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Regular Article

Rivaroxaban: Quantification by anti-FXa assay and influence on coagulation tests A study in 9 Swiss laboratories ☆

L.M. Asmis [a,*], L. Alberio [b], A. Angelillo-Scherrer [c], W. Korte [d], A. Mendez [e], G. Reber [f], B. Seifert [g], H. Stricker [h], D.A. Tsakiris [i], W.A. Wuillemin [j]

[a] Division of Hematology, University Hospital and University of Zurich, Zurich, Switzerland
[b] Department of Hematology and Central Hematology Laboratory, Inselspital University Hospital and University of Berne, Berne, Switzerland
[c] Service and Central Laboratory of Hematology, Centre Hospitalier Universitaire Vaudois and University of Lausanne, 1011 Lausanne, Switzerland
[d] Institute for Clinical Chemistry and Hematology, Cantonal Hospital St Gallen, St Gallen, Switzerland
[e] Center for Laboratory Medicine, Cantonal Hospital Aarau, Aarau, Switzerland
[f] Laboratory for Special Hemostasis, University Hospital of Geneva, Geneva, Switzerland
[g] Division of Biostatistics, Institute of Social and Preventive Medicine, University of Zurich, Switzerland
[h] Division of Surgery, Regional Hospital La Carita, Locarno, Switzerland
[i] Diagnostic Hematology, University Hospital of Basel, Basel, Switzerland
[j] Division of Hematology and Central Laboratory of Hematology, Cantonal Hospital Lucerne, University of Berne, Switzerland

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ABSTRACT

Introduction: Rivaroxaban (RXA) is licensed for prophylaxis of venous thromboembolism after major orthopaedic surgery of the lower limbs. Currently, no test to quantify RXA in plasma has been validated in an inter-laboratory setting.
Our study had three aims: to assess i) the feasibility of RXA quantification with a commercial anti-FXa assay, ii) its accuracy and precision in an inter-laboratory setting, and iii) the influence of 10 mg of RXA on routine coagulation tests.
Methods: The same chromogenic anti-FXa assay (Hyphen BioMed) was used in all participating laboratories. RXA calibrators and sets of blinded probes (aim ii.) were prepared *in vitro* by spiking normal plasma. The precise RXA content was assessed by high-pressure liquid chromatography-tandem mass spectrometry. For ex-vivo studies (aim iii), plasma samples from 20 healthy volunteers taken before and 2 – 3 hours after ingestion of 10 mg of RXA were analyzed by participating laboratories.
Results: RXA can be assayed chromogenically. Among the participating laboratories, the mean accuracy and the mean coefficient of variation for precision of RXA quantification were 7.0% and 8.8%, respectively. Mean RXA concentration was 114 ± 43 μg/L .RXA significantly altered prothrombin time, activated partial thromboplastin time, factor analysis for intrinsic and extrinsic factors. Determinations of thrombin time, fibrinogen, FXIII and D-Dimer levels were not affected.
Conclusions: RXA plasma levels can be quantified accurately and precisely by a chromogenic anti-FXa assay on different coagulometers in different laboratories. Ingestion of 10 mg RXA results in significant alterations of both PT- and aPTT-based coagulation assays.

Introduction

Rivaroxaban (RXA) is an oral, direct, specific inhibitor of factor Xa [1]. Its chemical structure and small size (435.9 g/Mol) enable it to fit into the active site pocket of FXa, thereby directly blocking FXa-mediated serine protease activity. No co-factor protein is necessary for this inhibition to occur. Due to its chemical properties RXA binds only to FXa, it does not interact with thrombin or other serine proteases involved in coagulation. The 10 mg per os application once daily is licensed in more than 100 countries worldwide for the prevention of venous thromboembolism (VTE) in patients after orthopaedic surgery (elective hip and knee surgery).

Therapeutic drug monitoring (TDM) can be defined as the individualization of drug dosage by maintaining drug concentrations within a predefined prophylactic or therapeutic range [2]. RXA dose not require monitoring since it has a predictable pharmacokinetic which is proportional to the pharmacodynamic effect [3,4]. Single dosing with no monitoring was successfully used in all the phase III studies performed so far [5–8]. However, there are situations

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* Corresponding author at: Unilabs Zurich, Dufourstr 90, 8034 Zurich, Switzerland. Tel.: +41 58 864 5858; fax: +41 58 864 5859.
E-mail address: lars.asmis@unilabs.com (L.M. Asmis).

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such as emergency surgery or planned invasive procedures in patients taking RXA, control of therapy adherence, putative RXA intoxication or treatment failure, as well as bleeding events in patients taking RXA, when treating physicians may want to know the RXA plasma level. For these purposes a widely available, validated test for RXA quantification is desirable. High-performance liquid chromatography-tandem mass spectrometry (HPLC-MS/MS) is a validated method to quantify RXA [9]. However, HPLC-MS/MS is not available at most medical laboratories.

Samama et al. have published data and a recent review describing the problems associated with RXA quantification using global coagulation tests such as the activated partial thromboplastin time (aPTT) and prothrombin time (PT) according to Quick [10,11]. Barret et al. have shown that chromogenic anti-factor Xa (FXa) assays are superior to prothrombin time based assays for the measurement of direct FXa inhibitors including RXA [12]. However, no commercial laboratory assay has been validated for the determination of plasma levels of RXA in an inter-laboratory setting thus far.

We hypothesize that a commercial chromogenic anti-FXa assay employing RXA calibrators would produce reproducible and precise RXA measurements in a real-life clinical setting. We tested this hypothesis among the 9 member laboratories of the RIVAMOS group (see Acknowledgements). Aims of this study were defined as follows: i) to assess the feasibility of a commercial chromogenic anti-FXa assay for RXA quantification, ii) to assess the accuracy and precision of this assay on the different coagulometers of the RIVAMOS group utilizing human plasma spiked with RXA *in vitro* as calibrators, and, finally iii), to assess the influence of 10 mg of RXA on routine coagulation tests in the different laboratories using plasma samples from 20 healthy male volunteers taken 2–3 hours after RXA ingestion.

Methods

Preparation of calibrators and blinded samples

Stock solutions of RXA were prepared in 100% DMSO. Calibrators (CAL) and blinded samples were obtained by spiking human plasma with RXA stock solution (final DMSO concentration was ≤2% v/v). RXA concentrations in CAL, blinded samples (aim ii) and samples from the ex-vivo studies (aim iii) were assessed at Dr Rohde's lab at Bayer HealthCare by HPLC-MS/MS [9]. CAL were labelled with the concentrations as determined by HPLC-MS/MS. Blinded samples were labelled with letters A-H. Both CAL and blinded samples were shipped at −20° to participating laboratories.

Analytics

The chromogenic anti-FXa assay used, Biophen Heparin 6 (Hyphen BioMed, Neuilly-sur Oise, France; reference 221006), is CE labelled for the measurement of heparins. This is a one stage assay that utilizes endogenous antithrombin. It is an automated kinetic method during which a constant amount of exogenously added bovine FXa is inhibited by anticoagulants in the sample to be tested. Non-inhibited FXa cleaves a FXa-specific chromogenic substrate, producing a yellow signal that is detected at 405 nm. Preparation of the standard curve was left to laboratory discretion.

For the HPLC-MS/MS method, lower level of quantification (LLOQ) of RXA is reported at 0.500 µg/l by Rohde et al. [9]. For the chromogenic anti-FXa measurement of RXA, the limit of detection (LOD) was determined by calculating the mean optical density of 7 independent blank values, subtracting 3 SD and converting that value into a RXA concentration using a HPLC-MS/MS validated standard curve (Fig. 1B). Laboratory platforms used by the participating RIVAMOS laboratories are provided in Table 1. Reagents are specified in Fig. 3 for each assay. Innovin, Pathromtin, Multifibren, Innovance, Thromborel, Berichrom, Thromboclotin are

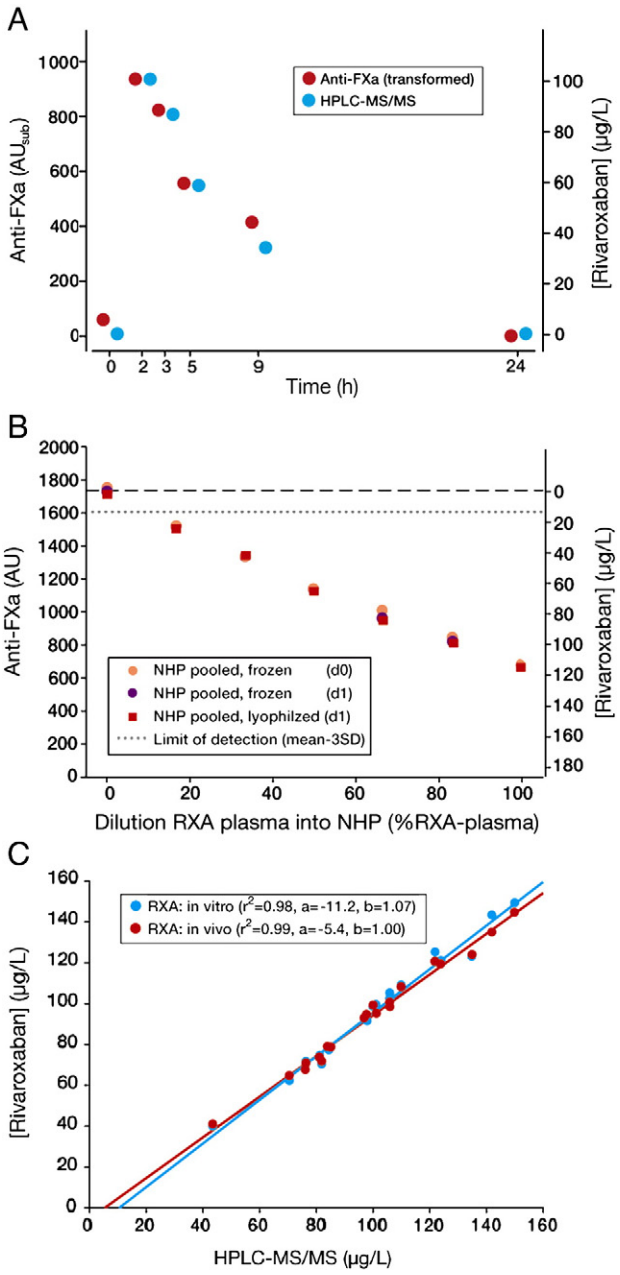

Fig. 1. Comparing chromogenic anti-FXa assay and HPLC-MS/MS measurements. **(A)** The time course of anti-FXa levels (red circles) and HPLC-MS/MS measurements (blue circles) were compared in a volunteer following the ingestion of 10 mg RXA. For better comparability, the anti-FXa results were transformed (individual optical density values were subtracted from the maximal value: y = 1649-x; these modified units were designated as arbitratry units$_{subtrated}$ (AU$_{sub}$)) to get a curve with a "positive" peak. For better visibility values from the same time point were placed adjacently and not superimposed. **(B)** Limit of detection (LOD) and dilution with different preparations of normal human plasma. Plasma from a volunteer obtained 2 hours after ingestion of 10 mg RXA was diluted using normal human plasma to dilutions of 0%, 16%, 33%, 50%, 66%, 83% and 100%. The percentage specifies the amount of volunteer plasma containing RXA. One series of diluted plasmas was measured at day 0 (orange dots), the same series was measured again one day later (day1, purple dots). The same dilutions of the volunteer plasma was prepared with lyophilized plasma from a commercial source (Siemens Healthcare; red square). **(C)** Correlation between RXA quantifications derived from the chromogenic anti-FXa assay (laboratory G) and those obtained by HPLC-MS/MS. Linear regressions for two different standard curves are depicted (y = a + bx; r^2: coefficient of determination, a: intersection with the Y axis, b: slope,). Blue circles indicate measurements derived from calibrators that were prepared by adding RXA *in vitro* to plasma. Red circles indicate measurements based on calibrators that were prepared by diluting RXA containing volunteer plasma.