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ARTICLE

Development of immediate-release formulation with reliable absorption of rivaroxaban in various meal regimes

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Abstract

The bioavailability of rivaroxaban at the higher doses (15 and 20 mg) is considerably reduced when the drug is administered on an empty stomach. This can lead to inadequate anticoagulant effect, and therefore, it is recommended to use the higher doses at fed state. However, proper posology may represent a barrier for some patients. Therefore, the aim of this study was to evaluate innovative rivaroxaban-containing formulations designed to eliminate the food effect to ensure reliable absorption and thus to improve patient adherence with the treatment. Three prototypes (Cocrystal, HPMCP and Kollidon) with rivaroxaban were developed and their bioavailability and food effect in comparison to the reference product was tested in open label, randomized, single oral dose, crossover studies, where test products were administered under fasting and fed conditions and the reference product was administered under fed conditions. Comparable bioavailability for all tested prototypes both under fed and fasting conditions was demonstrated as the 90% confidence intervals of the geometric mean ratios for area under the concentration–time curve remained within the standard acceptance range of 80.00%–125.00%. An innovative immediate release form of rivaroxaban with no food effect on drug bioavailability has been developed, which may represent an important step toward increasing adherence, improving treatment outcome and reducing health care costs.

Study Highlights

WHAT IS THE CURRENT KNOWLEDGE ON THE TOPIC?

The bioavailability of rivaroxaban at the higher doses is considerably reduced when the drug is administered on an empty stomach. This can lead to inadequate anticoagulant effect, and therefore, it is recommended to use the higher doses at fed state. However, proper posology may represent a barrier for some patients.

WHAT QUESTION DID THIS STUDY ADDRESS?

The clinical program was focused on the development of innovative rivaroxaban-containing formulations with diminished food effect to ensure reliable absorption and thus to improve patients' adherence with the treatment.

WHAT DOES THIS STUDY ADD TO OUR KNOWLEDGE?

An innovative immediate release form of rivaroxaban with eliminated effect of food on drug bioavailability has been developed and bioequivalence irrespective of food administration with reference formulation under fed conditions has been documented in healthy human subjects.

HOW MIGHT THIS CHANGE CLINICAL PHARMACOLOGY OR TRANSLATIONAL SCIENCE?

Improved drug bioavailability may represent an important step toward increasing adherence to therapy and subsequently improving treatment outcomes and reducing health care costs spent for cardiovascular complications related to inadequate anticoagulation.

INTRODUCTION

The introduction of direct oral anticoagulants (DOACs) to the market in 2010 meant a great step forward in anticoagulant therapy.[1] In comparison with vitamin K antagonists, DOACs offer more predictable pharmacokinetics and pharmacodynamics, faster onset and resolution of the action, lower potential for interactions, and lower requirements for monitoring of anticoagulation effect.[2–4]

Rivaroxaban (Xarelto®; Bayer Pharma AG, Germany) is the second approved DOAC after dabigatran.[5] Its mechanism of action is the interruption of the coagulation cascade by blocking of activated coagulation factor X, followed by the inhibition of the conversion of prothrombin to thrombin.[6]

Rivaroxaban tablets are currently available in 4 strengths—2.5, 10, 15, and 20 mg with dose-specific indications. The lowest dose, 2.5 mg, is used in combination with aspirin for the prevention of coronary artery disease or peripheral artery disease, 10 mg rivaroxaban is indicated for the prevention of deep venous thrombosis following hip or knee replacement surgery and the prevention of venous thromboembolism, 15 mg dose of rivaroxaban is used for the treatment of deep venous thrombosis and pulmonary embolism, and 20 mg is given to the patients suffering from non-valvular atrial fibrillation.[7]

The pharmacokinetics of rivaroxaban is characterized by rapid absorption with C_{max} achieved 2 to 4 h after the administration, volume of distribution in steady state of 50 L, and a significant binding to serum albumin of 92%–95%. Approximately, one-third of rivaroxaban dose is eliminated in an unchanged form by kidneys, the remaining two-thirds are metabolized into inactive products in the liver. A half of these inactive metabolites then undergoes renal excretion, while the other half undergoes hepatobiliary excretion.[4,8]

Both the anticoagulant effect and exposure were described as dose-dependent but only if the drug is administered at fed state. The effect of food on rivaroxaban bioavailability is an atypical phenomenon dependent on the administered dose. For lower doses up to 10 mg, the absolute bioavailability of rivaroxaban is high (80%–100%) regardless of food administration. However, the bioavailability of the highest 20 mg dose reaches more than 80% if administered with food and decreases to only 66% when the drug is administered on an empty stomach.[2,4,9] The decreased bioavailability can lead to inadequate anticoagulant effect and treatment failure, which may be fatal, therefore, it is recommended to use the higher doses of 15 and 20 mg at fed state.[7] Even this seemingly uncomplicated need to administer rivaroxaban after a meal may be a barrier for some patients with respect to proper posology.

Our aim was to develop innovative rivaroxaban-containing formulations with a diminished food effect to ensure reliable absorption and thus to improve patient adherence with the treatment. Our initial hypothesis was that inadequate bioavailability of rivaroxaban in fasted state is caused by suboptimal solubility which could be alleviated via modification of drug substance (Cocrystal) or solid-state characteristics (amorphous solid dispersions with HPMCP or Kollidon). We report

pharmaceutical and clinical development of novel rivaroxaban formulations lacking the food effect.

MATERIALS AND METHODS

Preparation of formulations

Cocrystal prototype

Rivaroxaban Cocrystal was homogenized with lactose monohydrate, sodium cross carmellose, sodium lauryl sulfate, silicified cellulose, and sodium stearyl fumarate. The homogenized mixture was compacted to obtain a granulate. More detailed information regarding preparation and characterization of this prototype has been published.[10]

HPMCP prototype

The solid solution of rivaroxaban and HPMCP was prepared by spray-drying process. The weight ratio of the prepared solid solution was 1:3 (rivaroxaban:HPMCP). The rivaroxaban and the polymer in the mentioned weight ratio were dissolved in organic solvent/s (e.g., 2,2,2-trifluoroethanol). The prepared solution was spray dried using lab scale spray dryer Büchi B 290 with the following process parameters: inlet temperature of 90–100°C, feed rate of 1 kg/h, air atomization pressure of 40 m^3/h and aspiration 85%. The product was then dried in vacuum oven at 45°C. The dried material was homogenized with lactose monohydrate, sodium cross carmellose, sodium lauryl sulfate, silicified cellulose, and sodium stearyl fumarate. The homogenized mixture was compacted to obtain a granulate.

Kollidon prototype

The solid solution of rivaroxaban, Kollidon VA64, and Polysorbate 80 was prepared by hot melt extrusion process. The weight ratio of the prepared solid solution was 1:4.5:0.055 (rivaroxaban:Kollidon VA64:Polysorbate 80). All the components of solid solution were homogenized in high-shear mixer. The homogenized mixture was then extruded using lab scale hot melt extruder Three-Tec, 12 mm with the following process parameters: temperature of heating segments of 140–200°C, screws speed of 50 rpm and feed rate of 200 g/h. The extrudate was then milled and homogenized with lactose monohydrate, sodium cross carmellose, sodium lauryl sulfate, silicified cellulose, and sodium stearyl fumarate. The homogenized mixture was compacted to obtain a granulate.

Reference

For dosing the rats, the original drug product (Xarelto, Bayer AG, Leverkusen, Germany) was crushed into powder and filled into the 9el gelatin capsules (Harvard Apparatus, Holliston, USA) as a reference formulation. Rivaroxaban content in one capsule was 4 mg.

In clinical studies, Xarelto® 20 mg film-coated tablets (Bayer Pharma AG), were used as a reference product.

For the clinical study, the final granulates of all tested prototypes were filled into hypromellose capsules with rivaroxaban content in one capsule of 20 mg, while for the preclinical study, the final granulates of all tested prototypes were filled into 9el gelatin capsules with rivaroxaban contents of 4 mg per capsule.

Dissolution experiments

Dissolution pH shift experiment was conducted using standard USP 2 dissolution bath equipped with a paddle apparatus using equipment from Sotax (Aesch, Switzerland). The medium was preheated to 37°C and the stirrer speed was 75 rpm. The experiment was initiated in 600 mL of 10 mM HCl pH 2 and after 30 min, concentrated FaSSIF buffer was added to the dissolution vessel to obtain 900 mL of FaSSIF buffer, pH 6.5. Formulations were administered directly into the dissolution vessel. The weight of rivaroxaban corresponded to 20 mg. Liquid samples (500 µL) were collected in predefined timepoints, filtered through a 0.45-µm filter, immediately diluted with 500 µL of MeOH, and analyzed on Waters Acquity UPLC system equipped with PDA detector and XBridge C18 column (3.5 µm; 4.6 × 50 mm). The following gradient of 30 mM $NH_4H_2PO_4$ pH 6.8/MeOH at flow rate 1.2 mL/min was used: linear change from 80/20 to 5/95 (0–2.5 min) followed by linear change to starting conditions (2.5–3 min) followed by steady state for 1 min to re-equilibrate. The data were processed using the Empower software.

Preclinical study

Animals

The experiment was conducted in accordance with the Guiding Principles for the Use of Animals at the Charles University, First Faculty of Medicine. Every effort was made to minimize animal suffering. The experimental animal project was approved by the Ministry of Education, Youth and Sports, Czech Republic under No. MSMT-11957/2021-4.

Male Wistar Rats were purchased from Velaz (Prague, Czech Republic). They were housed under standard conditions with 12-h light–dark cycle, temperature of 22 ± 2°C, relative humidity of 50 ± 10% and fed ad libitum by a standard granulated diet and water.

Experimental design and procedures

As the superiority of this design was previously confirmed,[11] a randomized, single dose, laboratory blinded, two-period, two-sequence, crossover study was conducted in rats to compare bioavailability of each new formulation with a reference product.

Totally, 36 adult rats were included into the study, 12 animals to test each formulation. The rats were randomly assigned into study groups according to the randomization protocol. There were six study groups of six rats each. Dosing sequences in each group were as follows: Reference—Cocrystal prototype (group 1), Cocrystal prototype—Reference (group 2), Reference—HPMCP prototype (group 3), HPMCP prototype—Reference (group 4), Reference—Kollidon prototype (group 5), Kollidon prototype—Reference (group 6).

Three days before the first dosing (period 1), right *v. jugularis* of each rat was cannulated. Anesthesia for cannulation was induced by 2.5%–5% isoflurane (IsoFlo 250 mL, Zoetis, Czech Republic) and maintained by a combination of xylazine (Rometar 20 mg/mL inj sol, Bioveta, Czech Republic) 5 mg/kg and ketamine (Narkamon 100 mg/mL inj sol, Bioveta, Czech Republic) 100 mg/kg intramuscularly. Ketoprofen (Ketodolor 100 mL inj, LeVet Beheer B. V., Netherlands) 5 mg/kg was administered subcutaneously at the end of the surgery. Jugular vein catheters 3 Fr (Instech Laboratories, Plymouth Meeting, USA) were subsequently flushed with 200 µL of physiological saline, 50 µL of diluted heparin (1250 IU/mL) (Heparin Léčiva 1 × 10 mL/50KU inj, Zentiva, k. s., Czech Republic), and finally sealed with mixture of glycerol with heparin (250 IU/mL).

After 3 days of recovery (period 1), one capsule of rivaroxaban formulation was administered orally to each rat, using X-9el dosing syringe (Torpac, Farfield, USA). Immediately after drug dosing, each rat was given 1 mL of water by oral gavage. Food was removed 4 h before the dosing and the rats did not have access to it for another 4 h to achieve fasted state. Blood samples were collected via catheters before dosing and at following timepoints: 0.5, 1, 1.5, 2, 2.5, 3, 4, 6 and 8 h after drug administration. Totally 100 µL of blood was taken at each timepoint, followed by intravenous administration of physiological saline (100 µL) and a mixture of saline with heparin (1250 IU/mL, 50 µL). The blood samples were centrifuged (4500 × *g*, 4°C, 10 min), serum was stored at −80°C until analysis. After a 48-h washout period, the procedures (period 2) were identical, but with a different formulation than in the first period according to the randomization.

Bioanalytical methods

Rivaroxaban concentration in serum was determined by sensitive and fully validated UHPLC–MS/MS (ultra-high performance liquid chromatography–tandem mass spectrometry) with an isotopically labeled internal standard as we previously described.[10] Briefly, measurements were carried out on the Nexera X3 UHPLC coupled with Triple Quad 8045 MS (Shimadzu, Kyoto, Japan). Poroshell 120 SB-AQ column (100 × 2.1 mm; 2.7 µm; Agilent Technologies, Santa Clara, CA, USA), thermostated at 40°C, was used for the analysis. The mobile phase consisted of 0.1% formic acid in deionized water (Solvent A) and acetonitrile with 0.1% formic acid (Solvent B). The flow rate of the mobile phase was 0.4 mL/min, and the injection volume was 1 µL. The optimized gradient elution was carried out as follows (min/% B): 0/30, 3.0/70, 4.0/70, 4.5/30, and 7.0/30. The samples were kept at 5°C. The tandem mass spectrometry measurement was performed in selected reaction-monitoring mode (SRM) using positive electrospray ionization. SRM transitions of 436.1 > 145.0 (Q1 pre-bias −12 V, Q3 pre-bias −27 V and collision energy −30 V) and 440.1 > 145.0 (Q1 pre-bias −22 V, Q3 pre-bias −25 V and collision energy −25 V) were monitored for rivaroxaban and rivaroxaban-d4, respectively. The ion source was set as follows: nebulizing gas flow: 3 L/min, heating gas flow: 10 L/min, interface temperature: 300°C, desolvation line temperature: 250°C, heat block temperature: 400°C, and drying gas flow: 10 L/min. 60 µL of 100% acetonitrile (containing rivaroxaban-d4 as internal standard at a concentration of 50 ng/mL) was added to 20 µL of serum, shaken (vortex) and centrifuged (8 min/8750 rcf). 40 µL of supernatant was transferred into the chromatographic vial.

Clinical study

Human subjects

Two clinical studies were reviewed and approved by Institutional Review Board of ACDIMA Center for Bioequivalence and Pharmaceutical Studies (Amman, Jordan). These studies were conducted between July 2021 and September 2021 at ACDIMA Center, in accordance with the Good Clinical Practice, applicable regulatory requirements, and the ethical principles that origin in the

Declaration of Helsinki. All subjects gave informed consent prior to study participation.

The eligibility of the subjects was based on medical history, vital signs, physical examination, and laboratory tests. Healthy Caucasian males, aged between 18 and 40 years with a body mass index between 18 and 30 kg/m^2 were eligible to participate in these studies. Subjects were ineligible if they had history of hypersensitivity and/or contraindication to the study medication, positive test results for hepatitis B/C or human immunodeficiency virus, history, or presence of chronic diseases. Subject's kidney and liver functions tests, and coagulation tests were within normal range. Subject was not vegetarian or a heavy smoker (smokes more than 10 cigarettes per day), did not suffer an acute illness 1 week before dosing, did not have a history of or concurrent abuse of alcohol or illicit drugs. Subject did not take a prescription medication within 2 weeks. Caffeine/xanthine and grapefruit were restricted 2 and 7 days prior to study, respectively, and any time during the study.

Experimental design and procedures

The bioavailability and food effect of HPMCP and Kollidon prototypes in comparison to Reference was tested in an open label, randomized, single oral dose, three-treatment, five-sequence and five-period crossover study with a washout interval of 7 days between dosing, where test products were administered under fasting and fed conditions and the reference product was administered under fed conditions (clinical study 1). Subjects were equally distributed to the five possible sequences (T1T2T3T4R, T2T4RT3T1, T3RT2T1T4, T4T3T1RT2, RT1T4T2T3; T1 for HPMLC prototype under fasting conditions, T2 for Kollidon prototype under fasting conditions, T3 for HPMLC prototype under fed conditions, T4 for Kollidon prototype under fed conditions and R for Reference under fed conditions) using the SAS 9.4 software (SAS Institute, NC, USA). Similarly, the bioavailability and food effect of the Cocrystal prototype was compared with the Reference in an open label, randomized, single oral dose, two-treatment, three-sequence and three-period crossover study with a washout interval of 7 days between dosing, when test product were administered under fasting and fed conditions and reference product was administered under fed conditions (clinical study 2). Subjects were equally distributed to the three possible sequences (RT1T2, T1RT2, T1T2R, T1 for Cocrystal prototype under fasting conditions, T2 for Cocrystal prototype under fed conditions and R for Reference under fed conditions). After an overnight fast of 10–12 h and in the morning of the second day of each study period, the study products were administered orally in a randomized fashion with 240 mL of water to the subjects that have taken the treatment under fasting condition. Whereas for the subjects that have taken the treatment under fed condition, in the morning of the second day of each study period, standardized breakfast was served, and the study products were similarly administered orally in a randomized fashion with 240 mL of purified water. Blood samples were collected into lithium heparin tubes pre-dosing (zero time) and at 0.33, 0.67, 1.00, 1.33, 1.67, 2.00, 2.33, 2.67, 3.00, 3.50, 4.00, 4.50, 5.00, 5.50, 6.00, 8.00, 10.00, 12.00, 16.00, 24.00, 36.00, and 48.00 h post-dosing. The blood samples were placed in an ice bath until centrifugation at 2688 × g for 5 min at 10°C. Supernatant plasma was transferred to polypropylene tubes and stored at −20°C.

Bioanalytical methods

The bioequivalence studies were conducted in ACDIMA Center for Bioequivalence and Pharmaceutical Studies; Amman, Jordan. Rivaroxaban concentration in plasma was determined as follows. A volume of 200 µL of human plasma was spiked with 20 µL of rivaroxaban-d4 (internal standard at a concentration of 6 µg/mL); 1000 µL of acetonitrile was added to each sample, vortexed and then centrifuged for 5 min at 3428 rcf at 5°C. Supernatant was measured by HPLC-MS/MS on Infinity 1260 LC (Agilent Technologies, Germany) coupled with Triple Quad 4500 mass spectrometer detector (AB Sciex, Canada). SVEA Plus-C18 column (150 × 4.6 mm; 5 µm; Nanologica AB, Sweden), thermostated at 40°C, was used for the analysis. The mobile phase consisted of deionized water and acetonitrile in a ratio of 80/20 (*v*/*v*) with 0.1% trifluoracetic acid with flow of 0.8 mL/min under isocratic elution. The injection volume was 10 µL. The samples were kept at 5°C. The tandem mass spectrometry measurement was performed in selected reaction-monitoring mode (SRM) using positive electrospray ionization. SRM transitions of 436.0 > 144.8 and 440.0 > 144.8 were monitored for rivaroxaban and rivaroxaban-d4, respectively. The ion source temperature was set to 550°C and ion spry voltage to 5500 V. The method was validated according to the Guidance for Industry, Bioanalytical Method Validation, FDA,[12] Guideline on Validation of Bioanalytical Methods, EMA[13] and applicable Good Laboratory Practices (GLP).

Data analysis and statistics

Due to the higher variability in the weight of rats, measured rivaroxaban serum concentrations in animal study were normalized to the weight of each rat before further

processing. In the clinical study, absolute concentration values were used. Comparison of mean concentration in each sampling time was performed using paired *t*-test in GraphPad Prism version 9.1.0. (GraphPad Software, San Diego, CA, USA). Significance was considered at $p \leq 0.05$. Phoenix WinNonlin 8.3 (Certara, Princeton, USA) was used for pharmacokinetic and equivalence analysis. Pharmacokinetic parameters—area under the concentration–time curve from zero to last measured concentration (AUC_{last}), area under the concentration–time curve from zero to infinity (AUC_{∞}), maximum serum concentration (C_{max}) and time to maximum serum concentration (t_{max}) were evaluated. The natural logarithmic transformation of AUC_{last}, AUC_{∞} and C_{max} was used for all statistical inference. AUC_{last} was calculated using the trapezoidal rule, while C_{max} and t_{max} were taken directly from the observed data. AUC_{∞} was calculated from the sum of AUC_{last} and extrapolated AUC from last measured concentration to infinity, calculated as C_{last}/terminal elimination rate constant, where terminal elimination rate constant was estimated for each subject and for each treatment via linear regression of at least three last points at the terminal phase of the log-concentration versus time curve. The ln-transformed pharmacokinetic parameters were analyzed using an ANOVA (analysis of variance) model where the effects of subject, treatment, period, and sequence were included as the fixed factors. The 90% confidence interval for the ratio of least-squares means between each test and reference products was calculated. Descriptive statistics included arithmetic mean, median, maximum and minimum values, and standard deviation (SD).

RESULTS

Dissolution experiment

To mimic transfer of rivaroxaban from a fasting stomach to a fasting intestine, a pH shift experiment was designed. The results of the large volume (from 600 to 900 mL) pH shift (from pH 2 to pH 6.5) dissolution experiment are presented in Figure 1. This experiment captures well the differences in behavior of tested formulations. For the dissolution of Reference (Xarelto) containing crystalline rivaroxaban, the change of the media volume and composition (pH, bile concentration) does not seem to play a significant role and only around 50% of administered drug is dissolved by the end of the experiment. However, for the test prototypes, the situation is more interesting. The increase in volume of dissolution media allows complete dissolution of the Cocrystal prototype and dissolution up to 85% for the Kollidon prototype. The dissolution of HPMCP prototype in the first stage of experiment is limited by the polymer matrix as HPMCP-HP55 is poorly soluble in acidic pH. The pH shift to neutral pH then allows complete dissolution of this prototype. As the test prototypes are essentially supersaturating systems, it is possible that after media change, some newly formed nanostructures passed through the filters and reached the collecting vials thus increasing concentration in the measured samples – notice that for some timepoints, fraction dissolved exceeds 100%. To account for the experimental error, general trends were confirmed by separate experiment where UV probes were used (data not shown). However, such an experiment cannot be used to show precise dissolution data as phthalate present in HPMCP prototype absorbs light. Overall, this

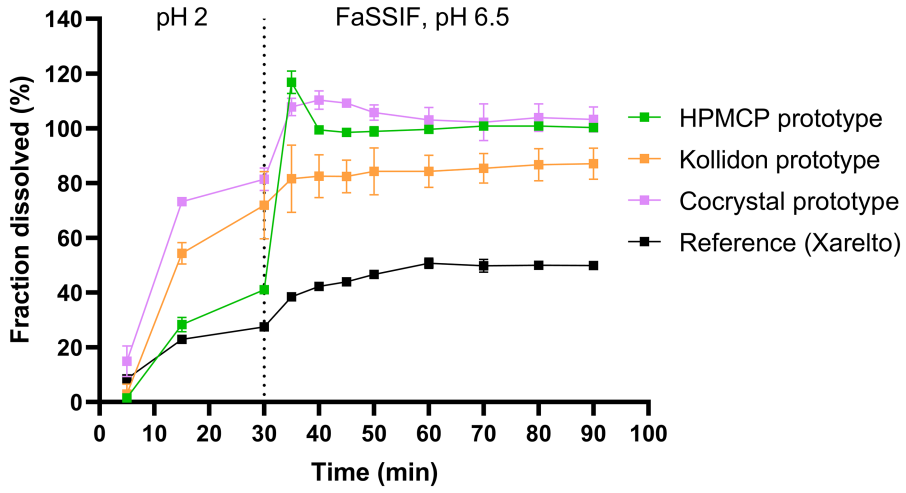

FIGURE 1 Mean ± SD in vitro dissolution profiles of reference and tested formulations in the large volume (from 600 to 900 mL) pH shift (from pH 2 to pH 6.5) dissolution experiment.

experiment shows the potential of each formulation to solubilize rivaroxaban and maintain the achieved effect upon transfer to the intestine-mimicking medium.

Preclinical study

Pharmacokinetic profiles from all 36 rats were collected (six rats per group). Rivaroxaban serum concentrations before dosing were all below the limit of quantification (1 ng/mL), excluding carry-over effect and ensuring that the washout period was sufficient.

Detailed information about rivaroxaban pharmacokinetic parameters and time–concentration profiles from bioavailability study with Cocrystal prototype in rats has been described previously.[10] Pharmacokinetic parameters and profiles from studies with HPMCP and Kollidon prototypes are summarized in Table 1 and Figure 2.

While C_{max} and AUC_{last} values observed after administration of Cocrystal prototype were approximately twice higher than after the reference product, both pharmacokinetic parameters obtained after the administration of HPMCP and Kollidon prototypes were approximately four-fold higher in comparison with reference formulation. Moreover, the 90% CI excluded the null difference as well as standard equivalence margins 80%–125% in all tested formulations. There was no significant difference in the t_{max} values between Reference and Cocrystal prototype, and between Reference and HPMCP prototype ($p = 0.6625$ and $p = 0.5748$, respectively), while Kollidon prototype showed significantly shorter t_{max} value than the reference ($p = 0.0424$). It means that Cocrystal and HPMCP prototypes have similar rate of absorption as original drug product and increased bioavailability, while the Kollidon prototype has increased both rate of absorption and bioavailability.

TABLE 1 Pharmacokinetic parameters and results of bioequivalence comparison of rivaroxaban formulations in preclinical study under fasting condition.

Formulation	C_{max} (ng/mL.g)	T/R C_{max} (%)	AUC_{last} (ng/mL.min.g)	T/R AUC_{last} (%)	t_{max} (h)
Reference (Xarelto)	0.913 ± 0.316	N/A	257.0 ± 101.7	N/A	3.52 ± 2.22
HPMCP prototype	3.628 ± 1.817	369.1 (293.3–464.5)	915.8 ± 386.7	349.1 (270.5–450.6)	3.10 ± 1.20
Reference (Xarelto)	1.051 ± 0.293	N/A	266.3 ± 61.61	N/A	4.44 ± 2.34
Kollidon prototype	4.628 ± 3.886	345.2 (248.6–479.3)	1206.0 ± 928.7	368.7 (274.2–495.7)	2.62 ± 1.24

Note: Values are given as arithmetic mean ± SD or geometric least-square means ratio with 90% confidence interval. Information about rivaroxaban pharmacokinetic parameters from bioavailability study with Cocrystal prototype in rats has been described previously.[10]

Abbreviations: R, reference formulation; T, tested formulation.

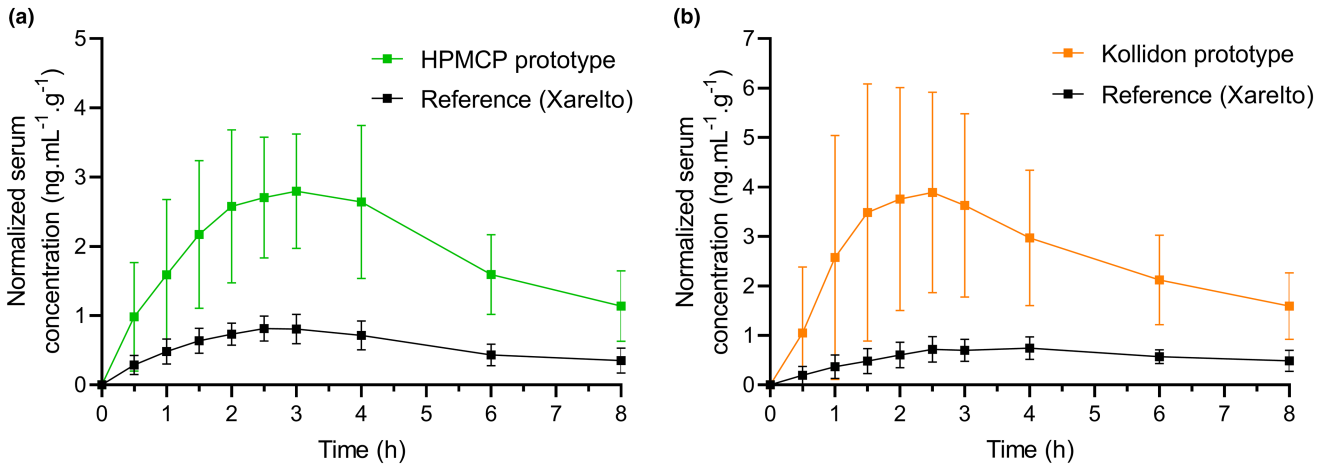

FIGURE 2 Mean (95% confidence interval) rivaroxaban pharmacokinetic profiles following single oral dose of rivaroxaban 4 mg formulated as Reference (Xarelto), HPMCP prototype (a) and Kollidon prototype (b) to rats. Information about rivaroxaban pharmacokinetic profiles from bioavailability study with Cocrystal prototype in rats has been described previously.[10]

	Study 1 ($n = 19$)		Study 2 ($n = 20$)	
	Mean ± SD	Median (range)	Mean ± SD	Median (range)
Age (years)	28.9 ± 6.7	28.0 (18–38)	27.2 ± 5.7	28.5 (18–38)
Body weight (kg)	70.7 ± 12.8	70.0 (52.0–92.0)	69.9 ± 10.7	68.0 (55.0–90.0)
Height (m)	1.73 ± 0.07	1.73 (1.57–1.89)	1.75 ± 0.06	1.75 (1.63–1.89)
Body mass index (kg/m^2)	23.6 ± 3.7	22.9 (19.0–29.7)	22.8 ± 3.0	21.7 (18.8–29.4)

TABLE 2 Summary of subjects' demographic data in clinical studies.

Clinical study

A total of 20 subjects were enrolled to the clinical study 1, of which 19 subjects were included in the statistical analysis (one subject withdrew before the administration of reference product) and 17 subjects completed all periods. There were 21 participants enrolled to the clinical study 2, of which 20 were included to the statistical analysis (one subject withdrew after first drug dosing), and 19 subjects completed all phases. Demographic data of subjects in both clinical studies are summarized in Table 2. Summary of adverse events/adverse drug reactions is given in Table S1.

Summary of rivaroxaban pharmacokinetic parameters following single administration of 20 mg dose in various formulations under fasted or fed conditions is given in Table 3.

The 90% CIs for the ratios of C_{max}, AUC_{last} and AUC_{∞} were within the standard bioequivalence criteria (80%–125%) except for Kollidon and Cocrystal prototypes under fasting condition. Pharmacokinetic profiles from clinical studies are presented in Figure 3a–c.

DISCUSSION

We have developed an innovative dosage form of rivaroxaban with no effect of food on drug bioavailability. The HPMCP prototype has demonstrated comparable bioavailability of the highest 20 mg strength administered both at fasted as well as fed states with reference formulation of the same strength administered at fed state. The other two prototypes, i.e. Kollidon and Cocrystal drug formulations have shown similar extent of exposure at both fasted and fed states with the reference formulation administered at fed state, but the absorption rate was slightly higher, as reflected by exceeding the standard bioequivalence limits for C_{max} in the fasted state in the clinical study. Overall, the HPMCP prototype delivers reliable drug exposure after more convenient posology regardless of timing of drug administration and food consumption resulting in simplified therapy.

Non-adherence to prescribed therapeutic regimen is induced by multiple factors, but a demanding medication regimen belongs among major reasons limiting patients' willingness to adhere with the treatment.[14] Adherence to rivaroxaban, as well as other DOACs, has been shown to significantly improve prognosis of patients with atrial fibrillation/flutter in terms of reduced risks of ischemic stroke, deep vein thrombosis and pulmonary embolism.[15,16] Although rivaroxaban development included efficacy studies where Xarelto was administered with food, there are no studies that specifically address the exact effect of the meal timing or composition with regards to patient outcomes. A few case reports of serious consequences of therapeutic failure of rivaroxaban due to nutrition-related drug malabsorption have been reported.[7,17] In general, the non-adherence to any treatment is high with 30%–50% of patients being considered non-compliant, which results in worse therapeutic outcome, high number of hospital admissions, and huge economic costs.[18–20] Specifically for rivaroxaban, a retrospective study showed treatment non-adherence due to inadequate meal content in 33.6% of patients and incorrect dosing in 6.9% of patients.[21] Therefore, simplification of rivaroxaban posology is potentially an important step toward increasing adherence to the therapy and subsequently improving treatment outcomes and reducing health care costs spent related to inadequate anticoagulation. Our food-independent rivaroxaban capsule alleviates issues caused by meal-related non-adherence and thus ensures more reliable safety and efficacy for patients compared with standard rivaroxaban products.

The ability to predict in vivo performance of new rivaroxaban-containing formulations represent a major challenge.[22] Rivaroxaban is a BCS Class II drug substance, for which dissolution is the rate-limiting step in drug absorption. Therefore, conventional in vitro drug release data reflecting biologically relevant conditions should be predictive for in vivo plasma concentration; however, this approach could not be applied in our case with formulations where rivaroxaban solubility is enhanced. We used atypical dissolution media and in vitro method to mimic key in vivo controlling processes for rivaroxaban dissolution, and the presented in vitro data corresponds well

TABLE 3 Pharmacokinetic parameters and results of bioequivalence comparison of rivaroxaban formulations under fasting and fed conditions in clinical studies.

	Formulation	C_{max} (ng/mL)	T/R C_{max} (%)	AUC_{last} (ng. h/mL)	T/R AUC_{last} (%)	AUC_{∞} (ng. h/mL)	T/R AUC_{∞} (%)	t_{max} (h)
Study 1	Reference (Xarelto) Fed condition	329.86 ± 54.86	N/A	2587.2 ± 454.2	N/A	2618.5 ± 455.4	N/A	3.29 ± 1.33
	HPMCP prototype Fasting condition	360.93 ± 87.32	107.8 (97.6–119.2)	2472.2 ± 528.6	95.1 (89.9–100.5)	2496.7 ± 525.8	94.9 (90.1–100.0)	3.12 ± 0.88
	HPMCP prototype Fed condition	347.19 ± 57.97	106.2 (96.0–117.6)	2692.8 ± 672.9	103.4 (97.7–109.5)	2714.3 ± 670.3	103.0 (97.7–108.7)	4.15 ± 1.23
	Kollidon prototype Fasting condition	391.98 ± 107.16	118.5 (106.8–131.5)	2571.6 ± 546.0	101.5 (95.8–107.5)	2623.0 ± 528.5	102.5 (97.1–108.2)	2.65 ± 0.79
	Kollidon prototype Fed condition	333.51 ± 68.12	99.9 (90.4–110.4)	2560.9 ± 477.6	98.9 (93.6–104.6)	2589.7 ± 480.4	98.9 (93.8–104.2)	4.18 ± 1.33
Study 2	Reference (Xarelto) Fed condition	338.45 ± 63.42	N/A	2578.7 ± 559.8	N/A	2597.8 ± 560.7	N/A	2.98 ± 1.35
	Cocrystal prototype Fasting condition	432.82 ± 111.72	124.9 (115.0–135.6)	2694.7 ± 730.5	103.1 (96.8–109.8)	2719.2 ± 732.2	103.3 (97.0–110.0)	2.13 ± 0.97
	Cocrystal prototype Fed condition	362.38 ± 76.86	105.7 (97.6–114.5)	2578.7 ± 559.8	102.0 (95.9–108.4)	2597.8 ± 560.7	102.0 (95.9–108.4)	2.98 ± 1.35

Note: Values are given as arithmetic mean ± SD or geometric least-square means ratio with 90% confidence interval.

Abbreviations: R, reference formulation; T, tested formulation.

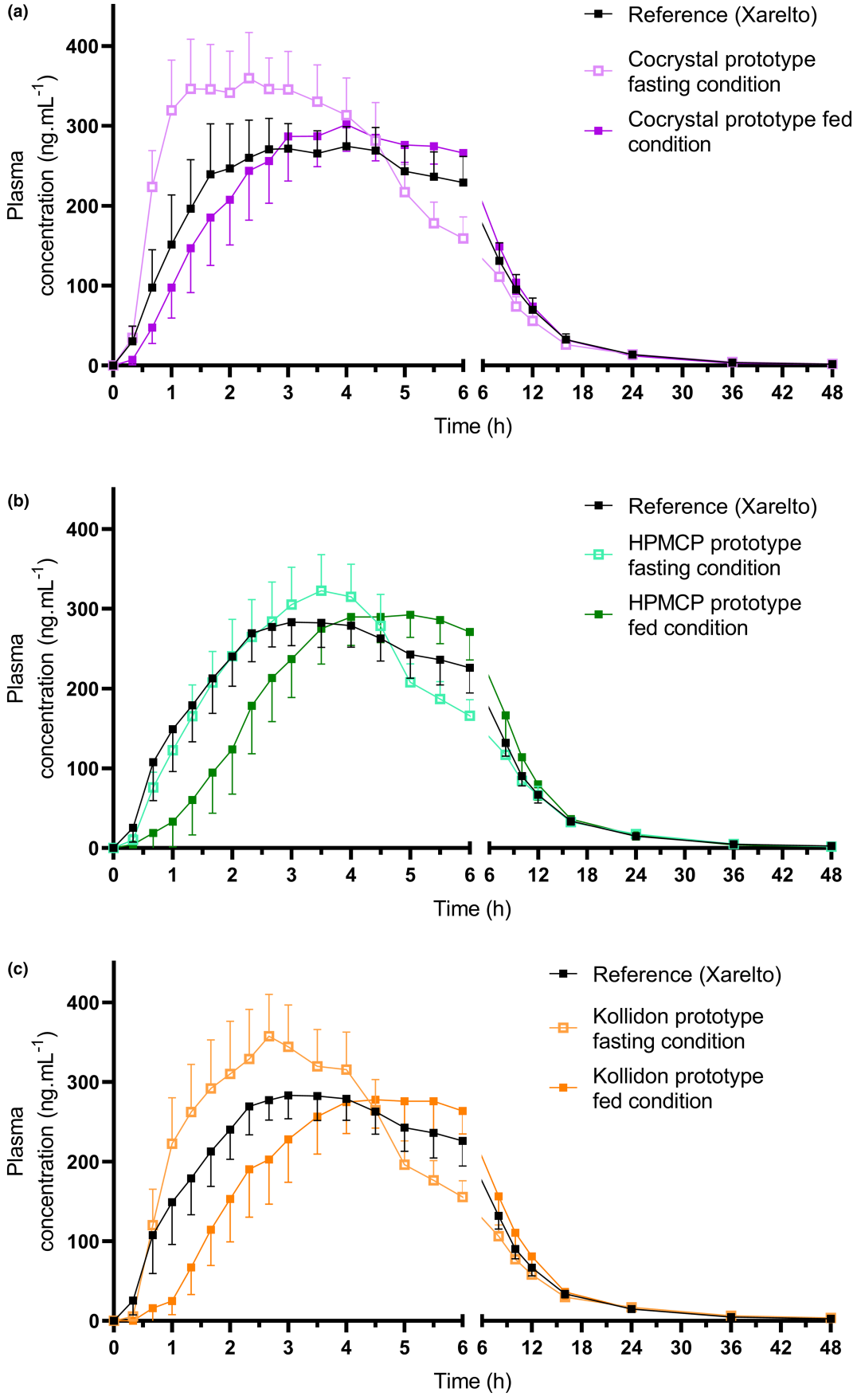

FIGURE 3 Mean (95% confidence interval) rivaroxaban pharmacokinetic profiles following single oral dose of rivaroxaban 20 mg formulated as Reference (Xarelto), Cocrystal (a), HPMCP (b), and Kollidon prototype (c) under fasting or fed conditions to human subjects.

to the comparative bioavailability data in rats. However, sufficient predictability for the true in vivo formulation performance in humans has not been achieved using this methodology. This may be caused by local precipitation of rivaroxaban and thus reduced bioavailability of the drug from reference formulation due to considerably higher dose used in the preclinical study (10 mg/kg). This dose has been based on previously reported animal data that reported even lower serum concentrations compared with those in man after 20 mg dosing.[23] The difference in solubilization capacity for such a high dose in limited volume in the rat gastrointestinal tract is likely an explanation for the insufficient predictability to human bioavailability study data, as well as the previously published discrepancy between the expected exposures after the human equipotent dose correction factor of 6:1 and the 10 mg/kg vs 20 mg doses.[23,24] Nevertheless, the rodent data do show improved bioavailability of our tested prototypes versus the crystalline reference and thus this methodology can be considered as a useful tool in the early phases of pharmaceutical development.

CONCLUSION

An innovative immediate release form of rivaroxaban with eliminated effect of food on drug bioavailability has been developed and bioequivalence irrespective of food administration with reference formulation under fed conditions has been documented in healthy human subjects. Improved drug bioavailability may represent an important step toward increasing adherence to therapy and subsequently improving treatment outcomes and reducing health care costs spent for cardiovascular complications related to inadequate anticoagulation.

AUTHOR CONTRIBUTIONS

M.Š., J.R., P.K., To.Kř., Te.Kr., I.O., Ja.Bo., and O.S. wrote the manuscript. Ja.Bo. and T.H. designed the research. P.K., To.Kř., Te.Kr., Jo.Be., and I.O. performed the research. Ja.Bo., M.Š., J.Š., T.H., and O.S. analyzed the data.

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CONFLICT OF INTEREST STATEMENT

JaBo, TK, IO, JoBe and TH are employees of Zentiva, k.s., Prague, Czech Republic, that sponsored the development program. All other authors declared no competing interests for this work.

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REFERENCES

1. Ganetsky M, Babu KM, Salhanick SD, Brown RS, Boyer EW. Dabigatran: review of pharmacology and management of bleeding complications of this novel oral anticoagulant. *J Med Toxicol*. 2011;7:281-287.
2. Kvasnicka T, Malikova I, Zenahlikova Z, et al. Rivaroxaban—metabolism, pharmacologic properties and drug interactions. *Curr Drug Metab*. 2017;18:636-642.
3. Escolar G, Carne X, Arellano-Rodrigo E. Dosing of rivaroxaban by indication: getting the right dose for the patient. *Expert Opin Drug Metab Toxicol*. 2015;11:1665-1677.
4. Mueck W, Stampfuss J, Kubitza D, Becka M. Clinical pharmacokinetic and pharmacodynamic profile of rivaroxaban. *Clin Pharmacokinet*. 2014;53:1-16.
5. Chen A, Stecker E, Warden BA. Direct oral anticoagulant use: a practical guide to common clinical challenges. *J Am Heart Assoc*. 2020;9:e017559.
6. Mazzone PM, Capodanno D. Low dose rivaroxaban for the management of atherosclerotic cardiovascular disease. *J Thromb Thrombolysis*. 2023;56:91-102.
7. Food and Drug Administration. Xarelto: highlights of prescribing information; section: Indications and usage. 2021 Accessed July 24, 2023. https://www.accessdata.fda.gov/drugsatfda_docs/label/2021/022406s036,202439s036lbl.pdf
8. Kreutz R. Pharmacodynamic and pharmacokinetic basics of rivaroxaban. *Fundam Clin Pharmacol*. 2012;26:27-32.
9. Genis-Najera L, Sanudo-Maury ME, Moquete T. A single-blind, randomized, single-dose, two-sequence, two-period, crossover study to assess the bioequivalence between two oral tablet formulations of rivaroxaban 20 mg in healthy Mexican volunteers. *Clin Pharmacol Drug Dev*. 2022;11:826-831.
10. Hrinova E, Skorepova E, Cerna I, et al. Explaining dissolution properties of rivaroxaban cocrystals. *Int J Pharm*. 2022;622:121854.
11. Kralovicova J, Bartunek A, Hofmann J, et al. Pharmacokinetic variability in pre-clinical studies: sample study with abiraterone in rats and implications for short-term comparative pharmacokinetic study designs. *Pharmaceutics*. 2022;14:643.
12. US Department of Health and Human Services Food and Drug Administration, Center for Drug Evaluation and Research (CDER). Guidance for Industry, Bioanalytical Method Validation. Accessed June 23, 2023. https://www.fda.gov/files/drugs/published/Bioanalytical-Method-Validation-Guidance-for-Industry.pdf
13. Committee for Medicinal Products for Human Use, European Medicines Agency (EMA). Guideline on Validation of Bioanalytical Methods (EMEA/CHMP/EWP/192217/2009 Rev. 1 Corr. 2.). Accessed June 23, 2023. https://www.ema.europa.

eu/en/documents/scientific-guideline/guideline-bioanalytical-method-validation_en.pdf
14. Kardas P, Lewek P, Matyjaszczyk M. Determinants of patient adherence: a review of systematic reviews. *Front Pharmacol*. 2013;4:91.
15. Deshpande CG, Kogut S, Laforge R, Willey C. Impact of medication adherence on risk of ischemic stroke, major bleeding and deep vein thrombosis in atrial fibrillation patients using novel oral anticoagulants. *Curr Med Res Opin*. 2018;34:1285-1292.
16. Yao X, Abraham NS, Alexander GC, et al. Effect of adherence to oral anticoagulants on risk of stroke and major bleeding among patients with atrial fibrillation. *J Am Heart Assoc*. 2016;5:e003074.
17. Beyer-Westendorf J, Siegert G. Of men and meals. *J Thromb Haemost*. 2015;13:943-945.
18. Osterberg L, Blaschke T. Adherence to medication. *N Engl J Med*. 2005;353:487-497.
19. Dunbar-Jacob J, Erlen JA, Schlenk EA, Ryan CM, Sereika SM, Doswell WM. Adherence in chronic disease. *Annu Rev Nurs Res*. 2000;18:48-90.
20. Munger MA, van Tassell BW, LaFleur J. Medication nonadherence: an unrecognized cardiovascular risk factor. *MedGenMed*. 2007;9:58.
21. Leiter MR, Packard KA, Qi Y, Krueger SK. Improved dosing and Administration of Rivaroxaban when prescribed by a cardiologist. *Heart Int*. 2019;13:24-27.
22. Kushwah V, Arora S, Tamas Katona M, Modhave D, Frohlich E, Paudel A. On absorption modeling and food effect prediction of rivaroxaban, a BCS II drug orally administered as an immediate-release tablet. *Pharmaceutics*. 2021;13:283.
23. Izuma H, Oka F, Ishihara H, et al. Thrombolysis with rt-PA under rivaroxaban anticoagulation in a hypertensive rat model of intraluminal middle cerebral artery occlusion. *J Stroke Cerebrovasc Dis*. 2018;27:2761-2767.
24. U.S. Department of Health and Human Services, Food and Drug Administration, Center for Drug Evaluation and Research (CDER). Guidance for Industry: Estimating the Maximum Safe Starting Dose in Initial Clinical Trials for Therapeutics in Adult Healthy Volunteers. 2005 Accessed December 15, 2023. https://www.fda.gov/media/72309/download

SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

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