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Relationship of edoxaban plasma concentration and blood coagulation in healthy volunteers using standard laboratory tests and viscoelastic analysis.

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Abstract

The capability of viscoelastic measurement parameters to screen anticoagulation activity of edoxaban in relation to its plasma concentrations was evaluated in 15 healthy male volunteers. Blood samples were drawn before the oral administration of edoxaban 60 mg and at 2, 4, 6, 8, and 24 h after administration. At each time point, standard coagulation tests were performed, blood viscoelastic properties were measured with thromboelastometry device ROTEM® delta analyzer (Instrumentation Laboratory, Werfen, Barcelona, Spain), and edoxaban plasma concentrations were measured. Our primary interest was the possible correlation between edoxaban plasma concentrations and values for ROTEM ExTEM® and FibTEM®; we also studied the correlation of edoxaban plasma concentrations with the results of standard coagulation tests. We saw the effect of a single dose of edoxaban most clearly in Clotting Time (CT) of ROTEM ExTEM® and FibTEM®. Changes in these parameters correlated significantly with edoxaban plasma concentrations up to 6 h from the ingestion of the drug. Activated partial thromboplastin time, prothrombin time and anti-Factor Xa were also affected. Peak changes were observed at 2 and 4 h after administration of edoxaban. The changes were mostly reversed after 8 h. In conclusion, ROTEM® CT correlates significantly with edoxaban plasma concentrations and can be used to estimate the effect of edoxaban. ROTEM® should be considered as part of the assessment of coagulation, with the big advantage of being readily available on site.

Keywords: Factor Xa Inhibitors, Blood Coagulation Tests, Thrombelastography, Healthy Volunteers, Drug Monitoring

Introduction

Antithrombotic prophylaxis in certain patient populations is necessary for the prevention of stroke and other systemic embolic events. The conventional anticoagulant agent warfarin carries the risk of bleeding and therefore regular therapeutic monitoring is needed. The recently developed direct oral anticoagulants (DOAC) apixaban, dabigatran, rivaroxaban, and edoxaban are alternatives to warfarin and their use has been increasing.¹ Their anticoagulant effect is more predictable and need not be monitored on a regular basis. However, in case of individual variation (due to, for example, drug-drug interactions, genetic factors, age, body size, renal function) in the effect on coagulation,^{2, 4} specific tests are needed to measure the effect of the non-vitamin K oral anticoagulants. Standard laboratory coagulation tests, such as activated partial thromboplastin time (APTT) or thrombin time (TT) and non-specific anti-Factor Xa (anti-FXa) are used for general screening, but rapid point-of-care tests for use in emergency situations would be desirable.³

The aim of this study was to investigate the usefulness of viscoelastic measurement parameters in screening the anticoagulant effect after a typical dose of edoxaban. We were primarily interested in the possible correlation of edoxaban plasma concentrations and ROTEM ExTEM® and FibTEM® values at defined time points. We also studied the possible correlation of edoxaban plasma concentrations and standard coagulation tests.

Methods

Subjects

This study was conducted according to the revised Declaration of Helsinki of the World Medical Association and International Conference on Harmonisation-Good Clinical Practice (ICH GCP) guidelines for good clinical trial practice. Prior to commencement of this study, the study protocol, patient information and informed consent form were approved by the investigational review board of the Hospital District of Southwest Finland (protocol number 13693, decision number TP2/016/18) and the Finnish Medicines Agency. The eligible volunteers gave written informed consent and were ascertained to be healthy by clinical examination, medical history and routine laboratory tests. The clinical phase of this study was conducted in Turku University Hospital (Turku, Finland).

The inclusion criteria for the volunteers were age 18 – 40 years and body mass index (BMI) 20 - 35. The criteria for exclusion were: a previous history of intolerance to the study drugs or to related compounds or additives; history of bleeding disorder or similar; past or present alcoholism, drug abuse, psychiatric or psychological or emotional problems likely to invalidate informed consent; concomitant drug therapy of any kind for at least 15 days prior to study; special diet or lifestyle habits which would compromise the conditions of the study or interpretation of the results; participation in any other studies involving investigational or marketed drug products concomitantly or within 1 month prior to entry into this study; existing or recent significant disease; smoking for one month before the start of the study and during the whole study period; a “yes” answer to any one of three substance abuse questions;⁵ blood donation within the 4 weeks prior to and/or during the study.

Subjects potentially eligible for the study were approached approximately 2 weeks before the study day for information, preliminary assessment of eligibility criteria and for obtaining informed

consent. We performed a brief medical check-up, including measurement of basic vital parameters (blood pressure and heart rate), and we performed basic laboratory blood tests: blood count and thrombocytes (CBC), fibrinogen (P-Fibr) and ionized calcium (fS-Ca-Ion). The risk of participants developing drug dependency was considered low as evaluated by the three substance abuse questions in Finnish translation.⁵

Study design and sampling

An open, longitudinal observational study design was used. Participants fasted overnight before the study day. Venous blood samples (5 mL each) were drawn from a venous plastic cannula placed in a forearm vein. Subjects were preferably sitting for 4 h after the administration of the study drug. They were given a standardized meal 4 h and snack 8 h after the administration of edoxaban. Participants were discharged home after the 8- h blood sample but returned for withdrawal of the last sample the following morning, 24 h after the administration of the study drug. The investigator called each subject 12 h after the administration of edoxaban to ask about any adverse events. The participation of each subject was complete after the last blood sample.

Blood samples for standard coagulation measurements (APTT, TT) and ROTEM® were collected in citrate containing tubes (BD Vacutainer™ REF 363048, 2.7 mL of blood). Blood samples for plasma concentration of anti-FXa were collected in tubes containing buffered citrate, theophylline, adenosine and dipyridamole (BD Vacutainer™ REF 367599, 4.5 mL of blood). Blood samples for plasma concentration of edoxaban were collected in ethylenediaminetetraacetic acid (EDTA) tubes (Greiner Bio-One VACUETTE® REF 456043, 6 mL of blood). A qualified study nurse was in charge of handling the blood samples. Blood samples were drawn before the oral administration of edoxaban 60 mg (baseline) and 2, 4, 6, 8, and 24 h after administration (Table 1). Altogether 70 mL of blood was taken from each participant.

Coagulation measurements

One investigator (MH) performed thromboelastometry tests at the study site using a ROTEM® delta analyzer (Instrumentation Laboratory, Werfen, Barcelona, Spain) with ExTEM® and FibTEM® reagents, according to the manufacturer's instructions.⁶

The ROTEM® delta analyzer (Instrumentation Laboratory, Werfen, Barcelona, Spain) allows global assessment of hemostatic function in a timely manner and is mainly used to evaluate the coagulation process in whole blood rather than plasma, covering the process from initial platelet-fibrin interaction to eventual clot lysis. The sample is placed in a cuvette, and a rotating pin suspended in the sample. When the blood starts to clot, the pin's movement is increasingly restricted. The rotation of the pin is recorded by an integrated computer (Figure 1^{7,8}).⁹

ExTEM® is a screening test for the (extrinsic) hemostasis system. It is used for assessment of Factors VII, X, V, II, I, platelets and fibrinolysis. Coagulation is activated via the physiological activator tissue factor. In the FibTEM assay, coagulation is activated as in ExTEM. It provides information on the fibrin part of the clot, by eliminating the contribution of the platelets through irreversible inhibition by cytochalasin D. The clot is dependent therefore only on fibrin formation and polymerization.

Standard coagulation tests (APTT, TT and Anti-FXa) were analyzed in Turku University Hospital's laboratory (Tykslab, Turku, Finland), using standard methods.

We calculated values for the areas under the different coagulation measurement (ExTEM, FibTEM, APTT, TT and Anti-FXa) versus time curves (AUC) using the same methodology as for edoxaban and M4 plasma concentration (see "Pharmacokinetic measurements").

Plasma drug concentrations

The blood samples drawn for the plasma drug concentration measurement of edoxaban and its major metabolite (M4) were separated within 30 min by centrifugation for 10 min at 1000 g at room temperature and stored at -70 °C until analysis. The plasma concentrations of edoxaban and M4

were analyzed at the Department of Clinical Pharmacology, University of Helsinki. Analytical standard edoxaban was purchased from Toronto Research Chemicals (North York, ON, Canada), edoxaban M4 and internal standard (IS) edoxaban-D6 from Clearsynth (Clearsynth Labs Ltd, Mumbai, India).

Analytes and IS were analyzed using liquid chromatography–tandem mass spectrometry (LC-MS/MS). Before analysis, 100 µL sample aliquots and standard/quality control (STD/QC) plasma samples spiked with edoxaban and M4, were mixed with 300 µL acetonitrile containing IS. In order to precipitate proteins, samples were kept in the tubes for 10 minutes and briefly vortexed before centrifugation (21000 g, 15 min at 10 °C). After centrifugation, 300 µL of the supernatants were evaporated to dryness under air flow at 50 °C. Thereafter, the dried samples were reconstituted with 75 µL of 50% methanol prior to LC-MS analyses.

The analytes were separated on an Atlantis T3 column (3 µm, 2.1x100 mm + 2.1x10 mm, Waters, Milford, MA) by liquid chromatography (Nexera X2, UHPLC system, Shimadzu, Kyoto, Japan) at a flow rate of 0.2 mL/min with 0.05% formic acid as mobile phase A and methanol as mobile phase B. The gradient programme for mobile phase B was 30% 0-1 min, 30-52.5% 1-7 min, followed by 90% flush of the column for 1.5 min and balancing at 30% before next injection. The analytes and IS were quantified with an API3000 mass spectrometer (Sciex, Toronto, ON, Canada) operated in electrospray positive ionization mode (ESI +) using the multiple reaction monitoring transitions of $[M+H]^+$ m/z 548.3-366.3 for edoxaban, m/z 521.3-339.1 for edoxaban M4 and m/z 554.3-372.3 for the IS. The analytical method was validated in-house. The intra-day repeatability was assessed by analyzing six parallel samples of each QC level. The concentrations of QC samples were 4, 20 and 250 ng/mL for edoxaban and 2, 10 and 125 ng/mL for M4, the intra-day coefficients of variation were $\leq 3.0\%$ for edoxaban and $\leq 2.1\%$ for M4, and the accuracies were between 101-104% for edoxaban and 100-104% for M4. The edoxaban standard curve was linear between 0 and 700 ng/mL with correlation coefficient values of ≥ 0.989 and a lower limit of quantitation of 1 ng/mL.

For the metabolite M4, the standard curve was linear between 0 and 350 ng/ml with correlation coefficient values of ≥ 0.994 and a lower limit of quantitation of 0.4 ng/mL. The inter-day ($n = 4$) coefficients of variation of the QC samples were $\leq 4.6\%$ for both analytes, and the accuracies were between 103-104% and 95-101% for edoxaban and M4, respectively.

Pharmacokinetic measurements

Actual sampling times were used for pharmacokinetic calculations. The C_{\max} and t_{\max} were observed directly from the data. The area under the edoxaban or M4 plasma concentration–time curve during 0-24 h (AUC_{0-24h}) was estimated by means of the trapezoidal rule. We used the linear trapezoidal rule for increasing values and the logarithmic trapezoidal rule for decreasing concentrations. The pharmacokinetic analysis was performed with the WinNonlin program (version 4.1; Pharsight Corp, Mountain View, CA).

Statistics

It was calculated that 13 subjects would be needed to be able to detect a Pearson correlation coefficient of 0.70 between edoxaban peak plasma concentration and ROTEM® parameters using a two-tailed test with a level of significance of 0.05 and power of 80%. Eventually, 15 healthy non-smoking Caucasian male volunteers were recruited to compensate for possible drop-outs during the study.

The changes in blood coagulation were analyzed with repeated measures analysis of variance, and the values at each time point (2, 4, 6, 8 and 24 h) were compared to the baseline using Dunnett's adjustment. The correlations between viscoelastic measurement parameters and conventional coagulation parameters and edoxaban plasma concentration were analyzed with Pearson correlation coefficients. We considered P values of 0.05 or less as significant and a correlation coefficient of at least 0.50 as significant. Statistical analyses were done with SAS System for Windows, version 9.4 (SAS Institute Inc., Cary, NC).

Results

Fifteen healthy non-smoking volunteers were recruited, with a median age of 25 years (18 – 40 years). All subjects completed the whole study according to protocol, and no adverse effects were observed.

A single 60 mg dose of edoxaban caused distinct changes in the values for CT-ExTEM and CT-FibTEM (Figure 2). When compared to baseline, CT-ExTEM was significantly prolonged at 2, 4, 6 and 8 h ($p < .001$) and at 24 h ($p < .007$) post dose. CT-FibTEM was significantly prolonged from baseline at 2, 4, 6 and 8 h ($p < .001$) and at 24 h ($p < .021$). In contrast, neither MCF-ExTEM ($p < .641$) nor MCF-FibTEM ($p = 0.094$) showed significant changes after a single dose of edoxaban (Figure 2).

When compared to baseline, APTT was prolonged at 2, 4, 6 and 8 h ($p < .001$) and at 24 h ($p < .003$). TT was decreased as well at 2, 4, 6 and 8 h ($p < .001$). Anti-FXa was also significantly affected at 2, 4, 6, 8 and 24 h ($p < .001$). These changes are displayed in Figure 3.

We quantified plasma concentrations of edoxaban and its metabolite M4 at 2, 4, 6, 8 and 24 h after edoxaban intake. There were huge interindividual variations in peak edoxaban plasma concentration among subjects (Figure 4).

Correlation analysis

We found a strong correlation ($p < .001$) between CT-ExTEM and edoxaban plasma concentration at 2 ($r = 0.90$), 4 ($r = 0.84$) and 6 h ($r = 0.77$) and a significant correlation ($p < .05$) at 8 h ($r = 0.52$) after edoxaban administration. Similarly, there was a strong correlation ($p < .001$) between CT-FibTEM and edoxaban plasma concentration at 2 ($r = 0.86$) and 4 h ($r = 0.78$) and a significant correlation ($p < .05$) at 6 h ($r = 0.67$) after the administration of edoxaban.

There was a significant correlation ($p < .05$) between edoxaban plasma concentration and APTT values up to 8 h after edoxaban. There was also a significant correlation between plasma edoxaban concentration and change from baseline in anti-FXa for the whole 24 h period, with $p < .001$ at 6 and 8 h and $p < .05$ at 2, 4 and 24 h after edoxaban.

A further correlation analysis was performed to examine the possible relationship between the edoxaban and M4 AUC and the AUC values calculated for the coagulation measurements: ExTEM, FibTEM, APTT, PT ja anti-FXa (Figure 5 and 6). We found the strongest correlation ($r = 0.91$) between the edoxaban plasma concentration–time AUC and anti-FXa AUC (Table 2).

Discussion

Edoxaban is an orally available, reversible, direct inhibitor of Factor Xa. The absolute bioavailability of edoxaban is estimated to be 62% for the 60 mg dose. Edoxaban is rapidly absorbed, with maximum concentrations appearing 1 to 2 (-3) hours after tablet intake. Recovery to pre-dose values is dose-dependent, with return to baseline by 24 to 36 hours post dose in all persons. Edoxaban is mainly eliminated unchanged through multiple renal and non-renal pathways. Non-renal elimination includes both metabolism and biliary excretion of unchanged drug.¹⁰⁻¹² Transport proteins, especially P-glycoprotein (P-gp), are considered to play a crucial role in edoxaban pharmacokinetics.¹³ Edoxaban is metabolized by hydrolysis by enzyme carboxylesterase, by conjugation and by oxidation by cytochrome P450 3A4/5. Edoxaban has three active metabolites. M4 is created by carboxylesterase-1 (CES1):¹¹ exposure to it is less than 10% of the exposure to the parent compound in healthy subjects. For other metabolites, exposure is less than 5% of exposure to the parent compound. The terminal elimination half-life of edoxaban is 10 to 14 hours.¹⁴

The pharmacokinetics of edoxaban in persons with normal renal and hepatic functions are fairly well known, but impaired hepatic or, especially, renal function delays the elimination of the drug.^{10,11} The pharmacokinetics can also differ in the case of emergencies (e.g. overdose, trauma, bleeding, urgent surgery). The recommendation for the patients undergoing surgery is to interrupt anticoagulation at least 24 h before a procedure.

In this study, the effect of a single dose of edoxaban on blood coagulation was quantified in all coagulation measurements. As expected from the pharmacokinetics of edoxaban, the maximum changes were observed 2-4 h after its administration and they were still significant 24 h post dose in most of the coagulation parameters.¹⁰

The use of DOAC does not require routine monitoring due to a fairly small interindividual variation in the pharmacokinetics of these drugs. On the other hand, individual factors, such as

drug-drug interactions, body size and disease states, can cause marked variability in their pharmacokinetics and pharmacodynamics, and, in the case of emergency situations (severe bleeding, urgent surgery, overdose), the patient's coagulation status and the remaining effect of the drug have to be assessed as quickly as possible.¹⁵ We can use three types of tests for the assessment of anticoagulation intensity in patients treated with a DOAC: (1) drug levels, (2) coagulation tests with specific calibrators or standards that can provide accurate estimates of drug levels, and (3) routine coagulation tests that can provide qualitative information (e.g. presence or absence of the drug and crude estimates of whether drug levels are likely to be high or low). Specific tests are still not routinely available in many hospitals. Even where available, they may not be available around the clock and, as they are not bed-side techniques, there may be delays in obtaining the results.

Standard laboratory tests (except FXa) are neither specific to a particular drug, nor sufficiently sensitive and therefore not reliable.^{16,17} PT and APTT show poor sensitivity to the effects of apixaban, although they are affected by rivaroxaban, dabigatran and edoxaban.¹⁸ However, the correlation is poor and a normal APTT and/or PT/international normalized ratio (INR) does not exclude a significant anticoagulant effect.¹⁹ Intrinsic FXa activity is sensitive to the effect of edoxaban and its activity decreases concentration- dependently as edoxaban plasma concentrations increase up to 440 ng/mL.²⁰

According to the guidelines on management of bleeding in patients receiving DOAC, the coagulation status should be evaluated by complete blood count, standard laboratory tests (PT/INR, APTT), specific assays for anti-Factor Xa activity, as well as liver and renal function tests. Point-of-care (POC) tests are not included in the guidelines, because there are insufficient data available on the relationship between drug plasma concentration and POC results.^{15,21}

We found that standard laboratory tests, especially anti-FXa, could also detect the effect of a single dose of edoxaban. Our anti-FXa assay was not specifically adjusted for the effect of edoxaban. It is recommended that anti-FXa should be checked at the time of peak and trough levels of the DOAC.

For edoxaban, the peak (the highest concentration of the drug) is 1-2 h after ingestion and the trough (the lowest concentration of the drug before the administration of the next dose) is typically 24 h post dose. [https://www.practical-haemostasis.com/Miscellaneous/anti_xa_assays.html] Anti-FXa < 0.1 U/mL is deemed to be the value when there is no significant remaining anticoagulant effect of edoxaban.^{22,23}

The PT and/or APTT may be normal, despite a significant anticoagulant effect of edoxaban, and so they are not useful tests to assess 'on-therapy' concentrations of edoxaban.²⁴

In the present study, the correlations between edoxaban plasma concentration and CT-ExTEM and CT-FibTEM values were strong up to 8 hours after edoxaban intake. In an in vitro study, Seyve et al. showed that CT-ExTEM is affected as long as the plasma concentration of edoxaban is above ~100 ng/mL.¹⁶ We also observed both prolonged CT-ExTEM and CT-FibTEM with lower plasma concentrations of edoxaban. The mean edoxaban plasma concentration 8 h after edoxaban intake was 82 [66-133] ng/mL.

Anti-FXa results correlated to edoxaban concentrations up to 24 h after edoxaban intake, which is in agreement with the finding that FXa activity decreases dose-dependently as edoxaban plasma concentrations rise from 0 up to 440 ng/mL.²⁰ Therefore, we think that anti-FXa test can quantify low edoxaban plasma concentrations better than CT-ExTEM or CT-FibTEM ones. The advantage of ROTEM® is its rapidity – CT results are usable essentially immediately, whereas the analysis of anti-FXa takes longer, results being available in 1 to 2 hours.

We saw marked interindividual variation in the time from the edoxaban ingestion to its peak plasma concentration, as well as in the time points of maximal change in coagulation parameters, although our study group was homogenous and included only 15 healthy male subjects. Because the pharmacokinetics of edoxaban are similar in male and female patients when renal function is taken into account, and because there is a linear correlation between edoxaban concentrations and anti-FXa activity, it is reasonable to assume that our data can be extrapolated to women. The biggest

individual variations we observed in CT-ExTEM, the maximum change in CT-ExTEM varying from 90 to 260 s. On the other hand, the maximum change in APTT showed the lowest individual variability (28-41 s). This variability is likely due to differences in drug absorption, metabolism and elimination. In any case, it is likely that pharmacokinetic variability is greater in the actual patient population.

Conclusion

ROTEM® tests, especially CT-ExTEM, can be used to estimate the effect of edoxaban. The results are available within just a few minutes and a prolonged ROTEM®-CT signifies a clinically relevant edoxaban effect on FXa. ROTEM® should be considered as a part of assessment of coagulation, with the big advantage of being readily available on site.

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Statement Regarding Accessibility of data

The dataset supporting the conclusions of this article is available on request by contacting the corresponding author.

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Figure 1. Coagulation cascade and coagulation tests. Activated partial thromboplastin time (APTT) measures the speed of coagulation by the intrinsic pathway, while prothrombin time (PT) evaluates the speed of clotting by the extrinsic pathway. Anti-Factor Xa activity (anti-FXa) shows how much the investigated drug inhibits the activated coagulation Factor X. ROTEM® delta analyzer (Instrumentation Laboratory, Werfen, Barcelona, Spain) enables a global assessment of hemostatic function in a timely manner. It gives information on the whole coagulation process in whole blood. Clotting time (CT) measures the time between the start of the analysis and the start of clot formation. Maximum clot firmness (MCF) gives information about the strength of the clot. Modified from Yada and Nogami, 2020; Hoffman, 2003.

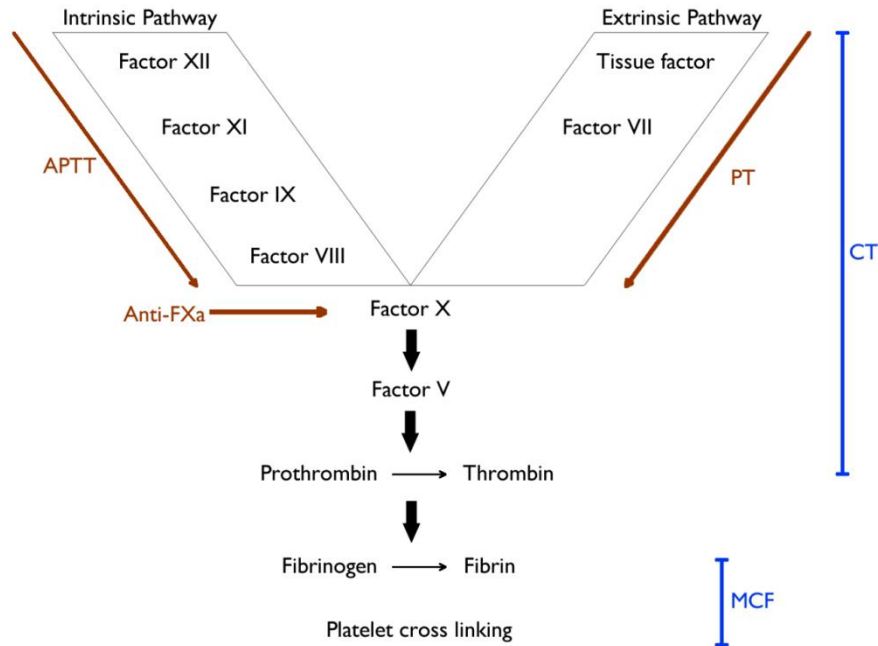
Figure 2. CT-ExTEM, CT-FibTEM, MCF-ExTEM and MCF-FibTEM at 2, 4, 6, 8 and 24 h after a single 60 mg dose of edoxaban. Each gray line represents an individual subject and the blue line represents the mean value.

Figure 3. APTT, TT and anti-FXa at 2, 4, 6, 8 and 24 h after a single dose of 60 mg edoxaban. Each gray line represents an individual subject and the blue line represents the mean value. Anti-FXa measurement was not calibrated for the study drug edoxaban and values exceeding 2 IU/mL were reported as “> 2 IU/mL”.

Figure 4. Edoxaban and metabolite M4 plasma concentrations at 2, 4, 6, 8 and 24 h after a single dose of 60 mg edoxaban. Each gray line represents an individual subject and the blue line represents the mean value.

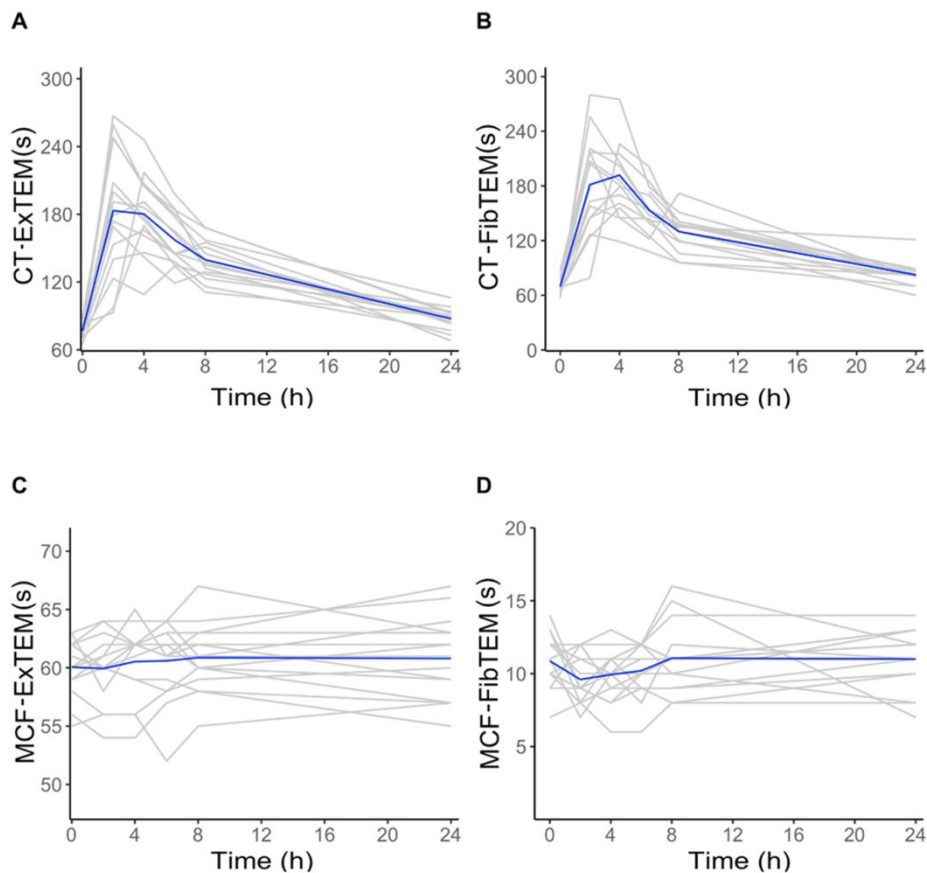
Figure 5. Correlations among plasma concentration of edoxaban and parameter values in ROTEM ExTEM® and FibTEM® and changes in standard coagulation tests, correlation of parameters in every time point are expressed in one plot.

Figure 6. Correlation between edoxaban plasma concentrations and changes from baseline in coagulation measurements (CT-ExTEM, CT-FibTEM, anti-FXa and APTT) after a single dose of edoxaban in the course of the study. Broken line represents correlation of 0.50. Anti-FXa measurement was not calibrated for the study drug edoxaban and values exceeding 2 IU/mL were reported as “> 2 IU/mL”.

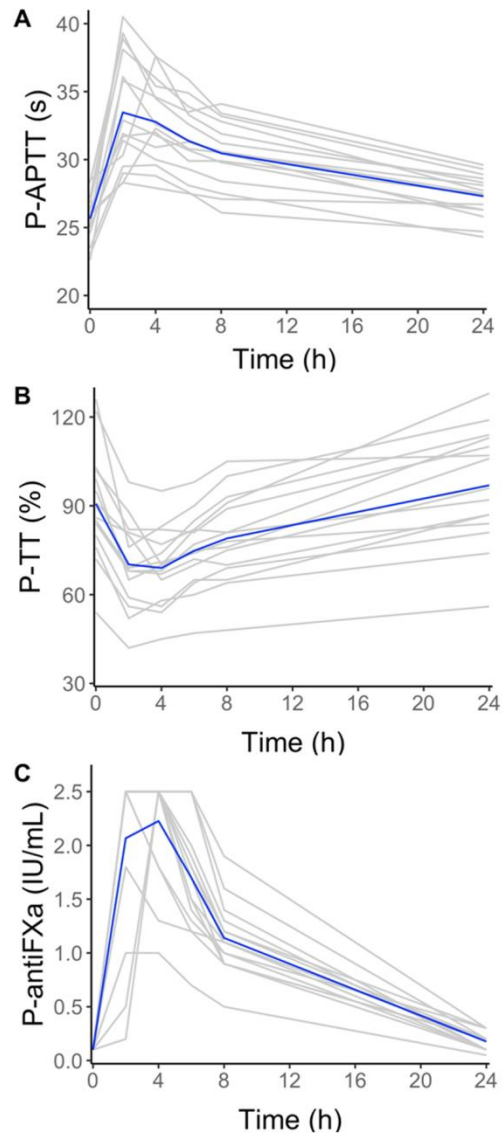


Coagulation cascade and coagulation tests. Activated partial thromboplastin time (APTT) measures the speed of coagulation by the intrinsic pathway, while prothrombin time (PT) evaluates the speed of clotting by the extrinsic pathway. Anti-Factor Xa activity (anti-FXa) shows how much the investigated drug inhibits the activated coagulation Factor X. ROTEM® delta analyzer (Instrumentation Laboratory, Werfen, Barcelona,

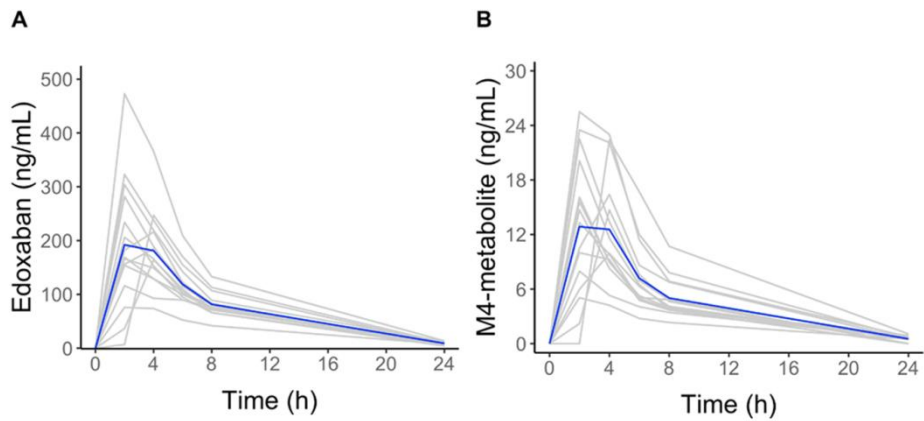
Spain) enables a global assessment of hemostatic function in a timely manner. It gives information on the whole coagulation process in whole blood. Clotting time (CT) measures the time between the start of the analysis and the start of clot formation. Maximum clot firmness (MCF) gives information about the strength of the clot. Modified from Yada and Nogami, 2020; Hoffman, 2003.



CT-ExTEM, CT-FibTEM, MCF-ExTEM and MCF-FibTEM at 2, 4, 6, 8 and 24 h after a single 60 mg dose of edoxaban. Each gray line represents an individual subject and the blue line represents the mean value.

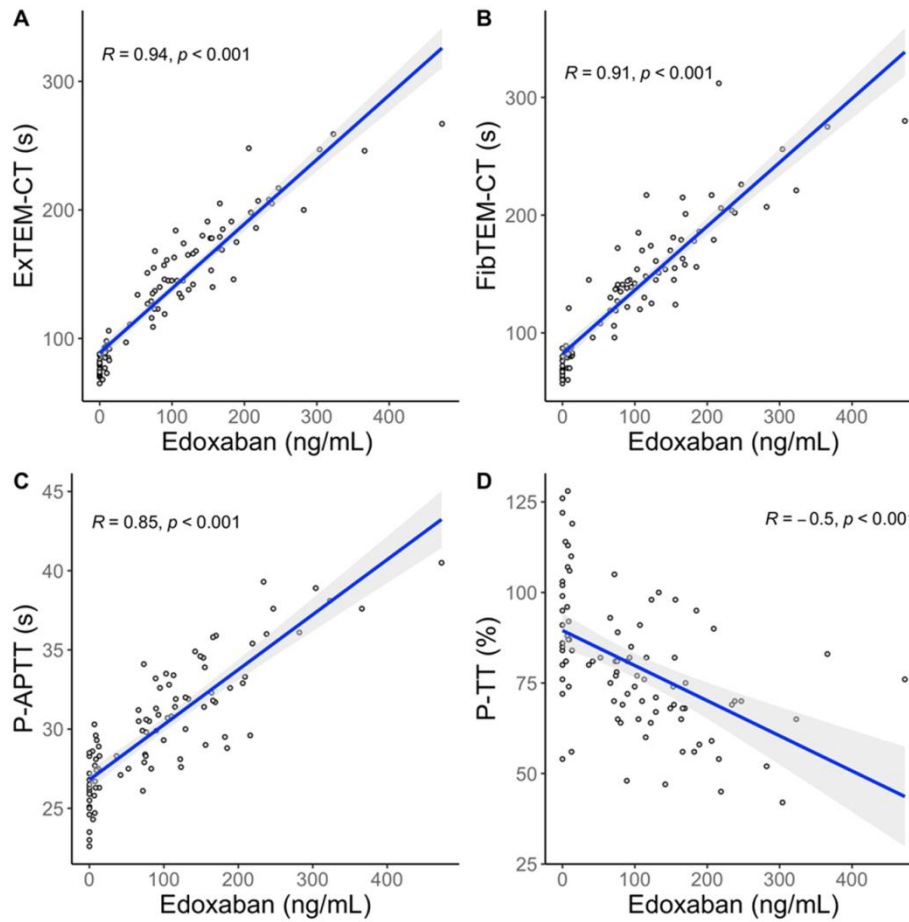


APTT, TT and anti-FXa at 2, 4, 6, 8 and 24 h after a single dose of 60 mg edoxaban. Each gray line represents an individual subject and the blue line represents the mean value. Anti-FXa measurement was not calibrated for the study drug edoxaban and values exceeding 2 IU/mL were reported as "> 2 IU/mL".

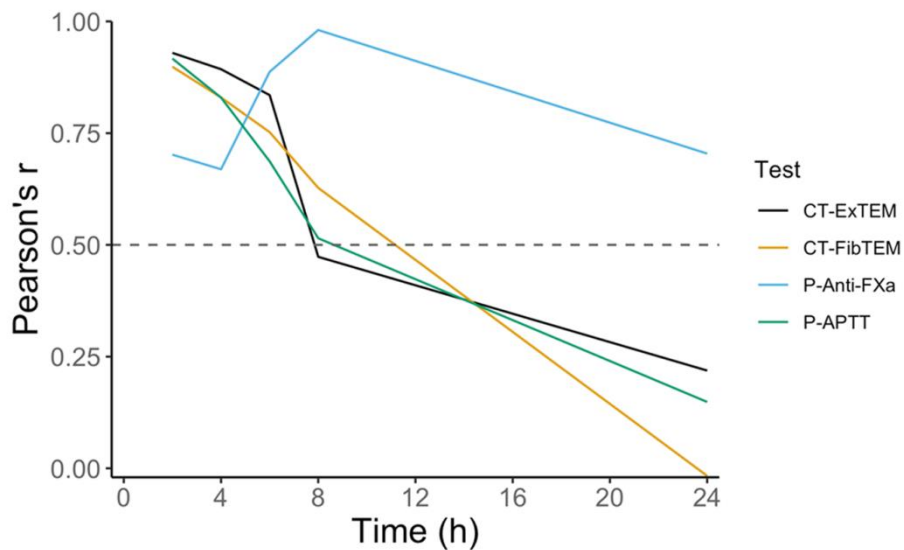


Edoxaban and metabolite M4 plasma concentrations at 2, 4, 6, 8 and 24 h after a single dose of 60 mg edoxaban. Each gray line represents an individual subject and the blue line represents the mean value.

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Correlations among plasma concentration of edoxaban and parameter values in ROTEM ExTEM® and FibTEM® and changes in standard coagulation tests, correlation of parameters in every time point are expressed in one plot.



Correlation between edoxaban plasma concentrations and changes from baseline in coagulation measurements (CT-ExTEM, CT-FibTEM, anti-FXa and APTT) after a single dose of edoxaban in the course of the study. Broken line represents correlation of 0.50. Anti-FXa measurement was not calibrated for the study drug edoxaban and values exceeding 2 IU/mL were reported as " \geq 2 IU/mL".

Table 1. EdoROT - Study day schedule

Time course (h)	0	1	2	4	6	8	24
Edoxaban 60 mg ingestion		x					
Blood sample							
APTT	x		x	x	x	x	x
PT	x		x	x	x	x	x
AntiFXa	x		x	x	x	x	x
Thrombin time	x		x	x	x	x	x
ROTEM® (ExTEM®, FibTEM®)	x		x	x	x	x	x
Edoxaban plasma concentration			x	x	x	x	x

Table 2. Correlation between the values for edoxaban and M4 AUC and the AUC values for the coagulation measurements (ExTEM, FibTEM, EXTEM, APTT, PT, AntiFXa).
 Table 2. Correlation between the values for edoxaban and M4 AUC and the AUC values for the coagulation measurements (ExTEM, FibTEM, EXTEM, APTT, PT, AntiFXa).

AUC values for the coagulation measurements	AUC values for edoxaban and M4 concentrations			
	Edoxaban	<i>P</i>	M4	<i>P</i>
CT-ExTEM	0.77	0.001	0.47	0.078
CT-FibTEM	0.57	0.025	0.29	0.289
MCF-EXTEM	0.54	0.038	0.34	0.217
MCF-FibTEM	0.36	0.187	0.20	0.484
APTT	0.52	0.047	0.56	0.030
PT	0.07	0.796	0.21	0.448
AntiFXa	0.91	< 0.001	0.76	0.001

