Regular Article

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

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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.

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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

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 $\leq 2\%$ 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
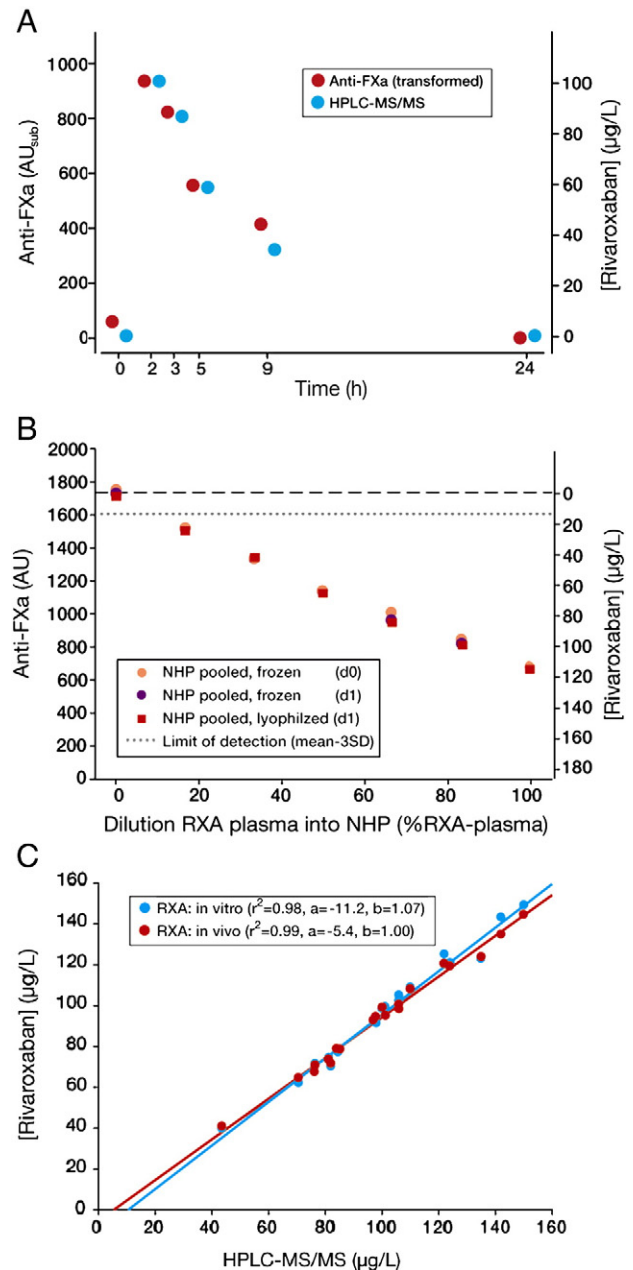
Fig. 1. Comparing chromogenic anti-FXa assay and HPLC-MS/MS measurements. **(A)** The time course of anti-FXa levels (red circles) and HPLC-MS/MS measurements (blue circles) were compared in a volunteer following the ingestion of 10 mg RXA. For better comparability, the anti-FXa results were transformed (individual optical density values were subtracted from the maximal value: y = 1649-x; these modified units were designated as arbitrary units$_{subtracted}$ (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.

Table 1
Coagulometers used in the participating laboratories.

Laboratory	Company	Model
A	Siemens	BCS-XP
B	Roche	STA-R
C	Siemens	BCS-XP
D	Siemens	BCS-XP
E	Sysmex	CA7000
F	Sysmex	CA1500
G	Roche	STA-R
H	Instrumentation Laboratory	ACL Top 700
I	Siemens	BCS

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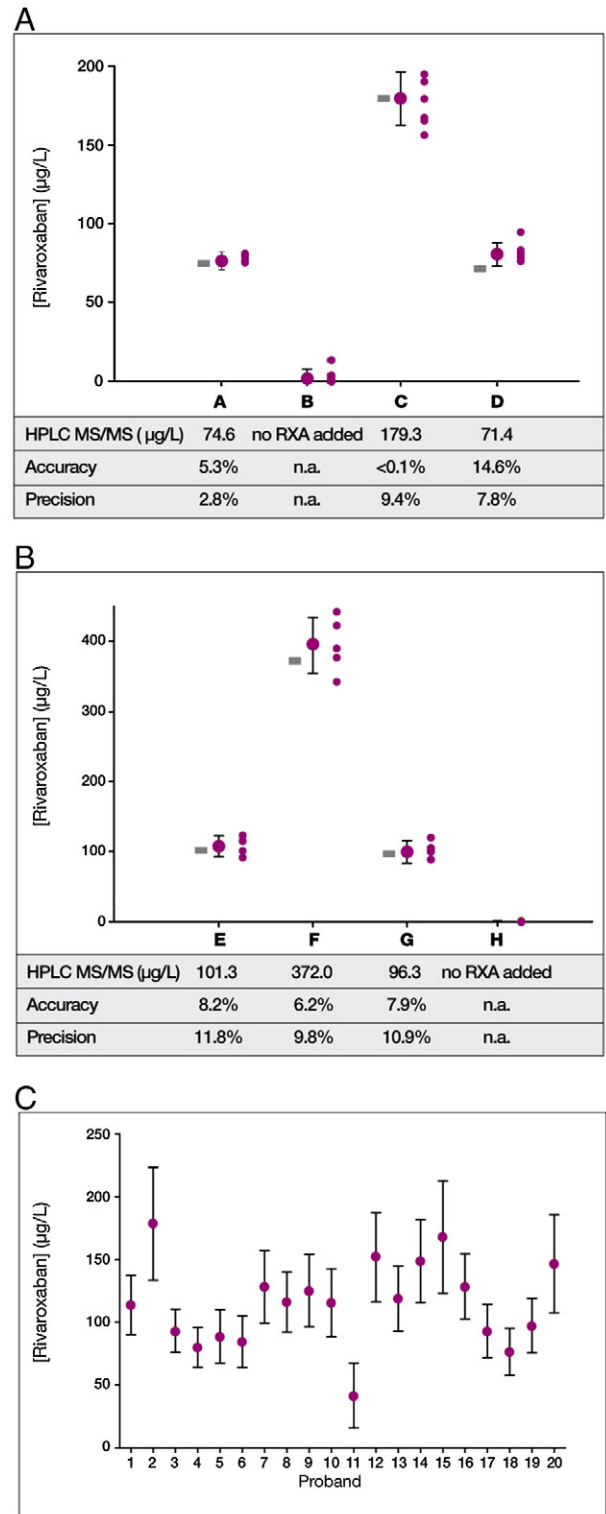
Volunteers

Following ethical approval through the relevant board at the senior author's institution, 21 healthy male volunteers were recruited and informed consent was obtained. Before and 2 to 3 hours after the ingestion of 10 mg of RXA per os up to 100-120 mL of citrated venous blood were drawn as previously described [13]. Plasma samples were aliquoted, snap-frozen and shipped to participating labs by courier at $-20\ °C$.

Preparatory studies

In order to evaluate aim i), we first measured RXA containing plasma samples from a volunteer having ingested 10 mg RXA with the chromogenic anti-FXa assay and compared these results to HPLC-MS/MS measurements (Fig. 1A). Secondly, we investigated the effect of dilutions with buffers (data not shown) and various preparations of plasma (frozen vs. lyophilized) on the anti-FXa measurements (Fig. 1B). Thirdly, utilizing the chromogenic anti-FXa assay, we determined the LOD for RXA as described above (mean-3SD method, Fig. 1B). Fourthly, we compared a standard curve obtained with RXA containing plasma probes prepared with a stock solution of RXA in DMSO with the curve obtained with RXA containing plasma from a volunteer after RXA ingestion; the RXA concentration of the latter plasma probe had been assessed by HPLC-MS/MS (Fig. 1C). Fifthly, we assessed whether, and down to which concentration, DMSO as a vector influenced routine coagulation tests (not shown). Finally, a first quality control experiment was performed with a sample containing an unknown concentration of RXA. This sample was sent out to the participating laboratories for RXA quantification (not shown).

In vitro studies

Three rounds of independent quality control experiments were performed using calibrators and samples that were spiked with RXA *in vitro* or stemmed from volunteers who had ingested RXA. The first round with a single blinded sample (as described above) was performed to ascertain the feasibility of the project (aim i.). The following two experiments (aim ii.) each involved a set of labeled CAL and a set of four blinded samples (Fig. 2A: samples A-D and Fig. 2B: samples E-H). Each set of blinded samples had one negative sample, two samples with identical RXA concentrations that were within the range of the supplied calibrators, and one sample with a high RXA concentration requiring sample dilution. CAL and blinded samples were prepared in laboratory I. Laboratory F, where anti-FXa testing was not offered, and laboratory I, which was not blinded were not included in the quality control analysis performed to

	A	B	C	D
HPLC MS/MS (µg/L)	74.6	no RXA added	179.3	71.4
Accuracy	5.3%	n.a.	<0.1%	14.6%
Precision	2.8%	n.a.	9.4%	7.8%

	E	F	G	H
HPLC MS/MS (µg/L)	101.3	372.0	96.3	no RXA added
Accuracy	8.2%	6.2%	7.9%	n.a.
Precision	11.8%	9.8%	10.9%	n.a.

Fig. 2. Blinded quality control trials (*in vitro*) and RXA quantification (ex-vivo). In two rounds ((**A**) and (**B**)) of blinded quality control testing, four samples of normal human plasma spiked with RXA *in vitro* were distributed per round. The participating labs were unaware of the RXA concentration in the samples. The RXA concentration measured by HPLC-MS/MS is specified under each sample and shown as a grey bar in the figure. Purple points with error bars of the standard deviation represent the mean of all submitted results obtained using the chromogenic anti-FXa assay. The purple points represent the measurements of each participating lab. Accuracy (in %) and coefficient of variation of precision are specified under each sample. Not applicable (n.a.). **(C)** RXA plasma concentration measured with the validated anti FXa assay in 20 volunteers: purple points indicate the mean ($+/-$ standard deviation) of the RXA concentration measurements obtained in eight laboratories (Table 1: all labs, except Lab F)) for the 20 volunteers described under *Volunteers & Methods*.

assess accuracy and precision. Laboratories were requested to report RXA concentrations in µg/L.

Ex vivo studies

Plasma samples from 20 volunteers drawn before and 2–3 hours after RXA ingestion were utilized to measure RXA plasma concentrations with the newly validated anti-FXa assay (Fig. 2C) and to determine the influence of RXA on routine coagulation tests (aim iii.; PT, aPTT, thrombin time, fibrinogen, D-Dimer, FII, FV, FVII, FVIII, FIX, FX, FXI, FXIII; Fig. 3). Not all laboratories performed all tests (see reagent specifications in the respective graphs in Fig. 3).

Data handling and statistics

Results were entered into spreadsheets (Excel 2003, Microsoft Corp., Seattle, WA). Graphs were produced using Sigma Plot for Windows, Version 11.0 (Systat Software Inc., Chicago, IL). Accuracy in % was calculated by dividing the difference between the mean of anti-FXa quantifications and the respective HPLC-MS/MS result by the HPLC-MS-MS result and multiplying the result by 100; e.g. sample A in Fig. 2A: $[(78.6-74.6)/74.6] \times 100 = 5.3\%$. The coefficient of variation (CV) for inter-laboratory precision of *in vitro* measurements was assessed by dividing the standard deviation of 7 anti-FXa measurements (one from each blinded participating laboratory that performed anti-FXa testing) by the mean of these measurements and multiplying the result by 100; e.g. sample A in Fig. 2A: $(2.1/78.5) \times 100 = 2.8\%$. Missing data were not extrapolated and not included in statistical analyses. The difference between the results of the coagulation tests in the plasma samples before and after ingestion of 10 mg of RXA was analyzed using a mixed effects analysis of variance (ANOVA) with fixed factor "time" and random factors

"volunteer" and "laboratory". Inter laboratory reliability was assessed using a three way random ANOVA. Inter-laboratory reliability is the ratio of the sum of variance components not depending on laboratory and the sum of all variance components [14]. All analyses were performed using SPSS 17 (SPSS Inc., Chicago, IL). P-values ≤ 0.05 are considered statistically significant. Reliability was not assessed for anti-FXa measurements before vs after drug ingestion, due to missing data.

Results

Preparatory studies

Measurements of RXA by the chromogenic anti-FXa assay and by HPLC-MS/MS showed very similar time courses after ingestion of 10 mg RXA as illustrated in Fig. 1A. Both chromogenic and HPLC-MS/MS measurements of RXA were more variable when RXA was diluted in buffer vs. plasma (data not shown). Fig. 1B shows the LOD for RXA measurements (about 14 µg/L, determined in lab I) and illustrates the effect of various preparations of plasma (frozen vs lyophilized) on anti-FXa measurements. Standard curves based on RXA diluted in plasma using a stock solution of RXA in DMSO and those using plasma from a volunteer after RXA ingestion were nearly super-imposable (Fig. 1C). DMSO as a vector influenced routine coagulation tests. We observed detectable prolongations of coagulation times for aPTT and PT at values >2% DMSO (data not shown). Finally, a first quality control experiment with a blinded sample that was send out to participating laboratories showed reproducible results (not shown). In summary these results demonstrate the feasibility of RXA quantification with a commercial chromogenic anti-FXa assay (aim i.) without further modifications.
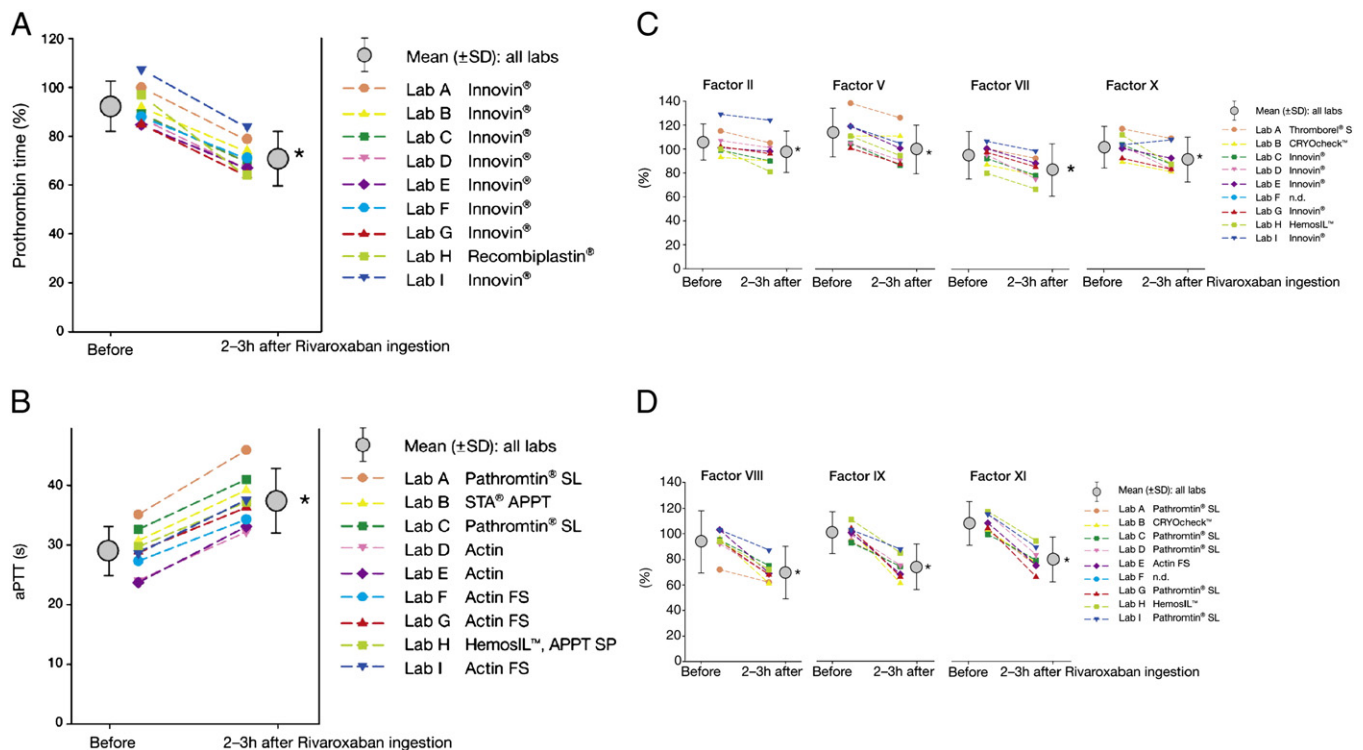
Fig. 3. Influence of RXA on coagulation tests. (A) Prothrombin time (PT). **(B)** activated partial thromboplastin time (aPTT). **(C)** PT-based factor determinations: Factors II, V, VII, X. **(D)** aPTT-based factor determinations: Factors VIII , IX , XI. Grey circles indicate the mean of all measured results of all participating labs and the error bars indicate the mean SD for all labs. Labs and reagents used by the labs are specified in the legend, n.d. for not determined in this lab. The asterix indicates a statistically significant difference (before vs. after, p<0.05) determined by ANOVA (*Volunteers and Methods*). Results for thrombin time, fibrinogen, D-Dimer, and FXIII were not significantly different (before vs after RXA). These graphs are not shown.

In vitro studies

Results of two quality control experiments with blinded samples in seven participating labs (lab F did not offer anti-FXa testing, lab I was unblinded, see above) are shown in Fig. 2A and B. RXA concentrations in the plasma probes measured by HPLC-MS/MS for the respective samples are given under each sample. Accuracy (aim ii) was high with values of 5.3%, <0.1%, 14.6% (sample A, C, D) and 8.2%, 6.2% and 7.9% (samples E, F, G), respectively. The mean result for accuracy was 7.0% (range: 0.01%-14.6%). Inter-laboratory precision (aim ii) was good with CV of 2.8%, 9.4%, 7.8% as well as 11.8%, 9.8% and 10.9%, respectively. Mean CV for inter-laboratory precision was 7.9% (range: 2.6-10.5%). Accuracy and precision were not calculated for negative controls (samples B + H).

Ex vivo studies

Blood samples were taken from 20 healthy volunteers before and 2–3 three hours after ingestion of 10 mg of RXA. Values for RXA concentrations in the baseline samples were below LOD. After ingestion of 10 mg per os, the mean RXA concentration obtained by anti-FXa measurement was $114 \pm 43\,\mu g/L$. For HPLC-MS/MS the mean result was $100 \pm 26\,\mu g/L$. Fig. 2C shows the mean RXA levels in the 20 volunteers 2–3 hours after the intake of 10 mg RXA measured by the anti-FXa assay in eight laboratories (laboratory F did not offer anti-FXa tests). Of note, RXA concentrations differ considerably between the volunteers and the variability among the eight laboratories is higher than in the quality control experiments.

To assess the influence of RXA on routine coagulation tests (aim iii) sets of 2×20 samples (before and 2–3 hours after ingestion of 10 mg RXA) were distributed to the nine participating laboratories. Each laboratory used its established assays as specified in Table 1 and Fig. 3. In lab F not all tests were offered. Fig. 3 illustrates the mean value per test before (grey symbol on left) vs after (grey symbol on right) ingestion of RXA. Error bars on the grey symbols represent the mean standard deviation among the 9 partcipating laboratories. Color-coded symbols in the middle depict the mean value of 20 measurements from a single laboratory as specified in the graph (error bars were omitted for better visibility).

RXA prolonged PT (Fig. 3A), INR (not shown) and aPTT (Fig. 3B) clotting times and the mean results from samples taken after vs. before ingestion differed significantly ($p \leq 0.05$). Thrombin time, fibrinogen and D-Dimer results were not affected by RXA. PT-based (Fig. 3C) and aPTT-based (Fig. 3D) specific coagulation factor tests were all influenced by RXA and the values differed significantly in plasma samples taken after and before RXA ingestion. FXIII analysis was not affected by RXA intake. As a tool for quality control, we assessed agreement of the measured values in the participating laboratories for a specific test by calculating inter-laboratory reliability (see *Volunteers and Methods: Data handling and statistics*). Minimal reliability is 0; maximal reliability is 1. Inter-laboratory reliability was medium to high for most tests and ranged from 0.5 to 0.9. High reliability was found for PT (0.81), INR (0.76) and aPTT (0.71) and most factor determinations. Thrombin time, D-Dimer and FII determination were exceptions with very low to low reliability values of 0.04, 0.34, 0.39, respectively. For thrombin time and D-Dimer this may be explained by differences in thrombin concentration and in assay formats and antibodies.

Discussion

RXA quantification

The results of this inter-laboratory evaluation demonstrate that reliable quantification of RXA in plasma is possible using a commercial chromogenic anti-FXa assay and a set of validated RXA calibrators

without further modifications. In experiments designed specifically to assess accuracy and precision (aim ii), the chromogenic anti-FXa results were compared to measurements made by HPLC-MS/MS, which is a validated method to quantify RXA [9]. The anti-FXa assay proved accurate (mean accuracy in quality control experiments 7.0%) and precise (mean CV 8.8%, Fig. 2A, B). In the *ex-vivo* experiments of aim iii measuring the RXA plasma concentrations in the 20 volunteers, accuracy was 14% (mean RXA by HPLC-MS/MS $100 \pm 26\,\mu g/L$ vs. $114 \pm 43\,\mu g/L$ by anti-FXa assay). The result we obtained by anti-FXa measurement is nearly identical to the one previously published by Freyburger et al., who found a mean concentration of $117 \pm 78\,\mu g/L$ in 40 patients 2 hours after ingestion of 10 mg RXA [15]. The higher variability of RXA measurements in the ex-vivo experiments (Fig. 2C) compared to the quality control experiments (Fig. 2A, B) could potentially be related to preanalytical issues including repeated freezing and thawing and how long the samples were maintained at room temperature. The range of the RXA plasma concentrations in the 20 volunteers (Fig. 2C), however, appears wide (43–150 μg/L, mean $100 \pm 26\,\mu g/L$; 2 C). This is in line with previously reported data [16] where it was demonstrated that a large range for RXA peak levels on day 1 of RXA intake has to be expected, with a saturation effect (and a narrower peak RXA range) on day 5.

Quantification of drug plasma levels can be necessary or desirable. There are three major sources of variability for drug effects: i) pharmacokinetic variability, which refers to drug dosing and plasma concentration, ii) pharmacodynamic variability, which refers to a drug's concentration at the receptor level and the response elicited, and iii) therapy adherence, which refers to a patient's ingestion of the drug. For vitamin K antagonists (VKA) drug monitoring in the form of INR testing is necessary, as VKA are characterized by both high pharmacokinetic and pharmacodynamic variability together with a narrow therapeutic window. For RXA, however, monitoring is not routinely required, but quantification can be desirable in special situations, such as emergency surgery or planned invasive procedures, putative RXA intoxication or treatment failure, as well as bleeding events in patients taking RXA. Indeed, data from phase II trials show pharmacokinetic characteristics and a pharmacodynamic profile with a large therapeutic window making monitoring unnecessary [16]. None of the completed randomized controlled phase III trials that have led to RXA registration for VTE prophylaxis in hip and knee surgery included a procedure for RXA "monitoring" or – as we prefer to refer to it – quantification [5–8]. RXA quantification is also not foreseen as a necessary measure in an ongoing RCT with prophylactic doses of RXA, Magellan study for VTE prophylaxis in medically ill patients, or clinical trials with other doses of RXA, such as Einstein DVT for treatment of deep vein thrombosis (20 mg), Einstein PE for treatment of pulmonary embolism (20 mg), Einstein Extension for extended anticoagulation (20 mg) or in the Atlas-ACS (TIMI-51) for secondary prevention after acute coronary syndromes (2.5/5 mg) and Rocket AF for atrial fibrillation (20 mg) [17].

RXA impact on coagulation assays

Rivaroxaban inhibits both free FXa as well as FXa in the prothrombinase complex bound to phospholipid bilayers in a highly specific manner [1]. According to the model of the coagulation cascade, RXA - in theory - should alter coagulation tests of both the extrinsic and intrinsic system, including aPTT, PT and specific factor tests. Tests of coagulation factors "below" the RXA inhibition site (FXa), such as thrombin time and determinations of fibrinogen, FXIII or D-Dimer levels, - in theory - should not be influenced by RXA. Our results confirm this (Fig. 3). Recently, Samama et al. have published data indicating that indeed PTT and PT are prolonged by RXA [10]. However, the range of lengthening, the slope of the resultant test curves, and the variability observed for the aPTT or PT reagent used

render these tests unsuitable for RXA quantification. Our study extends the data of Samama et al. i) by characterizing and validating a readily available chromogenic anti-FXa assay for RXA quantification in 8 different laboratories using different platforms and ii) by defining the influence of RXA on routine coagulation tests in 9 different labs in a group of volunteers large enough to permit statistical evaluation.

Limitations of the study

A limitation of our work is that we assessed only one chromogenic anti-FXa assay and that the assay is not specific for RXA. Heparins and other inhibitors of FXa in the sample will influence the test results. It is conceivable that other anti-FXa assays are also suited to quantitate RXA. Recently, results for a specific anti-FXa assay for RXA were published [18]. We choose the Biophen Heparin assay since all participating laboratories had implemented this assay already at the time of study initiation. The range of this assay covers RXA concentrations of up to 160 μg/L RXA, suitable to measure peak levels of RXA after intake of 10 mg RXA [19]. For DVT treatment and stroke prevention in patients with atrial fibrillation, 20 mg RXA od have been tested in the phase III studies (14). Sample predilution will be necessary to quantitate the peak concentrations of RXA in these patients. In our hands sample predilution was best performed using plasma, as dilution in buffer lead to more variable results.

Strengths of the study

Strong points of our study include the inter-laboratory setting with nine participating labs, the large sample size (9 laboratories measuring 40 samples utilizing up to 13 tests), the variability of implemented reagents, tests and test platforms, the use of a combined *in vitro* and *ex-vivo* approach, the implementation of a blinded quality control approach and the correlation of data with HPLC-MS/MS measurements.

The novel direct anticoagulants, including RXA, may lead us into a new era of anticoagulation [20]. This potential progress will not only bear therapeutic consequences but may very well require a revised approach regarding the interpretation of coagulation tests. We hypothesize that an INR of 2.0 in a patient taking VKA will have different implications regarding therapeutic efficacy and bleeding risk than an INR of 2.0 in a patient on RXA. In our study INR ranged from 1.0-1.5 (all labs utilized a recombinant tissue factor as activating agent with an ISI close to 1). As the work by Samama et al. suggests the highest observed INR in our study would be ≥2.0 utilizing a PT reagent with an ISI of 1.75 [10]. PT reagents have been artificially rendered insensitive to heparin through the addition of heparin neutralizing agents. Such reagents could be added to the anti-FXa assay to render it more specific for RXA. Furthermore one could consider rendering tests such as the PT insensitive to RXA to obtain a test devoid of the RXA influence. This could be achieved though addition of a RXA "quencher", similar to the herparin neutralizing agent. We project that this and related topics will give rise to intense research and debate in the imminent future.

In conclusion, we present a simple, accurate and precise chromogenic anti-FXa assay for RXA quantification, suitable for many hospital laboratories. Our data show that coagulation tests such as the prothrombine time, aPTT and derived coagulation factor assays will be altered following the ingestion of 10 mg of RXA p.o. The range and intensity of assay alteration will depend on the type of assay and coagulometer used.

Conflict of interest statement

Acknowledgements

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