - h Hemotec, Inc., Englewood, CO 80112
 - i Fisher Scientific, Pittsburgh, PA 15219
 - j VACUTAINER Systems, Division of Becton, Dickinson, and Company, Rutherford, NJ 07070

with heparin. This complex is able to neutralize factor Xa more rapidly than antithrombin III alone. Upon addition of excess factor Xa in the system, the amount of factor Xa neutralized after an appropriate time interval is proportional to the amount of heparin or heparin-like factor originally present in the plasma. The remaining factor Xa quantity is determined from the rate of release of p-nitronaline from the chromogenic substrate $\text{CH}_3\text{SO}_3\text{-D-Leu-Gly-Arg-pNA}$ (CBX 31.39) which is followed spectrophotometrically at 405 nm. The concentration of heparin or heparin-like substances in the sample was calculated from a standard curve determined with heparin calibrators.

The difference of the ACT measured at the cold temperature minus the ACT measured at the warm temperature for each patient was computed. The difference of the heparin concentration measured at the cold temperature minus the heparin concentration measured at the warm temperature for each patient was also computed. The resulting differences were analyzed with a one sided paired Student's t-test using Statistical Analysis System (SAS)^k. All results are described as mean plus and minus one standard deviation. The ranges are given in parentheses.

RESULTS

Seventeen patients (13 males, 4 females) undergoing first time cardiopulmonary bypass surgeries were investigated. Sixteen of them had coronary artery bypass grafting surgeries and one had aortic valve replacement surgery. The average age was 58 ± 12 years (range 36-78 years), the average weight was 86 ± 16 kg (range 66-115 kg). The average cooling time was 6 ± 3 minutes (range 3-11 min). The mean preoperative platelet count was 240 ± 73 K/mm³ (range 110-352 K/mm³) and the mean preoperative hematocrit was $39 \pm 4\%$ (range 29-46%). The average venous blood temperature was $32.9 \pm 0.9^\circ\text{C}$ before cooling, and $28.4 \pm 1^\circ\text{C}$ after cooling.

The mean ACT was 465 ± 75 seconds at a venous blood temperature of $32.9 \pm 0.9^\circ\text{C}$ prior to cooling and 499 ± 69

Figure 1: Average heparin and ACT at warm and cold temperatures

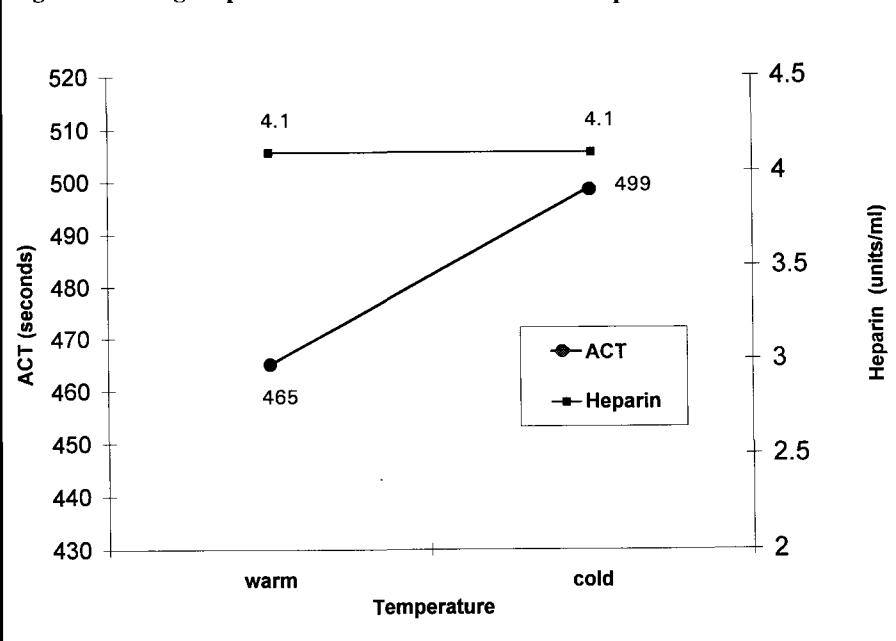
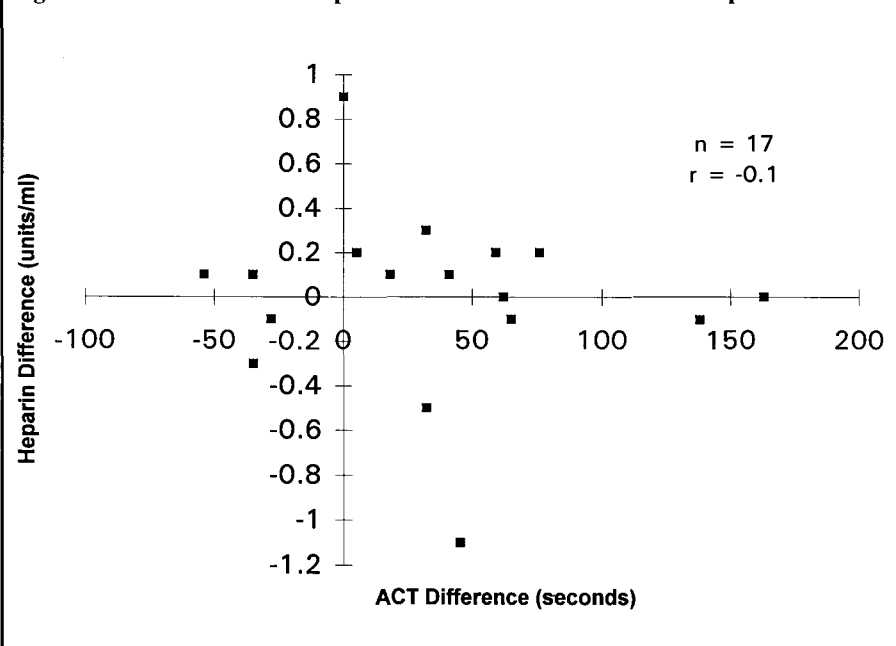


Figure 2: ACT difference vs. heparin difference at cold and warm temperatures



seconds at a venous blood temperature of $28.4 \pm 1^\circ\text{C}$ after cooling. The mean of the differences between ACTs measured at the cold and warm temperature was 34 ± 59 seconds ($p < 0.01$).

The mean ACTs and mean heparin concentrations at the warm and cold temperatures are displayed in Figure 1.

A scatter plot of the changes in ACT versus the changes in heparin concentration measured at the warm temperature minus the cold temperature is displayed in Figure 2 ($r = -0.1$).

^k SAS Institute, Inc., Cary, NC 27511

DISCUSSION

Few studies have investigated the effect of hypothermia on heparin anticoagulation (5, 6, 11, 12). Despotis et al reported a weak inverse correlation ($r = -0.2$) between temperature and kaolin or celite automated ACT determination (11). Cohen et al, using protamine titration to determine heparin concentration and the Hattersley's ACT technique found that ACT was prolonged and heparin concentration essentially unchanged with cooling during CPB (6). Unlike Cohen et al, the present study used a heparin anticoagulant activity factor Xa inhibition assay. This assay measures actual heparin activity and not just its concentration, as in the heparin protamine titration method. Paul et al reported that profound hypothermia (20°C) for 60 minutes in dogs could induce a constant release of a heparin-like factor which reacts as a specific inhibitor of factor Xa (12).

The heparin concentration remained unchanged in our study. Several possible explanations exist. First, the cooling time lasting only 3-11 minutes was not long enough to release a significant amount of heparin-like substance. Second, the cooling temperature, 33°C to 28°C, did not induce enough changes in the body to cause a significant release of heparin-like substance seen in Paul's study. Third, heparin-like substance was released at a similar rate as heparin metabolism.

Regardless of etiology, heparin or heparin-like substance anticoagulation concentration did not change with cooling. However, we found that the ACT became prolonged with cooling. The scatter plot (Figure 2) shows that the changes in ACTs and the changes in heparin concentration with cooling are independent of each other ($r = -0.1$). Judging by the prolonged ACTs and unchanged heparin concentration, it seems reasonable to conclude that the prolongation of the ACT is not due to a change in heparin anticoagulation concentration.

Because the heparin concentration remained unchanged during the short cooling period, the prolongation of ACTs can only be related to the cooling process itself. Hypothermia may induce some changes in platelet morphology (17, 18), function (19), and sequestration (20) that would slow down the blood clotting. Furthermore, the decreased temperature may alter the kinetics of enzymes that may also delay or slow down the initiation and propagation of the platelet plug and fibrin clot (21).

Because the ACT was prolonged while the heparin anticoagulation concentration remained unchanged while cooling, clinicians should be cautious when using the ACT as an indicator of heparin concentration during the CPB.

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