Thrombosis Research 135 (2015) 532–536

Contents lists available at ScienceDirect

Thrombosis Research

journal homepage: www.elsevier.com/locate/thromres

Regular Article

Measurement of dabigatran plasma concentrations by calibrated thrombin clotting time in comparison to LC-MS/MS in human volunteers on dialysis

Michael Schmohl, Dietmar Gansser, Viktoria Moschetti, Joachim Stangier *

Boehringer Ingelheim Pharma GmbH & Co. KG, Biberach, Germany

ARTICLE INFO

Article history:
Received 19 September 2014
Received in revised form 5 December 2014
Accepted 23 December 2014
Available online 2 January 2015

Keywords:
Method comparison
Dabigatran etexilate
Diluted thrombin time
HEMOCLOT® Thrombin Inhibitors assay
LC-MS/MS
end-stage renal disease

ABSTRACT

Introduction: Dabigatran etexilate is an oral direct thrombin inhibitor. Although routine anticoagulation monitoring with dabigatran is not usually required, a simple and precise laboratory test to measure dabigatran concentrations in patient plasma may be useful in certain clinical circumstances, such as emergency situations. The HEMOCLOT® Thrombin Inhibitors assay has demonstrated accurate and precise determination of dabigatran concentrations within a range of 50–500 ng/ml. The objective of this study was to assess comparability of dabigatran concentrations determined by HEMOCLOT® and by liquid chromatography/tandem mass spectrometry (LC-MS/MS) in plasma samples from human volunteers with end-stage renal disease (ESRD) undergoing regular haemodialysis (HD) during a Phase I study.
Materials and Methods: Overall, 304 plasma samples were obtained from seven ESRD patients in dabigatran steady-state for measurement by HEMOCLOT® (calibrated diluted thrombin time [dTT]) and by LC-MS/MS. Agreement of dabigatran concentrations was assessed by regression analysis and difference plots.
Results: The measurements of calibration standards of the HEMOCLOT® assay showed excellent precision with coefficients of variation <5%. Accuracy determined by analysis of two quality control samples was 90% and 111%. HEMOCLOT®-derived dabigatran plasma concentrations paralleled those obtained by LC-MS/MS. The mean ratio of the LC-MS/MS and dTT-derived concentrations was 0.955 (67% limits of agreement: 0.771–1.18).
Conclusions: The HEMOCLOT® Thrombin Inhibitors assay is suitable for measuring dabigatran plasma concentrations in volunteers with ESRD undergoing haemodialysis. The agreement between dabigatran concentrations determined by the HEMOCLOT® assay and the LC-MS/MS reference method met bioanalytical acceptance criteria.
© 2015 Elsevier Ltd. All rights reserved.

Introduction

Dabigatran etexilate (PRADAXA®, Boehringer Ingelheim, Ingelheim, Germany) is the oral prodrug of dabigatran, a direct thrombin inhibitor (DTI). It is approved for prevention of stroke and systemic embolism in patients with nonvalvular atrial fibrillation; for the treatment of DVT and PE in patients who have been treated with a parenteral (injectable) anticoagulant for 5–10 days, and to reduce the risk of recurrent DVT and PE in patients who have previously received anticoagulant therapy; and (outside of the United States) for the prevention of venous thromboembolism following total knee or hip replacement surgery. Because of its predictable pharmacokinetic (PK) and pharmacodynamic (PD) profile, dabigatran etexilate allows fixed dosing without the need for routine coagulation monitoring and dose adjustments [1–3]. Although routine assessment of dabigatran anticoagulant activity is not required in clinical practice, a standardised, accurate and precise laboratory test to measure dabigatran in patients' plasma would be useful in certain clinical circumstances, such as emergency situations [2]. Various assays have been described that can be used to assess activity of anticoagulant drugs; however, not all assays are appropriate in the context of dabigatran treatment [2].

Activated partial thromboplastin time (aPTT) is a clotting assay targeting the intrinsic and common coagulation pathway. Although aPTT might be useful when alternative tests are not available, its utility is limited by a curvilinear dabigatran concentration — response curve, flattening at concentrations ≥200 ng/ml dabigatran, and a lack of precision and standardisation [2,4]. Ecarin clotting time (ECT) utilises ecarin, a snake venom converting prothrombin into catalytically active meizothrombin, which can in turn be inhibited by dabigatran in a concentration-dependent manner. Although there

Abbreviations: aPTT, activated partial thromboplastin time; CI, confidence interval; CV, coefficient of variation; DTI, direct thrombin inhibitor; dTT, diluted thrombin time; ECT, ecarin clotting time; EDTA, ethylenediaminetetraacetic acid; ESRD, end-stage renal disease; HD, haemodialysis; INR, international normalised ratio; LC-MS/MS, liquid chromatography/tandem mass spectrometry; PD, pharmacodynamic(s); PK, pharmacokinetic(s); PT, prothrombin time; QC, quality control; TT, thrombin time; VKA, vitamin K antagonist.

* Corresponding author at: Drug Metabolism and Pharmacokinetics, Boehringer Ingelheim Pharma GmbH & Co. KG, 88397 Biberach an der Riss, Germany. Tel.: +49 7351 54 8443; fax: +49 7351 83 8443.
E-mail address: joachim.stangier@boehringer-ingelheim.com (J. Stangier).

http://dx.doi.org/10.1016/j.thromres.2014.12.021
0049-3848/© 2015 Elsevier Ltd. All rights reserved.

is a linear correlation between ECT and dabigatran plasma concentration, and the assay shows good sensitivity, its limited availability, the lack of standardisation and the lot-to-lot variability of reagents restrict its routine use in clinical practice [5,6].

Thrombin time (TT) also provides a direct measure of thrombin inhibition in a plasma sample and exhibits a linear dose–response relationship to dabigatran concentration. However, at plasma levels ≥600 ng/ml the time to clot formation frequently exceeds the measuring range of coagulometers, implying that sensitivity is too high for use in emergency situations. This, together with a lack of standardised reagents, also limits the utility of this test [2]. The prothrombin time (PT)/international normalised ratio (INR) test is not useful due to limited sensitivity and false-positive elevations of INR, and should not be performed for dabigatran.

The HEMOCLOT® Thrombin Inhibitors assay has been developed for measuring dabigatran plasma concentrations and is available for clinical testing in the EU and in other countries where the CE mark is sufficient. It has previously been shown to allow the precise and accurate determination of dabigatran concentrations within a range of 50–500 ng/ml [1]. In this study, we assessed the comparability of dabigatran concentrations determined by the HEMOCLOT® test method with those determined by validated LC-MS/MS (reference method) in patient plasma obtained from volunteers with ESRD who were participating in an open-label, single-centre phase I study with two fixed multiple dosing periods, as published elsewhere [7]. According to Khadzhynov et al., PK, PD and safety of multiple doses of dabigatran were assessed before, during and after 4-hour haemodialysis (HD) sessions on Days 1, 3 and 5 of each period. Dabigatran 150 mg was administered immediately after HD on Day 1, followed by 110 mg on Day 2 and 75 mg on Day 3 8 hours after HD. This trial showed that 48.8 and 59.3 % of dabigatran could be removed by HD with 200 and 400 ml/min targeted blood flow rate, respectively. Measurement of HEMOCLOT® diluted thrombin time (dTT) demonstrated a linear PK/PD relationship.

Materials and Methods

The presented data were collected in the context of an open-label, single-centre, multiple-dose phase I study to investigate the elimination, PK, PD, safety and tolerability of dabigatran (Pradaxa®, Boehringer Ingelheim, Ingelheim, Germany) in ESRD patients undergoing intermittent haemodialysis [7].

Study Population

304 plasma samples were obtained from seven ESRD patients aged 21–60 years without atrial fibrillation and with a body mass index of 18 to 30 kg/m^2, in steady-state conditions of dabigatran. Ethics committee approval and patients' written, informed consent were obtained. Patients were in a clinically stable condition for at least 4 weeks prior to study entry. Patients went sequentially through two dosing and elimination periods, each period consisting of dosing on days 1 to 3 and three 4-hour HD sessions on Days 1, 3 and 5. The two treatment periods were separated by a washout period of at least 6 weeks.

Sample Collection and Analysis

Twenty-six venous blood samples were collected at baseline (0 h, Day 1) and through 95 hour post dose until Day 5. For the PK analysis of free and total dabigatran (glucuronide acid conjugates of dabigatran included) by validated LC-MS/MS, blood samples were drawn into EDTA-containing collection tubes and placed on ice until centrifugation (3000 × g, 10 min at 4 °C) for plasma preparation. Samples were stored at −70 °C until analysis. Analysis was performed by validated LC-MS/MS methods at Nuvisan GmbH (Neu-Ulm, Germany). Samples for PD analysis were collected into sodium citrate tubes and placed on ice until centrifugation (2500 × g, 10 min at 4 °C). Plasma samples were stored at −20 °C until further analysis at Menal GmbH (Emmendingen, Germany). PK and PD samples were collected in parallel, at the same time points, for PK/PD correlation and methods comparison.

Diluted thrombin time was determined using the HEMOCLOT® Thrombin Inhibitors assay (Hyphen Biomed, France) according to the manufacturers' instructions. Pre-diluted test plasma (1:8) was mixed with normal pooled human plasma provided in the assay kit. Clotting was initiated by adding a constant amount of highly purified thrombin in the alpha form. Coagulation times were determined using a 10-channel coagulometer (MC10 plus). For calibration, dabigatran standards (Hyphen Biomed, France) were analysed in duplicate with each sample batch. Actual dabigatran concentration of plasma calibrators and controls provided with the kit was assessed by a validated LC-MS/MS method. Quality control (QC) samples (Hyphen Biomed, France) were measured in duplicate before, in the middle of and after each batch (n = 6 per batch).

Data Analysis

Analyse-it for Excel V2.21 (Analyse-it Software Ltd, UK) was used for data evaluation. Diluted thrombin clotting times were converted into concentration values (ng/ml) by means of linear least-squares regression analysis of the four-point calibration series measured with each batch. Using the intercept and slope of the calibration line, dabigatran concentrations were calculated as intercept + slope × coagulation time.

Total, within-day (repeatability) and between-day imprecision were assessed by analysis of calibration standards and QCs and expressed as coefficient of variation (CV). Accuracy of the HEMOCLOT® assay was determined by analysis of the QC samples and subsequent comparison of dabigatran concentrations to the target concentrations determined by LC-MS/MS.

Agreement of dabigatran plasma concentrations as determined by the dTT test method and the reference method LC-MS/MS was assessed by least-squares regression analysis and modified Bland–Altman difference plots. The mean reference/test ratio and 67% limits of agreement were calculated. The assessment of precision in a methods' comparability study followed the concept presented by Rocci et al. [9] for incurred sample analysis, i.e. the re-analysis of a given sample with the same analytical method. According to Rocci et al., the 67% limits of agreement of the ratio of sample results should be determined. These limits are interpreted as the range within which the ratio of sample results is expected to fall two thirds of the time. If the difference between any 2 repeat samples should be within 20% of each other, then these limits of agreement should be within 0.83 to 1.2. A range of 20% was considered acceptable for the comparison of two different analytical methods for the determination of dabigatran plasma levels. Of note, the concentration of free dabigatran plus its glucuronide acid conjugates (representing the pharmacologically active dabigatran) as determined by LC-MS/MS were used for comparison with the HEMOCLOT® assay [8].

Results

HEMOCLOT® Assay Precision and Accuracy

Total, within-day and between-day imprecision of the HEMOCLOT® assay was assessed by analysis of calibration standards (n = 28) and QC samples (n = 84) on 14 separate days with one run per day (Table 1). Coefficients of variation were below 5% for total, within-day (repeatability) and between-day imprecision of the calibration standards. Accuracy as determined by the analysis of QC samples at 122 and 297 ng/ml dabigatran was 90% and 111%, respectively. Total imprecision of QC samples (CV) was 7.0% at 297 ng/ml and 12.5% at 122 ng/ml.

Download English Version:

https://daneshyari.com/en/article/6001882

Download Persian Version:

https://daneshyari.com/article/6001882

Daneshyari.com