Correspondence

Stability of direct oral anticoagulants in whole blood and plasma from patients in steady state treatment

1. Introduction

Direct oral anticoagulants (DOACs) include the direct thrombin inhibitor dabigatran etexilate (hereafter termed dabigatran) and the direct factor X inhibitors rivaroxaban and apixaban [1]. Regular laboratory monitoring is not considered necessary [1,2], but measuring the anticoagulant activity is important in acute situations such as trauma, suspicion of intoxication, or prior to acute surgery or other invasive procedures [2,3].

The aim of this study was to investigate the influence of storage time and temperature on the concentration of DOACs as determined by functional haemostasis assays in samples from patients in steady state treatment.

2. Materials and methods

2.1. Patients

We included 60 patients in steady state DOAC treatment from March 2015 to December 2015, i.e. 20 dabigatran-treated patients, 20 rivaroxaban-treated patients and 20 apixaban-treated patients. Blood samples were obtained at Department of Cardiology, Aarhus University Hospital, Denmark. Laboratory analyses were performed at Department of Clinical Biochemistry, Aarhus University Hospital, Denmark. Inclusion criteria were ≥18 years and at least 4 days of dabigatran, rivaroxaban or apixaban treatment. After informed consent was obtained, citrated blood was collected by standard venipuncture using tubes containing 3.2% sodium citrate (Terumo, Leuven, Belgium). The study was approved by the Danish Data Protection Agency (1-16-02-137-15) and carried out in accordance with the Declaration of Helsinki.

2.2. Assays

Dabigatran activity was determined by a diluted thrombin time assay (Hemoclot®, Hyphen BioMed, Neuville-sur-Oise, France), named plasma-dabigatran. Rivaroxaban and apixaban activity was determined by specifically calibrated anti-factor Xa activity assays (Biophen Heparin (LRT)®, Hyphen BioMed, Neuville-sur-Oise,

France), named plasma-rivaroxaban and plasma-apixaban. All assays were performed using Sysmex CS2100i (Sysmex Europe GmbH, Norderstedt, Germany). The maximum between-run coefficient of variation (CV) of the assays at our laboratory was 10%. Results outside the range of quantification were excluded.

2.3. Study design

The design described below applied to each DOAC and the corresponding assay; plasma-dabigatran, plasma-rivaroxaban or plasma-apixaban. Acceptable stability was defined as 80–120% of the baseline result.

In order to investigate DOAC stability in citrated whole blood at room temperature (20–25 °C), blood samples were obtained from 15 patients treated with dabigatran ($n = 5$), rivaroxaban ($n = 5$) or apixaban ($n = 5$). Samples were left untouched for 30 min (baseline), 1 and 2 h at 20-25 °C prior to centrifugation (3163 G for 25 min at 20 °C) and analysis.

In order to investigate DOAC stability in citrated plasma after storage at room temperature (20–25 °C), refrigerated (5 °C (2–8 °C)) and frozen (-20 °C), blood samples were obtained from 30 patients treated with dabigatran ($n = 10$), rivaroxaban ($n = 10$) or apixaban ($n = 10$). Samples were left untouched at 20–25 °C for 1 h and then centrifuged. Plasma from each patient was pooled and divided into nine tubes. Tube 1 was analysed immediately and served as the baseline measurement. Tubes 2–5 were left untouched at 20–25 °C for 2 h, 4 h, 6 h or 8 h prior to analysis. Tubes 6-8 were left at 5 °C for 8 h, 24 h or 48 h prior to analysis. Tube 9 was stored at -20 °C for 1 month, thawed for 5 min in a 37 °C water bath, left for 10 min at 20–25 °C and analysed.

In order to investigate the freeze-thaw stability of DOACs in citrated plasma, blood samples were obtained from 15 patients treated with dabigatran ($n = 5$), rivaroxaban ($n = 5$) or apixaban ($n = 5$). Samples were left untouched at 20–25 °C for 1 h prior to centrifugation. Plasma from each patient was pooled and divided into four tubes. Tube 1 was analysed immediately and served as the baseline measurement. Tubes 2–4 were frozen and stored at -20 °C. At day 3–5, tubes 2–4 were thawed as described above. Tube 2 was analysed and tubes 3 and 4 were immediately refrozen. At day 8, the plasma in tube 3 and 4 was thawed as described above, and tube 3 was analysed. Tube 4 was immediately refrozen. At day 10-12, plasma in tube 4 was thawed as previously described and analysed.

2.4. Statistical analysis

As the data was not normally distributed, non-parametric statistical analysis was used. Descriptive analysis is presented as median and 95% confidence intervals (CI). Data analysis was performed using GraphPad Prism® (version 7.01, GraphPad software Inc., CA, USA).

Abbreviations: C, Celsius; DOAC, Direct oral anticoagulant;

Table 1

Stability of direct oral anticoagulants in citrated whole blood and citrated plasma obtained from patients in steady state treatment.

	Dabigatran	Rivaroxaban	Apixaban
Whole blood			
Stored at 20–25 °C	2 h	2 h	2 h
Plasma			
Stored at 20–25 °C	2 h	8 h	8 h
Stored at 5 °C	Not stable	48 h	48 h
Stored at −20 °C	30 days	30 days	30 days
3 freeze-thaw cycles	Not stable	Stable	Not stable

C: Celsius.

3. Results

The concentrations of DOACs in the blood samples was widely distributed within the analytical capabilities of the assays (data shown in supplementary material Figs. 1-3).

Table 1 provides an overview of the stability results, which are further commented below.

3.1. Stability of DOACs in whole blood

The 95% CI for dabigatran, rivaroxaban and apixaban samples remained within the predefined range of acceptance for stability at 80–120% of the baseline value when whole blood was stored at 20–25 °C for up to 2 h (Fig. 1A).

3.2. Stability of DOACs in plasma

The 95% CI for dabigatran samples was within the predefined acceptance range for up to 2 h at 20-25 °C (Fig. 1B). The 95% CI for rivaroxaban and apixaban samples remained within the range of acceptance for up to 8 h at 20-25 °C and up to 48 h at 5 °C (Fig. 1B). At −20 °C the 95% CI for all DOACs was within the acceptance range (Fig. 1B).

3.3. DOAC freeze-thaw stability

As shown in Fig. 1C, the lower 95% CI limits for dabigatran and apixaban samples were outside the range of acceptance after the first cycle, whereas the 95% CI for rivaroxaban samples remained within the range of acceptance through three freeze-thaw cycles.

The freeze-thaw stability of rivaroxaban was supported by the results from plasma samples kept at −20 °C for 1 month (Fig. 1B and C). The results for dabigatran and apixaban samples stored for 1 month at −20 °C suggested acceptable stability (Fig. 1B). The freeze-thaw stability results on dabigatran and apixaban did not remain within the acceptable range (Fig. 1C).

4. Discussion

This is the first study to report the stability of DOACs both in citrated whole blood and in citrated plasma using functional haemostasis assays on blood samples obtained from patients in steady state DOAC treatment.

Schmitz et al. [4] developed and validated a liquid chromatography tandem mass spectrometry (LC-MSMS) method for measuring DOACs and included stability testing of the plasma used for the assay. They used spiked (dabigatran, rivaroxaban or apixaban) citrated plasma obtained from healthy non-medicated individuals and showed that plasma was stable after 24 h at room temperature, three freeze-thaw cycles and 72 days at −80 °C. Their stability acceptance criterion was recovery of 80–120% of the baseline value. The present results support a comparable stability of rivaroxaban samples but an inferior stability of dabigatran and apixaban samples. Importantly, the stability results

obtained in the present study are representative of clinical practice, because we used blood samples obtained from patients in steady state DOAC treatment.

LC-MSMS is generally accepted as the gold standard for determination of the DOAC concentration in patients [5,6], but this method is not widely available for analysis 24 h a day. In contrast, the functional DOAC haemostasis assays are commercially available and far less laborious. The results obtained by functional DOAC tests are strongly correlated to the LC-MSMS results [5,6].

The determination of limits for acceptable stability must be balanced between the analytical capability of the assay and the clinical relevant difference. Regarding routine coagulation assays, Kemkes-Metthes [7] concluded that sporadic changes from baseline should be considered clinically relevant if they exceeded 15% in >10% of the samples. No such distinction has been made regarding DOAC assays due to limited knowledge of the relationship between DOAC plasma concentration and DOAC effect [8,9]. Considering the inter-individual variation of DOAC concentration [8,9] and the between-run CV below 10% in DOAC assays at our laboratory, we decided that the acceptable stability should be 80-120% of the baseline value. Acceptable stability of 80–120% was also used by Schmitz et al. [4]. However, considering the wide 'therapeutic' range of DOACs, even a 20% deviation from baseline may not be clinically relevant.

The main strength of this study was the use of whole blood and plasma from DOAC-treated patients instead of in vitro spiked samples. Additionally, we used commercially available functional haemostasis assays for measurement of DOAC activity. The present results can therefore be extrapolated to the laboratory practice of today. The limitations were the relatively limited number of patients in parts of the study and the different methodology used; the clotting assay for determining dabigatran activity and the chromogenic assays for determining rivaroxaban and apixaban activity.

5. Conclusion

Whole blood from patients treated with dabigatran, rivaroxaban or apixaban showed no differences in stability, whereas significant differences were observed regarding stability in plasma. Clinicians and laboratories should take this into consideration and adjust guidelines regarding sample collection, analysis and storage accordingly.

Supplementary data to this article can be found online at doi:10.1016/j.thromres.2016.10.023.