Plasma-Diluted Thrombin Time to Measure Dabigatran Concentrations During Dabigatran Etexilate Therapy

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Key Words: Dabigatran; Thrombin time; Thrombin inhibitor

Abstract

New anticoagulants, like the orally available direct thrombin inhibitor (DTI) dabigatran etexilate, have recently been introduced into the market for venous thromboembolic prophylaxis and for stroke prevention in atrial fibrillation. While dabigatran has been approved for use without the need for routine therapeutic monitoring, there are clinical scenarios in which monitoring can help guide clinical management. We report herein the application of a recently described plasma-diluted thrombin time (DTI assay) used to monitor intravenous DTI as a useful and easily implemented method to monitor oral DTIs.

Materials and Methods

All measurements were made on Diagnostica Stago analyzers (STA-R Evolution and STA-Compact, Parsippany, NJ). Measurements were made in triplicate using Diagnostica Stago STA Thrombin for the thrombin time and STA
PTT Automate for the activated partial thromboplastin time. Pooled normal plasma was purchased from Precision Biologic, Dartmouth, Canada. For the plasma-diluted thrombin time (DTI assay), 1 volume of citrated plasma was added to 3 volumes of pooled normal plasma. Next, 100 μL of this diluted plasma was added to 100 μL of STA thrombin reagent that contains approximately 1.5 NIH units of human thrombin per milliliter. The final thrombin concentration in the assay was approximately 0.75 NIH U/mL of human thrombin. The manufacturer reports the onboard instrument stability of STA thrombin after reconstitution to be 7 days. Controls run before and after each run were in control and similar in value.

Calibration material was obtained from Aniara (Mason, OH), Dabigatran Plasma Calibrator reference A222801, which consists of pooled normal plasma with known quantities of dabigatran measured using high-performance liquid chromatography.² By using this calibration material, we constructed a dose-response curve ranging from 0 to 500 ng/mL of dabigatran. An examination of the published literature of the pharmacology of dabigatran indicated that the expected peak steady-state concentration of dabigatran in patients with atrial fibrillation (150 mg, 2 times daily) was approximately 180 ng/mL with a trough of approximately 90 ng/mL (11.5 hours after last dose).³ Control plasma samples containing a usual concentration ± acceptance range of approximately 120 ± 50 and 300 ± 80 ng/mL (Aniara, Dabigatran Plasma Control Plasma reference A222701) was used for precision studies and run 9 or 10 times at each level.

Studies on human subjects were carried out according to the principles of the Declaration of Helsinki. The study was approved by the University of Washington (Seattle) Human Subjects Review Committee, under the Nonhuman Subjects Protocol.

Venous blood samples were anticoagulated by adding 2.7 mL of blood to 0.3 mL of 0.105 mol/L citrate. All samples were centrifuged immediately at 3,600 g for 2 minutes at room temperature, divided into aliquots, and frozen at –80°C until analyzed.

**Results**

The dose-response curve for the plasma-diluted thrombin time assay with dabigatran is linear with a correlation coefficient of $R^2 = 0.9808$ ($y = 0.3203x + 27.5$) [Figure 1]. With this equation, a dosing reference range was determined to be 56 to 85 seconds (rounded to the nearest second), which corresponds to plasma dabigatran levels of 90 to 180 ng/mL. For ease of use in our laboratory, a reference range encompassing peak and trough levels was chosen to be 70.2 to 195.1 ng/mL (50-90 seconds) (Figure 1). The mean ± SD within-run imprecision was 38.8 ± 0.8 seconds, with a coefficient of variation (CV) of 2.0% ($n = 10$; equivalent to 35 ng/mL); 75.6 ± 1.2 seconds and a CV of 1.6% ($n = 10$; equivalent to 150 ng/mL); and 131.8 ± 2.8 seconds with a CV of 2.1% ($n = 9$; equivalent to 326 ng/mL). The controls were determined to be within the acceptable range as specified by the manufacturer.² Back-calculation of concentrations for calibration standards with the linear regression equation demonstrated an average of 2.4% difference between the assigned and determined values.

A patient known to be undergoing chronic dabigatran therapy of 150 mg/mL twice daily was admitted to the medical facility for head bleeding after a traumatic accident. Owing to the nature of the hemorrhage and his ongoing DTI therapy, the clinical team requested a stat DTI assay for the patient. The result was determined to be 74 seconds or 145 ng/mL, which is well in the reference range at our medical center (50-90 seconds). The patient was reported to have taken his last dose approximately 8 to 12 hours before the sample was drawn.

**Discussion**

As previously demonstrated, the plasma-diluted thrombin time assay is useful for monitoring intravenous DTIs such as bivalirudin and argatroban.¹ The dose-response curves presented herein compare favorably with those previously published for argatroban and bivalirudin. Other methods proposed to measure dabigatran levels are the thrombin time (TT), ecarin clotting time, and TT by Hemoclot thrombin inhibitor
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assay (Aniara). These assays have linearity coefficients that range from 0.8568 to 0.9164 in multiple dose trials. Of the other methods, only the TT is commonly available in the United States for clinical use. At this time, studies addressing the correlation of dabigatran concentration with risk of bleeding (overdose) or breakthrough thrombosis (underdosing) are unavailable. The lack of such studies currently limits the predictive power of the DTI assay to determine hemorrhage/thrombosis risk; however, adoption of this assay would be a valuable tool to aid in carrying out such studies. We anticipate that with time, the market penetration of dabigatran as a replacement for warfarin will increase, as will the need for convenient and timely monitoring of DTI dosing. While no known antidote is available for the DTI class of medications, other interventions to reverse DTI-induced anticoagulation such as dialysis can be attempted and the amount of drug removed can be measured easily with the DTI assay. With access to appropriate pharmacodynamic and pharmacokinetic data and relevant calibration material, the DTI assay can easily be applied for use in the monitoring of orally available DTIs such as dabigatran etexilate.

References


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