Measurement of the Pharmacodynamic Effect of Dabigatran: Thrombin Clotting Time

J. Stangier, K. Wetzel, W. Wienen, K. Rathgen, J. van Ryn

Depts. Pharmacokinetics&Drug Metabolism, Pulmonary Research, Discovery Support, Clinical Research
Boehringer Ingelheim Pharma GmbH & Co KG, Biberach, Germany

Clinical Biomarker Team

Boehringer Ingelheim

INTRODUCTION

Dabigatran etexilate (Pradaxa®) is an oral direct thrombin inhibitor for the prophylaxis of thromboembolism in patients undergoing total knee or hip replacement approved in more than 40 countries worldwide including Europe and Canada.

Dabigatran etexilate is under advanced clinical investigation for the prevention of stroke in patients with atrial fibrillation.

Although clinical monitoring of dabigatran is not required, a standardized and validated laboratory method to measure the pharmacodynamic effect of dabigatran would be useful in e.g. emergency situations.

OBJECTIVE

The objective of this assay validation study was to determine whether the Hemoclot® Thrombin Inhibitor Assay [ACK002K, HYPHEN BioMed, France] is suitable for the assessment of blood coagulation time in patients receiving anticoagulant therapy with dabigatran etexilate.

METHODS

The Hemoclot® Thrombin Inhibitor Assay is a chronometric, standardized and calibrated commercially available coagulation assay proposed for the quantitative determination of hirudin and other thrombin inhibitors in citrated plasma. The assay is easy to perform and requires a standard ball coagulometer.

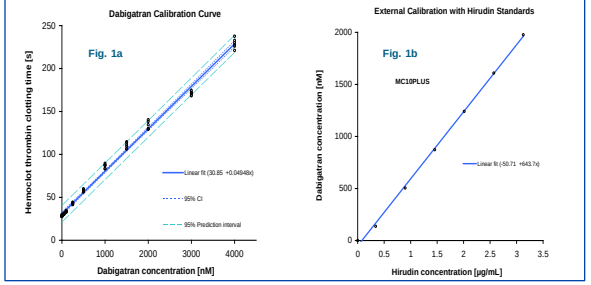
Hemoclot assay calibration curve linearity, inter- and intra-assay precision, and assay accuracy were investigated with dabigatran. Plasma sample stability and the feasibility of lyophilized dabigatran standards for assay calibration were assessed. Lyophilized standards were reconstituted with a pre-defined volume of aqua dest. to obtain calibration samples at concentrations covering the working range of the assay. Stabilities of native samples and lyophilized dabigatran standards were determined. A method comparison study investigated the Hemoclot® dabigatran assay performance in two independent laboratories.

Linear regression analysis was used to establish calibration curves for dabigatran and to obtain regression coefficients for back-calculation of dabigatran concentrations using the coagulation time of dabigatran plasma samples [Fig. 4a]. Coagulation time data are presented in box-and-whisker plots displaying the median, the 1st and 3rd quartiles, and minimum /maximum data. Total assay precision/within device/laboratory), repeatability (within run), between runs (if multiple runs were analyzed) and between day precision were determined. Validation data were evaluated with the analytical method evaluation software 'Analyse-it for Excel', Version 2.09, Analyse-it Software Ltd., Leeds LS277WZ, U.K.

RESULTS

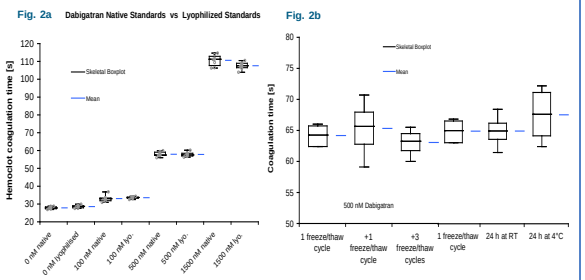
- A linear calibration curve was obtained with dabigatran concentrations of 0-4000 nM (1885 ng/mL). The slope of the calibration line indicated adequate sensitivity of the assay.[Fig.1a].
- Hemoclot® Thrombin Time displayed a close linear correlation of dabigatran and hirudin concentrations providing the possibility of external calibration with hirudin standards.

Hemoclot® Dabigatran Calibration Line and External Calibration with Hirudin



- Dabigatran citrated plasma samples for Hemoclot thrombin time determination were stable for at least 24 hrs at ambient temperature and for 4 freeze-thaw cycles [Fig.2a].
- Lyophilized dabigatran calibrators displayed comparable clotting times with freshly prepared standards and were stable for at least 4 hrs after reconstitution in water [Fig.2b].

Dabigatran Plasma Sample and Calibrator Stability

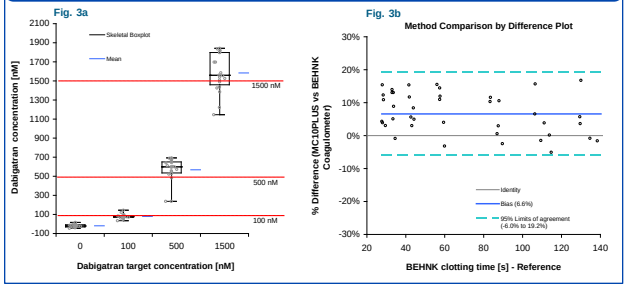


- Duplicate analysis of validation samples in two runs per day over 5 days demonstrated adequate precision of the Hemoclot® dabigatran assay [Tab.1].

Hemoclot® Dabigatran Assay Precision

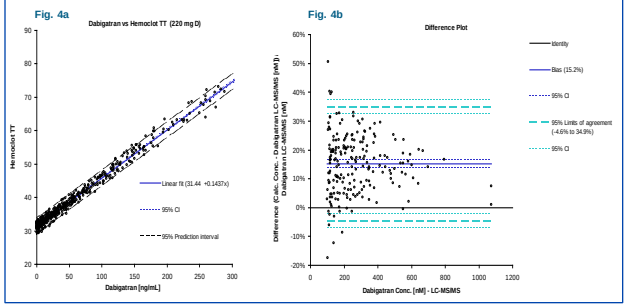
Dabigatran Concentration [nM]	Total precision % CV	Within run % CV	Between run % CV
Blank	2.9	2.1	2.0
100	4.7	2.6	4.0
500	12.0	3.1	10.0
1500	10.2	1.2	5.7

Hemoclot® Dabigatran Assay Accuracy and Robustness



- The bias of quality control samples was – 20.7% at 100 nM, 13.7% at 500 nM and 5.6% at 1500 nM dabigatran [Fig.3a]. The mean bias between two laboratories was 6.6.% [Fig.3b].
- The mean bias between back-calculated dabigatran concentration using the clotting time in a Phase I clinical study in subjects receiving 220 mg dabigatran etexilate and actual dabigatran concentrations determined by LC-MS/MS was 15.2%. [Fig.4b].

Hemoclot® Dabigatran Method Comparison with LC-MS/MS



CONCLUSION

The HYPHEN BioMed thrombin time coagulation assay is suitable for the quantitative assessment of dabigatran concentrations in human citrated plasma.

The Hemoclot Dabigatran assay covers a concentration range of up to 4000 nM dabigatran which is far beyond the maximum concentration expected in patients receiving dabigatran etexilate.

External calibration of the Hemoclot assay with hirudin for the determination of dabigatran concentrations expressed as 'hirudin equivalents' is possible. Direct calibration of the assay with stable, lyophilised dabigatran calibrators is also feasible.

Disclosures: All authors are employees of Boehringer Ingelheim

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