Establishing an institution-specific therapeutic range for heparin

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Abstract: The relationship between heparin concentration and activated partial thromboplastin time (aPTT) in pooled plasma was compared with that in patient samples to assess the feasibility of using heparin-spiked pooled plasma to determine a therapeutic range for aPTT. Blood samples were taken from 32 patients who had been receiving intravenous unfractionated heparin sodium for more than 24 hours. The samples were stored at 70 °C until anti-Xa assay within three months of collection. Pooled normal plasma was spiked with unfractionated heparin sodium to produce nominal anti-Xa concentrations of 0, 0.05, 0.1, 0.2, and 0.5 unit/mL. Heparin concentrations and aPTT values were measured, and the relationship between the two was determined by linear regression. For the ex vivo samples, the range of aPTT values corresponding to therapeutic heparin concentrations of 0.3–0.7 units/mL was 64–106 seconds, which corresponds to an aPTT range of 2.3–3.9 times the mean of the normal range (compared with the traditionally defined therapeutic range of 1.5–2.5 times the control value). For the in vitro samples, the aPTT range corresponding to heparin concentrations of 0.3–0.7 units/mL was 121–256 seconds, which corresponds to an aPTT range of 4.4–9.4 times the mean of the normal range.

Each institution should establish a therapeutic aPTT range by calibrating aPTT values against heparin concentrations from blood samples of patients receiving intravenous heparin.

Index terms: Angina; Anticoagulants; Blood levels; Heparin sodium; Methodology; Myocardial infarction; Protocols; Tests, laboratory


Heparin is an anticoagulant administered intravenously for the treatment of various thromboembolic diseases, such as deep-vein thrombosis, pulmonary embolism, unstable angina, and acute myocardial infarction. Many methods for monitoring therapy with unfractionated intravenous heparin have been investigated; however, measuring the activated partial thromboplastin time (aPTT) is currently the most commonly used method. The aPTT is a measure of the facilitation of antithrombin III's inhibition of thrombin, factor Xa, and factor IXa by heparin. Several clinical trials have demonstrated a relationship between subtherapeutic levels of heparin anticoagulation and recurrent thrombosis measured by either aPTT values or heparin concentrations. To reduce the risk of recurrent thrombosis and possible hemorrhage with heparin treatment, it is clearly important to monitor anticoagulation with aPTT values or heparin concentrations and make appropriate adjustments.

The therapeutic range for aPTT values has traditionally been defined as 1.5–2.5 times the control value. Because of differences in sensitivities to heparin among commercial brands of aPTT reagents and among heparin lots, any fixed aPTT range is unreliable in defining the therapeutic range for heparin. For example, the range of aPTT values corresponding to 1.5–2.5 times the control value may lead to very different levels of anticoagulation, depending on the reagents used. For many of the commercial aPTT reagents, a therapeutic level of anticoagulation is not achieved when the aPTT value is equal to 1.5 times the control value. The differences in aPTT reagent sensitivity are greatest among different manufacturer brands; however, there are significant differences even among different lots from the same manufacturer. This problem is analogous to using prothrombin times (PTs) or PT ranges for warfarin monitoring, a problem at least partially corrected by using the International Normalized Ratio (INR). Attempts to standardize aPTT values, however, have failed because of the wide variation among reagents. Manufacturers of aPTT reagents do not seem to be close to standardizing these reagents as has been done with PT reagents.

Heparin concentrations in the range of 0.3–0.7 units/mL measured by anti-Xa assays correlate well with clinical efficacy and are associated with less variability.
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in patients with thromboembolic events compared with aPTT levels. Heparin concentrations of 0.3–0.7 unit/mL measured by anti-Xa assays are approximately equivalent to heparin concentrations of 0.2–0.4 unit/mL measured by protamine titration. Heparin concentrations may also be more predictive of an effective level of anticoagulation compared with aPTT values. Although using heparin concentrations as a replacement for aPTT values may seem warranted, not all hospital laboratories are equipped to measure and report heparin concentrations on a clinical basis. The routine monitoring of heparin therapy with anti-Xa assays would also result in a substantial cost increase. An alternative to using heparin concentrations routinely to monitor treatment is to establish a therapeutic aPTT range by correlating aPTT values with heparin concentrations.

Given the problems associated with using a fixed aPTT range (1.5–2.5 times the control value or mean of the normal range) for determining the therapeutic range, it is now recommended that a therapeutic aPTT range be calibrated for each aPTT reagent at each institution. This therapeutic range for aPTT values should correlate with heparin concentrations of 0.3–0.7 unit/mL measured by an anti-Xa assay (0.2–0.4 unit/mL if a protamine titration assay is used). In view of the established benefits of achieving and maintaining adequate levels of anticoagulation, the purpose of this study was to show how the therapeutic range for aPTT values was established at our institution by correlating aPTT values with heparin concentrations. We also wanted to determine the feasibility of establishing the therapeutic range by using in vitro pooled normal plasma samples spiked with heparin compared with using samples from patients receiving intravenous heparin (ex vivo samples). If it could be demonstrated that the therapeutic aPTT range determined from in vitro plasma samples was similar to the range determined from ex vivo samples, it would be less costly and more practical to use only in vitro spiked plasma samples.

Methods

Our methods were adapted from a protocol recommended by Brill-Edwards et al. for establishing therapeutic aPTT ranges for heparin. The protocol was approved by our institution’s investigational review board, and informed consent was obtained from each patient before blood samples were taken.

Ex vivo samples. Thirty-two patients admitted to the coronary intensive care unit with unstable angina or acute myocardial infarction over a one-month period were evaluated. Only patients who gave informed consent and who had been receiving intravenous unfractioned heparin sodium (McGaw, Irvine, CA) for more than 24 hours were included in the study. Patients who had received thrombolytic therapy or warfarin, patients with a known coagulation disorder, and patients with known liver disease were excluded. One venous blood sample was obtained from each patient. Samples were drawn at least 6 hours after a bolus heparin dose or any dosage change in the heparin infusion, 12 hours after cardiac catheterization, 24 hours after angioplasty, or after two consecutive “therapeutic” values were achieved. The venous blood samples were drawn into tubes containing 3.8% sodium citrate and then centrifuged to obtain platelet-poor plasma. The samples were placed on ice and centrifuged within one hour of phlebotomy. Heparin concentrations (measured by an anti-Xa assay) and aPTT values were measured in duplicate on each plasma sample. The mean of the duplicate measurements was used in the analysis. The aPTT assays were performed immediately after the plasma was obtained. The plasma samples were then stored at –70 °C until the anti-Xa assays were performed, which was within three months of sample collection.

In vitro samples. Pooled normal plasma (Cryochek, Precision Biologicals, Dartmouth, Nova Scotia, Canada) was spiked with unfractionated heparin sodium (Elkins-Sinn, Inc., Cherry Hill, NJ) to produce nominal anti-Xa concentrations of 0, 0.05, 0.10, 0.20, and 0.50 unit/mL (samples spiked with heparin to produce anti-Xa levels of >0.5 unit/mL yielded aPTTs greater than 200 seconds). Heparin concentrations (measured by an anti-Xa assay) and aPTT values were measured in triplicate on each of the in vitro plasma samples. The mean of the three measurements was used in the analysis.

Laboratory assays. All aPTT values were measured with aPTT reagents (Platelin-L, Organon Teknika, Durham, NC) by using an MDA-180 coagulation-monitoring device (Organon Teknika). The mean of the normal range for this aPTT reagent was 27.2 seconds. Heparin concentrations were measured by using a chromogenic anti-Xa assay (Stachrom, American Bioproducts, Parsippany, NJ). All testing was performed according to the manufacturer’s recommendations.

Data analysis. For the ex vivo plasma samples, a heparin dose-response curve was developed by plotting aPTT values on the y-axis and heparin concentrations (anti-Xa units/mL) on the x-axis (Figure 1). The relationship between aPTT values and heparin concentrations (measured from the same sample) was analyzed by using a least-squares regression method. The 95% confidence interval (CI) was calculated for the slope and y-intercept values from the linear regression. The range of aPTT values corresponding to heparin concentrations of 0.3–0.7 anti-Xa unit/mL on the heparin dose-response curve was identified as the therapeutic range. The aPTT ratio was determined by dividing the lower and upper aPTT value in the therapeutic range by the mean of the laboratory control value (27.2 seconds). For the in vitro spiked plasma samples, the relationship between aPTT values and heparin concentrations (measured from the same sample) was also...
determined by linear regression. The actual measured heparin concentrations (anti-Xa unit/mL) were used in the analysis rather than the levels expected as a result of spiking the sample. A similar heparin dose-response curve was constructed for the in vitro spiked plasma samples and the 95% CI calculated for the slope and y-intercept values. The aPTT range corresponding to heparin concentrations of 0.3–0.7 anti-Xa unit/mL was identified and compared with the therapeutic range determined from the ex vivo samples. Confidence intervals were used to compare the slopes of the two regression lines. The level of significance was set at 0.05.

Results

Ex vivo samples. Thirty-two patients receiving unfractionated intravenous heparin met the inclusion criteria and had venous blood samples drawn for measurement of aPTT values and heparin concentrations. The heparin dose-response curve for the ex vivo samples (Figure 1) shows that the range of aPTT values corresponding to heparin concentrations of 0.3–0.7 anti-Xa unit/mL was 64–106 seconds, which is very different from the traditionally defined therapeutic range of 1.5–2.5 times the control value. At our institution, an aPTT value equal to 1.5 times the mean of the normal range would be 40.8 seconds (0.08 anti-Xa unit/mL), which would be considered subtherapeutic.

In vitro samples. Heparin concentrations and aPTT values were measured in pooled normal plasma spiked with heparin sodium to produce anti-Xa concentrations of 0, 0.06, 0.08, 0.22, and 0.47 unit/mL. Samples spiked with heparin to produce anti-Xa levels of >0.5 unit/mL had aPTT values that exceeded 200 seconds and were not included in the analysis. The heparin dose-response curve for the in vitro samples shows that the aPTT range corresponding to heparin concentrations of 0.3–0.7 unit/mL was 121–256 seconds, which corresponds to an aPTT range of 4.4–9.4 times the mean of the normal range.

Comparison of ex vivo and in vitro results. The heparin dose-response curves for the in vitro and ex vivo samples appear to be different. The 95% CI for the slope derived from the ex vivo samples (73.5–136.1) is significantly different from the 95% CI for the slope derived from the in vitro samples (240.0–399.8) because these intervals do not overlap ($p < 0.05$). This difference between the two regression lines indicates that the relationship between heparin concentrations and aPTT values is different depending on whether in vitro or ex vivo plasma samples are used. Because the slopes of the two regression lines are different, the aPTT ranges corresponding to 0.3–0.7 unit/mL are also different. It is thus clear that the in vitro samples do not accurately reflect the relationship between aPTT values and heparin concentrations in patients with thromboembolic disease being treated with intravenous heparin.

Discussion

The aPTT remains the most commonly used laboratory test for monitoring levels of anticoagulation with
unfractionated intravenous heparin therapy in patients being treated for thromboembolic disease. Because of differences in responsiveness to heparin associated with different aPTT reagents and reagent lots, a fixed aPTT range of 1.5–2.5 times the control value or times the mean of the normal range is not a reliable way to identify the therapeutic range for aPTT values. Because of these differences in the sensitivity of aPTT reagents, it is now recommended that each institution establish its own therapeutic range for aPTT values by calibrating aPTT values with plasma heparin concentrations. Heparin concentrations of 0.3–0.7 unit/mL measured by an anti-Xa assay (0.2–0.4 unit/mL if protamine titration is used) are associated with clinical efficacy.

The therapeutic range of 64–106 seconds used in our institution corresponds to an aPTT range of 2.3–3.9 times the mean of the normal range. A fixed aPTT range of 1.5–2.5 times the mean of the normal range in our hospital would give an aPTT range of 41–68 seconds, which is clinically very different from the range derived from the calibration with heparin concentrations (64–106 seconds). If a lower range were used, our patients treated with intravenous heparin would be inadequately anticoagulated and would be at significant risk for extension of thrombus or recurrent thrombosis.

Our results are similar to those of other investigators. Brill-Edwards et al. compared fixed aPTT ranges (1.5–2.5 times the control value) with therapeutic ranges determined from heparin dose-response curves from ex vivo samples. They evaluated five different reagents and found that a fixed range of 1.5–2.5 times the control value does not predict the therapeutic ranges calibrated from heparin concentrations. For all five reagents, an aPTT range of 1.5 times the control value was subtherapeutic. These authors concluded that it is inappropriate to use a fixed aPTT range for the therapeutic range and recommended establishing a new therapeutic range based on heparin concentrations for each new aPTT reagent.

Although it would be more practical and cost-effective to establish the therapeutic aPTT range from in vitro plasma samples spiked with heparin, we found this method to be unreliable. The therapeutic aPTT range determined from ex vivo samples was very different from the range derived from in vitro samples. Also, ex vivo rather than in vitro samples were used in the trials evaluating the relationship between clinical outcome and the maintenance of therapeutic levels of anticoagulation. There are many possible explanations for the different relationship between aPTT values and heparin concentrations when ex vivo samples were used compared with spiked in vitro plasma samples. The difference may result from elevated levels of procoagulant factors such as fibrinogen and factor VIII that occur in patients being treated for acute thromboembolic disease. Increases in fibrinogen and factor VIII may shorten the aPTT. Acutely ill patients also usually have elevated levels of a variety of acute-phase reactant proteins, some of which bind heparin, making the agent unavailable for its anticoagulant action. It is also possible that some of the differences between the in vitro and ex vivo results are due to the difference between the brand of unfractionated heparin sodium that was used for the patients (McGaw) and the brand used for the spiked plasma samples (Elkins-Sinn). However, other studies have demonstrated similar differences between ex vivo and in vitro results. It is clear from our results and from other studies that only ex vivo samples from patients being treated with intravenous heparin for thromboembolic disease should be used to determine the therapeutic range.

Because of the established benefits of achieving and maintaining a therapeutic level of anticoagulation, many institutions have developed heparin dosing nomograms to more quickly adjust the aPTT value into the therapeutic range. For some of these dosing protocols it is not stated whether the therapeutic aPTT range incorporated into the dosing nomogram is calibrated with heparin concentrations of 0.3–0.7 unit/mL (anti-Xa assay). In effect, the dosing nomogram may be adjusting heparin therapy into an aPTT range that is not therapeutic, depending on the heparin responsiveness of the aPTT reagents used.

At some point, low-molecular-weight heparins may replace unfractionated heparin, which would prevent any problems associated with laboratory monitoring. Although there are data to support the use of low-molecular-weight heparins for the treatment of some venous and arterial thromboembolic diseases, the cost of low-molecular-weight heparins in North America is 10–20 times the cost of unfractionated heparin. It is therefore difficult to justify the use of low-molecular-weight heparins in patients for whom the benefits of these agents are minimal compared with those of unfractionated heparin. In the outpatient setting, however, the use of low-molecular-weight heparins for the treatment of thromboembolic disease has the potential for cost savings through the avoidance of hospital charges. Unfractionated heparin will thus continue to be used for the treatment of thrombosis in the hospital setting for the foreseeable future, and aPTT standardization will remain a major problem.

Until manufacturers of aPTT reagents standardize these products with respect to heparin concentrations, it is important for each institution to establish a therapeutic range for aPTT values by using ex vivo samples. A new therapeutic range should be established whenever there is a change in aPTT reagents, reagent lot, or assay conditions (e.g., a new aPTT-measuring device). This therapeutic aPTT range may then be incorporated into a weight-based dosing nomogram for heparin. In our institution, the aPTT reagent lots are changed once a year. For each new reagent lot we repeat the process of standardizing aPTT with respect to heparin concentra-
tion to determine a therapeutic range for aPTT values. This aPTT therapeutic range is incorporated into our weight-based dosing nomogram for heparin, which is made available to health care professionals through our physician-order-entry system.

Conclusion

Each institution should establish a therapeutic aPTT range by calibrating aPTT values against heparin concentrations from blood samples of patients receiving intravenous heparin.

References