Evaluation of the Activated Partial Thromboplastin Time (aPTT) Sensitivity to Unfractionated Heparin Using Three Commercial Reagents: Implication for Therapeutic Range

Nisarat Opartkiattikul, M.D., Panutsaya Tientadakul, M.D., Wanida Wongtiraporn, M.D.

Department of Clinical Pathology, Faculty of Medicine Siriraj Hospital, Mahidol University, Bangkok 10700, Thailand.

ABSTRACT

At present, we are using the proposed therapeutic range for monitoring unfractionated heparin therapy which is the aPTT ratio of 1.5-2.5. However, the aPTT value is influenced by reagents and methods of detection. The College of American Pathologists and the American College of Chest Physicians recommended that site-specific validation of heparin therapeutic range should be established. The aim of this study was to determine the appropriate therapeutic range of unfractionated heparin therapy of our aPTT system by ex vivo study. For comparison, two other commercial reagents were also determined to observe the differences. Blood samples were drawn from 21 healthy blood donors who were not taking any medication and from other 24 patients suffering from either arterial or venous thrombosis, receiving continuous intravenous infusion of unfractionated heparin without concomitant oral anticoagulant therapy. Correlation coefficients between aPTT ratios and plasma heparin concentration varied between 0.722 (Actin FSL) to 0.817 (Actin FS). Calculated therapeutic ranges of aPTT ratios corresponding to the heparin level of 0.29 - 0.47 U/ml were 1.8 - 2.5, 1.9 - 2.5 and 2.7 - 4.6 for Actin FS, Actin FSL and Pathromtin SL, respectively. Therefore, the appropriate therapeutic range of our system obtained from this study might be aPTT ratio between 1.8 and 2.5 which is very closed to the ratio that we are using now.

Keywords: Unfractionated heparin; Therapeutic range; Activated partial thromboplastin time (aPTT)

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Heparin accelerates the inactivation of coagulation factor Xa and thrombin by antithrombin. It is widely used as an effective drug for prevention and treatment of thromboembolic condition. Adequate heparin treatment significantly decreases morbidity and mortality from acute thrombotic disease.[1] Unfortunately, heparin also causes hemorrhagic complications from over-anticoagulantion. Since each patient responses to unfractionated heparin differently, it needs monitoring and activated partial thromboplastin time (aPTT) is the common test used for this purpose. However, the aPTT value is influenced by reagents and methods of detection.[2]

The College of American Pathologists and the American College of Chest Physicians recommended that site-specific validation of heparin therapeutic range should be established.[3-4] This should be done by determining the aPTT that correlate with heparin concentration of 0.3 to 0.7 IU/ml by anti-Xa assay or 0.2-0.4 U/ml by protamine titration. However, this level of protamine assay was also reported to be equivalent to 0.29-0.47 IU/ml by chromogenic anti-Xa[2]. The samples should be obtained from patients who were receiving heparin for the treatment of thromboembolism *(ex vivo)*. The heparin concentration-derived therapeutic range has never been established in the Clinical Pathology Laboratory, Siriraj Hospital.

At the Siriraj Hospital, there are about forty to fifty patients per day who are receiving heparin therapy and need laboratory monitoring. At present, we are using the proposed therapeutic range for monitoring unfractionated heparin therapy which is the aPTT ratio of 1.5-2.5.[5-6] The aim of this study was to determine the appropriate therapeutic range of our aPTT system by *ex vivo* study as mentioned above. For comparison, two other commercial reagents were also determined to observe the differences.

MATERIALS AND METHODS

Population study

Blood was drawn from 21 healthy blood donors who were not taking any medication and from 24 patients suffering either from arterial or venous thrombosis, receiving continuous intravenous infusion of unfractionated heparin (Heparin Leo^{R}, LEO Pharmaceutical Products, Ballerup, Denmark) without concomitant oral anticoagulant therapy.

Correspondence to: Nisarat Opartkiattikul
E-mail: sinop@mahidol.ac.th

TABLE 1. Results of mean values of aPTT carried out using three different reagents in 21 healthy subjects

Reagent	Mean value (seconds) obtained from this study	Mean value (seconds) provided by the manufacturers
Actin FS	27.6 ± 2.0	30.5 ± 2.9
Actin FSL	32.8 ± 2.3	28.5 ± 2.1
Pathromtin SL	39.6 ± 3.0	32.8 ± 3.1

TABLE 2. Results of aPTT and aPTT ratio carried out using three different reagents in 24 patients' samples

Reagent	aPTT (seconds)	aPTT ratio
Actin FS	49.2 ± 20.7	1.8 ± 0.7
Actin FSL	61.8 ± 25.8	1.9 ± 0.8
Pathromtin SL	109.9 ± 82.5	2.8 ± 2.2

aPTT assays

Venous blood samples were collected into siliconised vacuum tubes (Vacutainer®, Beckton Dickinson, Switzerland), containing final concentration of 0.13 mol/l trisodium citrate (nine part of blood to one part of anticoagulant). Plasma was obtained after 1,500 g centrifugation for 15 minutes and stored in capped plastic tubes at -20°C until measurements which were performed within seven days after blood collection. APTT and heparin concentration were measured in the same patient' plasma.

APTT measurements were performed on an automated coagulometer (CA 500, Sysmex Co, Kobe, Japan), using three commercial reagents: Actin FS (soy phosphatides and ellagic acid, Dade Behring, Marburg GmbH, Germany) which was the reagent being used in our laboratory, Actin FSL (soy and rabbit brain phosphatides and ellagic acid, Dade Behring, Marburg GmbH, Germany) and Pathromtin SL (silica, Dade Behring, Marburg GmbH, Germany). The assays were performed according to the instructions provided by the manufacturers. Mean values were calculated for each reagent by measuring the aPTT of the 21 healthy blood donors.

Heparin measurement

Plasma concentration of heparin was measured by using an anti-factor Xa assay (Berichrom® Heparin, Dade Behring, Marburg GmbH, Germany) on a CA 500. In brief, after addition of dextran sulfate and antithrombin, the plasma sample was incubated with factor Xa. After 1 minute incubation at 37°C, chromogenic substrate was added, the solution was mixed and the absorbance was read in kinetic mode at 405 nm against plasma blank. The standard curve was performed by using He-parin Leo.

Statistical analysis

The statistical analysis was performed by using SPSS 10.0 program. Differences of aPTT assays performed by the three reagents of the same patient's sample were analyzed by paired t-test. A significant difference was determined at $p < 0.01$. The correlation between aPTT ratio and heparin concentrations measured in the same patient's sample was evaluated by linear regression and Pearson's correlation coefficient. Therapeutic ranges (as the ordinate on Y axis) that corresponded to heparin level of 0.3-0.7U/ml and 0.29 - 0.47 U/ml by anti-Xa assay were calculated by using regression equations.

RESULTS

As shown in Table 1, mean values for the healthy subjects of three different aPTT reagents ranged from 27.6 seconds for Actin FS to 39.6 seconds for Pathromtin SL. Each of the values was also different from those provided by the manufacturers. The mean values of each reagent were used to calculate for aPTT ratio of patients' samples.

Statistical analysis of aPTT values in subjects undergoing heparin therapy revealed by a significant difference among the three commercial reagents ($p < 0.01$) (Table 2). When we converted the aPTT values of the patients to aPTT ratio by dividing the patient's value with the mean value of the corresponding reagent, the difference was not significant between Actin FS and Actin FSL ($p = 0.195$), but it still showed significant difference between the results obtained from Actin FS and Pathromtin SL ($p = 0.001$).

Correlation coefficients (r) between aPTT ratios and plasma heparin concentration varied between 0.722 and 0.817. Fig 1 demonstrates correlation between aPTT ratios measured by the three different reagents and relative heparin concentrations with correlation coefficients (r). Ranges of aPTT ratios corresponding to the therapeutic range of heparin of 0.29 - 0.47 U/ml and 0.3 - 0.7 U/ml using the anti-factor Xa assay are shown in Table 3.

DISCUSSION

At present, monitoring of unfractionated heparin therapy by using anti-Xa assay for determining heparin concentration in plasma is not practical because it is very expensive. Almost all laboratories prefer to use aPTT, a global coagulation test that reflects the ability of the heparin-antithrombin complex to inactivate several coagulation factors.[3] The appropriate therapeutic range for each aPTT system is a critical factor for the safety of the patients receiving unfractionated heparin infusion. To determine the appropriate therapeutic range of our laboratory, the ex vivo study in frozen patients' plasma was conducted. It was found that the linear regression between heparin concentration in plasmas and aPTT ratios obtained from Actin FS, the reagent being used in our laboratory, had revealed the best correlation coefficients (0.817).

The heparin concentrations determined by anti-Xa assay that was equivalent to 0.2 - 0.4 μ/ml by protamine titration were 0.3 - 0.7 μ/ml and 0.29 - 0.47 U/ml. Its reason seemed to be the difference related to anti-Xa assay.[2-3] The heparin anti-Xa levels depend on many factors, including assay technique (chromogenic or clot-based), the use of exogenous anti-thrombin, instrument, the use of a standard heparin preparation and type of commercial reagent.[7] In this study, we calculated the appropriate therapeutic range by using both recommended

TABLE 3. Comparison of calculated therapeutic ranges of aPTT ratios of three reagents corresponding to a given plasma heparin concentration between 0.29 - 0.47 U/ml and 0.3- 0.7 U/ml as measured by anti-factor Xa. Linear regression and correlation coefficients (r) were given

Reagents	Linear regression	r	p	Calculated therapeutic ranges corresponding to heparin conc. 0.29-0.47 U/ml	0.3-0.7 U/ml
Actin FS	Y = 3.70 X + 0.72	0.817	< 0.001	1.8 - 2.5	1.8 - 3.3
Actin FSL	Y = 3.44 X + 0.89	0.722	< 0.001	1.9 - 2.5	1.9 - 4.2
Pathromtin SL	Y = 10.42 X - 0.33	0.790	< 0.001	2.7 - 4.6	2.8 - 7.0

TABLE 4. Comparison of calculated therapeutic ranges of aPTT reagents with the other two reports

	Actin FS	*r*	Actin FSL	*r*	Pathromtin SL	*r*
Heparin conc. 0.27 - 0.49 U/ml	1.8 - 2.5	0.82	1.9 - 2.5	0.72	2.7 - 4.6	0.79
Heparin conc. 0.3 - 0.7 U/ml	1.8 - 3.3	0.82	1.9 - 4.2	0.72	2.8 - 7.0	0.79
Manzato, et al	2.6 - 5.9	0.78	-		2.1 - 4.7	0.75
van den Besselaar, et al	1.5 - 1.7	0.66	2.1 - 2.6		1.7 - 1.8	0.42

heparin concentration. The calculated therapeutic ranges expressed as aPTT ratio of the three reagents were remarkably different from the reports of van den Besselaar et al. and Manzato et al. as shown in Table 4.[8-9] Our results confirmed that calculated therapeutic ranges, even with the same reagent, cannot be extrapolated to other centers. The explanation for the differences were the influence of instrumentation used, different blood collection systems and citrate concentrations.[10-13]

The optimal sample size that should be used to standardize an aPTT therapeutic range has not been clearly defined and a small sample size may not give the same results as a large one.[14-15] Our sample size was 24. Since it was tested by Kolmogorov-Smirnov test and found to be normal distribution, pair-t test was used for analyzing the data.

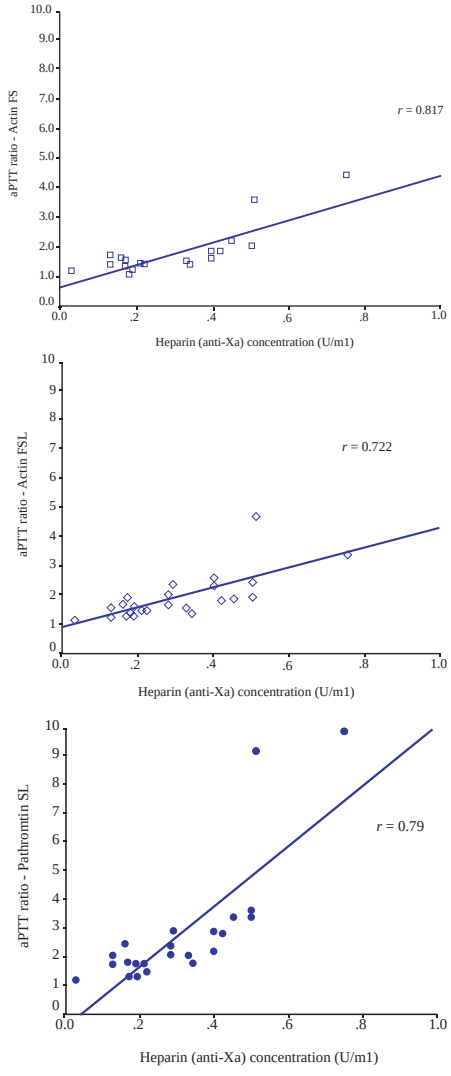

Fig 1. Correlation between anti-Xa determination of plasma heparin concentration and aPTT ratio in patients receiving parenteral heparin therapy using three reagents

In a single aPTT reagent, each lot had different responsiveness to heparin. Although the slope of the regression line was small, the intercepts were significantly different.[15] While the variability in heparin responsiveness among different lots of the same aPTT reagent was well documented,[16] its impact could depend on the type of reagent used.[17] The necessity to reevaluate the therapeutic range for each lot of heparin required further evaluation.[18]

Most laboratories, including us, prefer to use the proposed therapeutic range of aPTT ratios 1.5 to 2.5 in clinical practice. The results obtained from our study and those of other studies showed that this range could not be applicable for every reagent. When we calculated the therapeutic range by using heparin concentration of 0.29 - 0.47 U/ml, the appropriate therapeutic range of aPTT ratios for Actin FS and Actin FSL was closed to the proposed range, i.e., 1.8-2.5 and 1.9 - 2.5, respectively. But the ratios differed significantly from that of Pathromtin SL. The variability might be due to the combined effects of the different sensitivities of the reagents employed either to heparin or to the concentrations of some clotting factors, in particular of factor VIII.

Therefore, the appropriate therapeutic range of our system obtained from this study might be aPTT ratio between 1.8 and 2.5 which is very close to the ratio that we are now using. This range would be implemented for our patients who are receiving unfractionated heparin therapy at the Siriraj Hospital. However, the clinical relevant of this range should be studied in the future.

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บทคัดย่อ

การประเมินความไวของการทดสอบ activated partial thromboplastin time (aPTT) ต่อการติดตามขนาดของยาเฮปารินของน้ำยา 3 ยี่ห้อ เพื่อหาเกณฑ์การรักษาที่เหมาะสมสำหรับผู้ป่วย

นิศารัตน์ โอภาสเกียรติกุล พ.บ., พนัสยา เธียรธาดากุล พ.บ., วนิดา วงศ์ถิรพร พ.บ.

ภาควิชาพยาธิวิทยาคลินิก, คณะแพทยศาสตร์ศิริราชพยาบาล, มหาวิทยาลัยมหิดล, กทม 10700, ประเทศไทย.

เกณฑ์การรักษาเพื่อติดตามการให้ยาเฮปารินที่นิยมใช้กันโดยทั่วไปรวมทั้งในโรงพยาบาลศิริราชด้วยคือ ค่า aPTT ratio 1.5 ถึง 2.5 วิทยาลัยพยาธิแพทย์อเมริกันและวิทยาลัยแพทย์โรคทรวงอกแห่งประเทศอเมริกาได้แนะนำให้หาเกณฑ์การรักษาที่เหมาะสมสำหรับแต่ละห้องปฏิบัติการเอง เพราะค่าของการตรวจ aPTT ในแต่ละแห่งมีความแตกต่างกันแม้จะตรวจในพลาสมาเดียวกัน เนื่องจากน้ำยาที่ใช้และเครื่องมือที่ตรวจแตกต่างกัน คณะผู้วิจัยมีความประสงค์จะหาเกณฑ์การรักษาที่เหมาะสมสำหรับห้องปฏิบัติการพยาธิวิทยาคลินิก โรงพยาบาลศิริราช โดยใช้น้ำยา Actin FS และตรวจด้วยเครื่องอัตโนมัติ CA 500 นอกจากนี้ยังทำการศึกษาเปรียบเทียบกับน้ำยาอีก 2 ชนิด คือ Actin FSL และ Pathromtin SL ด้วย การศึกษาทำโดยนำเลือดของผู้บริจาคโลหิต 21 คน มาหาค่าเฉลี่ยของ aPTT ในคนปกติของน้ำยาแต่ละชนิด เพื่อใช้เป็นตัวหารในการคำนวณค่า aPTT ratio ของผู้ป่วย และนำเลือดของผู้ป่วยหลอดเลือดอุดตันที่กำลังได้รับยาเฮปารินทางหลอดเลือด 24 ราย มาหาค่าความเข้มข้นของเฮปารินด้วยการหาระดับ anti-Xa ด้วยวิธี chromogenic assay และหาค่า aPTT ด้วยน้ำยาทั้ง 3 ชนิด นำค่าที่ได้มาคำนวณหาความสัมพันธ์และสมการ พบว่า ค่าเกณฑ์การรักษาที่เหมาะสมของน้ำยา Actin FS คือ 1.8 ถึง 2.5 ซึ่งใกล้เคียงกับค่าที่ใช้อยู่ในปัจจุบัน ส่วนค่าเกณฑ์การรักษาที่เหมาะสมของน้ำยา Actin FSL คือ 1.9 ถึง 2.5 และของน้ำยา Pathromtin SL คือ 2.7 ถึง 4.6