Monitoring Heparin Anticoagulation with a Modified Whole Blood Thrombin Time

Adrienne Turner, Steven Thompson and Frank LaDuca
Research Division, International Technidyne Corp., Edison, N.J., and the Division of Cardiac Surgery, The Johns Hopkins Hospital, Baltimore, Md.

Keywords: thrombin time, heparin, anticoagulation, activated clotting time, dose-response

Introduction
Heparin anticoagulation is typically monitored during cardiopulmonary bypass (CPB) with the Activated Clotting Time (ACT) (1, 2, 3). The conventional plasma thrombin time (TT) which may be used to monitor low therapeutic heparin anticoagulation (4), is also sensitive to decreases of fibrinogen (5) and accumulation of Fibrinogen degradation products (6). The conventional plasma TT can not be used to monitor heparinization during CPB because of the high levels of heparin employed. The whole blood high dose thrombin time (HiTT) assay described reflects functional heparin levels and actual anticoagulation status, unlike protamine titration assays, which measure all heparin fractions. The test is useful as a heparin specific monitor in CPB patients. The test is unaffected by the protease inhibitor aprotinin (Trasylol), which has been given experimentally to patients to preserve platelet function and reduce post-operative blood loss. (7, 8).

The HiTT monitoring protocol is a Dose Response Curve, similar to that used for heparin monitoring with the ACT (9). For each patient the HiTT value is correlated to the patient's functional heparin concentration following the infusion of the bolus dose. The patient is then maintained within a HiTT target range, corresponding to a therapeutic heparin level for cardiac bypass. The HiTT value will change during the course of bypass surgery reflecting the consumption of functional heparin.

Methods

Assays:
The HiTT test tube contains a lyophilized preparation of human thrombin, snake venom (B. atrox) and protamine sulfate (Sigma Chemical Co., St. Louis, MO) in a stabilizing buffer contained in a Hemochron CA510 test tube. The ACT tubes consist of diatomaceous earth activator (Hemochron CA510). To assay, the HiTT tube is rehydrated with 0.5 ml of purified water at least 5 minutes prior to use and 1.5 ml of fresh blood is added to the test tube. Two ml of fresh blood are added directly to the ACT tubes. All tests are run on the Hemochron portable blood coagulation instrument.

In vitro studies: Blood was obtained from normal donors, free from medication by venipuncture. Blood was aliquoted and beef lung heparin (Upjohn Co., Kalamazoo, MI) was added to tubes in increasing amounts corresponding to 0, 2, 3, 4 units/ml heparin. From each aliquot HiTT and ACT assays were performed. In some samples, aprotinin (Sigma Chemical Co., St. Louis, MO) was added to determine the effect upon the HiTT assay. To determine the effect of the HiTT assay with different sources of heparin, Beef1 (Upjohn Co.), Beef2 (Organon Inc., W. Orange, NJ) and Porcine intestinal (Eli Lilly, Indianapolis, IN) were added to aliquots of fresh blood in amounts corresponding to 0, 2, 3, 4 units/ml.

Clinical studies: Studies were performed with cardiac surgery patients (CABG and valve replacement) receiving beef lung heparin (Organon). In a pilot study of 7 patients (3 male and 4 female) data was collected to evaluate therapeutic HiTT response corresponding to standard anticoagulation therapy. Therapy was not altered as a result of the HiTT assay. Beef lung heparin was infused empirically (300 units/kg) in a bolus amount. HiTT assays were performed in conjunction with ACT assays 5 minutes following the bolus heparin infusion and approximately 10 minutes after the beginning of bypass and approximately every 30 minutes while on bypass. The average length on bypass was 1.5 hours.

Correlation of HiTT, ACT and heparin concentration were determined using Lotus 1, 2, 3 linear regression. Heparin concentration in CPB patients was determined by dividing the post-bolus heparin dose by the blood volume derived from body weight and height (13).

Results

In vitro studies:
Correlation of HiTT to heparin concentration: The mean baseline HiTT value was 30 +/- 8 seconds in a normal population. As the heparin concentration increased the HiTT increased proportionally, showing a dose response...
sensitivity unique for each donor (Fig. 1). The heparin/HiTT sensitivity was not altered in the presence of aprotinin (Fig. 2).

HiTT response variability due to different heparin source: In a single normal donor population the HiTT/heparin dose-response is unique for each heparin source (Beef lung or porcine intestinal) (Fig. 3). For each heparin the HiTT remains linear and increases proportionally to the heparin concentration. Each donor displays a good correlation \( r = 0.90 \) and a unique dose response for the type of heparin applied.

**Clinical Studies:**

Correlation of the HiTT to ACT: In CPB patients the HiTT correlates well with the ACT. The dose response is unique for each individual. Some individuals demonstrate relative anticoagulant resistance while others demonstrate relative sensitivity (Fig. 4A-C). Consequently when the data is pooled a poor correlation of HiTT to ACT is observed \( r = 0.50 \).

Correlation of HiTT and ACT to heparin concentration: The heparin specificity of the HiTT and ACT were compared using the patients post-heparin bolus assay values. The mean post-bolus heparin concentration was 4.7 units/ml with a range of 4.0-5.7 units/ml. In pooled data the HiTT correlates well with the heparin concentration \( r = 0.87 \) (Fig. 5A) while the ACT does not correlate with the heparin concentration (Fig. 5B).

Clinical Application: The anticoagulant response of the CPB patients is summarized in Table 1. Based on these studies an acceptable target HiTT range of 107-144 seconds corresponds to a mean post bolus heparin concentration of 4.7 units/ml based on the infusion of an empirical heparin dose of 300 units/kg body weight. To monitor the CPB patient, the HiTT is used in a similar manner to Bull’s ACT methodology (9). During the bypass procedure the HiTT range (70-120 seconds) reflects the consumption of heparin. Additional heparin may be given intraoperatively to maintain the desired therapeutic range.

**Discussion**

Heparin sensitive assay:

A modified high dose thrombin time assay is described as an alternate means for monitoring heparin anticoagula-
The HiTT test system prevents interference from minor changes in fibrinogen, therefore making it much more sensitive to the true heparin level of the patient.

In laboratory studies the HiTT was unaffected by aprotinin. In patients receiving aprotinin the ACT becomes artificially elevated presumably through the interaction of the blood coagulation activator in the test tube with the aprotinin (12). Grossly elevated ACT values may not indicate actual patient heparin anticoagulation. The HiTT may prove to be an alternative method for monitoring heparin anticoagulation in these patients.

Application:
The clinical application of the HiTT is similar to monitoring heparin anticoagulation with the ACT (9) namely the use of dose-response method. Similar to ACT heparin dosing, the HiTT protocol suggests that for proper heparin anticoagulation the patient should be maintained within a certain therapeutic heparin level. In this study the therapeutic post-bolus heparin range was 4.0-5.7 heparin units/ml heparin with a mean post-bolus heparin concentration of 4.7 units/ml, which is consistent with our previous studies of 700 cardiac patients (10). The HiTT target range of the post-bolus, pre-bypass specimen evaluated (107-144 seconds) corresponded to a heparin level of 4.0-5.7 units. Lower HiTT values were observed while on bypass (70-120 seconds) while the comparative ACT values also declined. While the HiTT is sensitive to the degree of heparin anticoagulation induced by the complex of the patients ATIII cofactor with heparin, the ACT is sensitive to the level of heparin anticoagulation, as well as hypocoagulation induced by factor consumption and hemodilution. A high Grossly elevated ACT values may not reflect actual heparin anticoagulation while the HiTT assay is a true indicator of functional heparin anticoagulation status.

Individualized patient dose response curves provide a fingerprint of heparin anticoagulation following bolus infusion and while on bypass. A decline in the HiTT value indicates that additional heparin may be needed, especially if the heparin level falls below the therapeutic range. Since the desired HiTT target range is specific for the type (beef or porcine) of heparin infused (Fig. 3) it is important to identify acceptable target ranges in each institution through comparative HiTT/ACT studies.

Further studies are needed to identify HiTT response in patients previously identified as heparin resistant by ACT response characteristics. The HiTT affords the opportunity to clearly identify heparin anticoagulation in these patients.

Acknowledgements
The authors acknowledge the excellent technical support of the cardiac surgery, anaesthesia and perfusion teams who cooperated with this study.
References

11. Esposito RA, Colvin SB, Lackner H.: The role of the

Summary of clinical data:

<table>
<thead>
<tr>
<th>Infusion Dose</th>
<th>Post Bolus, Pre-Bypass specimen:</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean</td>
</tr>
<tr>
<td>Heparin Level (units/ml)</td>
<td>4.7</td>
</tr>
<tr>
<td>HiTT Response</td>
<td>125</td>
</tr>
<tr>
<td>ACT Response</td>
<td>601</td>
</tr>
</tbody>
</table>

During Bypass:

| HiTT Response | 95 | 70-120 |
| ACT Response | 555 | 473-674 |

Table 1. The anticoagulant response of CPB patients is summarized to determine an acceptable HiTT target range. Based on a standard infusion (300 units/kg) of beef lung heparin the post-bolus, pre-bypass HiTT range is 107-144 seconds, which corresponds to a therapeutic post-bolus heparin range of 4.0-5.7 units/ml. While on bypass the HiTT (70-120 seconds) will drop reflecting the consumption of heparin.