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The pharmacokinetics of darexaban are not affected to a clinically relevant degree by rifampicin, a strong inducer of P-glycoprotein and CYP3A4

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WHAT IS ALREADY KNOWN ABOUT THIS SUBJECT

- Darexaban is an oral direct factor Xa inhibitor developed as an antithrombotic for several indications. Several other new oral anticoagulants are metabolized via CYP3A4 and transported by P-glycoprotein, displaying clinically relevant drug interactions with rifampicin and ketoconazole.
- Darexaban is almost entirely metabolized to darexaban glucuronide, which is the main active moiety. *In vitro*, CYP3A4 metabolism is not involved in the formation or metabolism of darexaban glucuronide; and darexaban, but not darexaban glucuronide, is a P-glycoprotein substrate *in vitro*.

WHAT THIS STUDY ADDS

- The study shows that rifampicin does not affect the pharmacokinetic profiles of darexaban glucuronide and darexaban to a clinically relevant degree, suggesting that the potential for drug–drug interactions between darexaban and CYP3A4 or P-glycoprotein-inducing agents is low.
- This study was carried out to confirm the susceptibility of darexaban/darexaban glucuronide to CYP3A4 and P-glycoprotein induction *in vivo* in humans.

AIMS

We investigated the effects of rifampicin on the pharmacokinetics (PK) of the direct clotting factor Xa inhibitor darexaban (YM150) and its main active metabolite, darexaban glucuronide (YM-222714), which almost entirely determines the antithrombotic effect.

METHODS

In this open-label, single-sequence study, 26 healthy men received one dose of darexaban 60 mg on day 1 and oral rifampicin 600 mg once daily on days 4–14. On day 11, a second dose of darexaban 60 mg was given with rifampicin. Blood and urine were collected after study drug administration on days 1–14. The maximal plasma drug concentration (C_{max}) and exposure [area under the plasma concentration–time curve from time zero to time of quantifiable measurable concentration; (AUC_{last}) or AUC_{last} extrapolated to infinity (AUC_{∞})] were assessed by analysis of variance of PK. Limits for statistical significance of 90% confidence intervals for AUC and C_{max} ratios were predefined as 80–125%.

RESULTS

Darexaban glucuronide plasma exposure was not affected by rifampicin; the geometric mean ratio (90% confidence interval) of AUC_{last} with/without rifampicin was 1.08 (1.00, 1.16). The C_{max} of darexaban glucuronide increased by 54% after rifampicin [ratio 1.54 (1.37, 1.73)]. The plasma concentrations of darexaban were very low (<1% of darexaban glucuronide concentrations) with and without rifampicin. Darexaban alone or in combination with rifampicin was generally safe and well tolerated.

CONCLUSIONS

Overall, rifampicin did not affect the PK profiles of darexaban glucuronide and darexaban to a clinically relevant degree, suggesting that the potential for drug–drug interactions between darexaban and CYP3A4 or P-glycoprotein-inducing agents is low.

Introduction

Anticoagulants are widely used to prevent and treat venous thromboembolism, for stroke prevention in patients with atrial fibrillation and for the secondary prevention of thromboembolic events in patients with acute coronary syndrome. Amongst the oral anticoagulants, the only available agents for more than 60 years, until recently, were vitamin K antagonists (VKAs). Vitamin K antagonists are effective, for example, in preventing strokes in patients with atrial fibrillation. However, they have a number of drawbacks, including slow onset and offset of action, a narrow therapeutic window and a variable response due to several factors, such as diet, drugs and genetic polymorphisms [1]. Owing to this variable response, frequent coagulation monitoring is required. Even with monitoring, many patients are poorly controlled, which reduces the benefit of VKAs [2, 3]. Another related concern is the frequent reluctance of physicians to prescribe VKAs, even in appropriate patients for whom such treatment is indicated, due to the concern about the risk of bleeding [4]. In addition to orally acting VKAs, heparins (e.g. unfractionated heparin and low-molecular-weight heparin) have been the mainstays of treatment for patients with venous and arterial thromboembolic disease for the last half-century. These products, however, need to be administered parenterally, which is inconvenient for patients and has a negative impact on quality of life. The limitations of anticoagulants can result in poor anticoagulant control, which can lead to bleeding complications [5] or result in the underuse of appropriate anticoagulation therapy [6–9].

Owing to these drawbacks, a number of new oral anticoagulants have been developed, which target specific clotting factors. Amongst these, darexaban is a potent, oral, direct, competitive factor Xa (FXa) inhibitor that specifically and reversibly inhibits prothrombin activation induced by free and clot-associated FXa [10–13]. After oral administration, darexaban is rapidly absorbed and undergoes rapid and almost complete *O*-glucuronidation, forming the active metabolite darexaban glucuronide. The formation of darexaban glucuronide is mediated by multiple enzymes from the UDP-glucuronyltransferase (UGT) 1A family, predominantly UGT1A9 in the liver and UGT1A10 in the intestine, and to a lesser extent UGT1A7, 1A8 and 1A9 in the intestine [14]. The formation of darexaban glucuronide is rapid, with a mean observed time to maximal concentration (t_{max}) approximately 1–2 h after darexaban administration. The human mass balance study showed that darexaban glucuronide accounted for 86–96% of radioactivity in plasma (data on file). Other metabolites were minor, and not pharmacologically active. In addition, the plasma protein binding of darexaban and darexaban glucuronide was independent of concentration and ranged from 83.6 to 84.3 and from 73.9 to 77.0%, respectively (data on file). Based on these findings, it can be concluded that, whilst both darexaban and darexaban glucuronide are equipotent in terms of anti-FXa activity *in vitro* [11], it is darexaban glucuronide that is responsible for the antithrombotic effect *in vivo*.

Most notably, the major clearance pathways of darexaban glucuronide do not involve cytochrome P450 (CYP) pathways. In addition, in the human mass balance study, the average cumulative recovery of ^{14}C-radioactivity following an oral dose of darexaban amounted to 46.4% in urine and 51.9% in faeces, indicating that both elimination routes contribute to a similar extent to the overall clearance of darexaban and its glucuronide from the body (data on file). Darexaban, but not the primary active metabolite darexaban glucuronide, was found to be a substrate of the ABC-efflux transporter P-glycoprotein (P-gp, ABCB1) *in vitro* [15] The potential of darexaban as a clinically effective antithrombotic agent has previously been demonstrated in the prevention of venous thromboembolism in patients undergoing major orthopaedic surgery in two Phase II studies [16, 17].

Drug–drug interactions (DDIs) represent a significant and under-recognized medical risk [18], and patients receiving anticoagulation often require multiple concurrent medications. A number of commonly used therapeutic agents inhibit or induce the metabolizing enzyme CYP3A4 or the P-gp efflux transporter, which together play important roles in the absorption and clearance of many other drugs. Data from a recent study suggested that nearly half of the patients with atrial fibrillation who receive VKAs for anticoagulation are also prescribed P-gp-altering drugs [19], placing these patients at risk of experiencing clinically significant DDIs. In addition to the multiple well-described DDIs between P-gp/CYP3A4 inhibitors/inducers and VKAs such as warfarin [20], significant P-gp/CYP3A4-based DDIs have also been reported with the direct thrombin inhibitor dabigatran and the direct FXa inhibitor rivaroxaban [21, 22]. In light of this evidence, it is essential to assess fully the potential liability of darexaban/darexaban glucuronide to become the subject of P-gp/CYP3A4-based DDIs.

The current approach to testing for particular mechanisms of interaction (e.g. inhibition or induction of particular metabolic enzymes and/or drug transporters) between specific drugs has expanded to include the use of carefully selected drugs as general probes for the characterization of DDI mechanisms. This approach allows the specific results obtained to be generalized to other drugs and settings. The US Food and Drug Administration's draft guidance on drug interaction studies provides information on prototypical inhibitors and inducers of certain metabolic enzymes and transport proteins [23]. For example, the macrolide anti-tuberculosis drug rifampicin is recommended, because it is a potent activator of the human pregnane X receptor, a transcription factor that causes upregulation of many drug-metabolizing enzymes and transport proteins, including P-gp, CYP3A4, CYP1A2, CYP2B6, CYP2C8, CYP2C9, CYP2C19 and possibly CYP2D6

[24]. The objective of the present study was to investigate the effects of co-administration of a potent CYP3A4/P-gp inducer (rifampicin) on the pharmacokinetic (PK) profiles of darexaban and darexaban glucuronide. The safety and tolerability of darexaban administered alone and in combination with rifampicin were also evaluated.

Methods

Study design

This was an open-label, single-sequence study in healthy adult male subjects. The primary objective was to determine the effect of multiple once-daily doses of rifampicin on the single-dose pharmacokinetics of darexaban and darexaban glucuronide. The secondary objective was to evaluate the safety and tolerability of a single dose of darexaban alone and in combination with rifampicin.

A 60 mg dose of darexaban was selected because this dose is within the anticipated clinical dose range and has been previously used in several clinical trials [16, 17]. Rifampicin 600 mg once-daily dosing was used because this is a common and recommended regimen [23]. Subjects received a single oral 60 mg dose of darexaban on day 1 and a 600 mg once-daily dose of rifampicin on days 4–14, all in fasted conditions. On day 11, a second single 60 mg dose of darexaban was given in combination with rifampicin. All study medication was taken together at approximately 08.00 h. Intake of study drugs was supervised by study site personnel and confirmed by mouth check. Seven to 14 days after discharge, subjects returned to the study site for an end-of-study (EOS) visit, at which point safety parameters were assessed, including laboratory tests, vital signs, electrocardiogram (ECG) and adverse event (AE) reporting. Concomitant medications were also recorded.

The protocol and subjects' informed consent were reviewed and approved by the French competent authorities [Agence française de sécurité sanitaire des produits de santé (Afssaps), Saint Denis, France] and an independent Ethics Committee (Comité de Protection des Personnes Ile-de-France VIII, Boulogne-Billancourt, France) prior to the conduct of any study procedure. The study was conducted in accordance with the ethical principles set out in the Declaration of Helsinki, the applicable Good Clinical Practice guidelines, International Conference on Harmonization of Technical Requirements for Registration of Pharmaceuticals for Human Use guidelines and all applicable local laws and regulations. The study is registered on clinicaltrial.gov (NCT01406002).

Subjects

Healthy male subjects, aged 18–55 years old, with a body mass index between 18.5 and 30.0 kg m^{-2} were included. Subjects were not included in the study if they had alanine aminotransferase, aspartate aminotransferase or bilirubin above the upper limit of normal at repeated measures; any clinically significant history of asthma, eczema, any other allergic condition or previous severe hypersensitivity to any drug; abnormal blood pressure (systolic and/or diastolic blood pressure >140 and >90 mmHg, respectively) or used any prescribed or over-the-counter drugs (including vitamins, natural and herbal remedies, e.g. St John's wort) within 2 weeks prior to study start. Subjects with any of the following findings within 3 months prior to study start were excluded: use of any drugs of abuse; regular use of any inducer of liver metabolism (e.g. barbiturates, rifampicin); smoking of more than 10 cigarettes (or equivalent) per day; or alcohol consumption of more than 21 units per week (1 unit = 10 g pure alcohol) in any form. During the dosing period, all concomitant medication was prohibited except for occasional limited use of paracetamol (if required and approved by the investigator). Smoking, as well as any alcohol-, caffeine- or xanthine-containing beverages or grapefruit juice, was prohibited from at least 24 h before admission until study end.

Bioanalytical sampling and assays

Serial blood and urine samples for PK assessments of darexaban and darexaban glucuronide were collected predose and up to 72 h postdose following darexaban administration on days 1 and 11. Plasma and urine concentrations of both compounds were quantified using validated liquid chromatography–mass spectrometry/mass spectrometry methods according to current standards [23]. The lower limit of quantification (LLOQ) for darexaban and darexaban glucuronide was 2 and 10 ng ml^{-1}, respectively, for plasma and 5 and 100 ng ml^{-1}, respectively, for urine. For plasma, accuracy [expressed as relative error (%RE)] was between −5.5 and 1.7% for darexaban and between −2.8 and 2.3% for darexaban glucuronide. In addition, for plasma, the precision [(expressed as coefficient of variation (CV%)] was between 5.5 and 6.3% for darexaban and between 2.8 and 4.4% for darexaban glucuronide. For urine, accuracy (%RE) was between −8.8 and 1.6% for darexaban and between −3.5 and 1.0% for darexaban glucuronide. In addition, for urine the precision (CV%) was between 4.5 and 5.9% for darexaban and between 4.2 and 8.0% for darexaban glucuronide. All results were obtained in analytical runs that met all preset acceptance criteria. Blood samples for PK assessments of rifampicin were collected predose on days 8, 9, 10, 13 and 14, and predose through to 24 h postdose on day 11. Quantification of rifampicin in plasma was performed using a validated liquid chromatography–mass spectrometry/mass spectrometry method according to the same standards as for darexaban, with a LLOQ of 50 ng ml^{-1}. The %RE was between 4.0 and 17.0% and CV% was between 2.8 and 13.7%.

Pharmacokinetic assessments

The PK analysis set comprised all subjects who received at least one dose of study medication and had sufficient

plasma concentration data to enable the derivation of at least one PK parameter. Descriptive statistics were calculated for all PK parameters. Noncompartmental PK analysis for plasma darexaban and darexaban glucuronide concentrations was performed using WinNonlin Professional 5.2 (Pharsight Corporation, Mountain View, CA, USA).

Primary pharmacokinetic analysis The primary PK variables were darexaban glucuronide maximal plasma concentration (C_{max}) and total exposure [area under the plasma concentration–time curve from time zero to time of last quantifiable measurable concentration (AUC_{last}) or AUC_{last} extrapolated to infinity (AUC_{∞})]. The effect of rifampicin on the PK of darexaban glucuronide was assessed by analysis of variance (ANOVA) of the log-transformed darexaban glucuronide C_{max}, AUC_{last} and AUC_{∞} values. Based on the statistical model, point estimates and 90% confidence intervals (CIs) were obtained for the geometric mean ratios (GMR) of darexaban in combination with rifampicin relative to darexaban alone. Limits for statistical significance of 90% CIs for AUC and C_{max} ratios indicating a lack of a significant DDI were predefined as 80 and 125% (i.e. 0.8–1.25).

Secondary pharmacokinetic analyses Additional plasma and urine PK parameters for darexaban glucuronide included time to reach maximal plasma concentration (t_{max}), terminal elimination half-life ($t_{1/2}$), cumulative amount of drug excreted in urine (*Ae*) and renal clearance (CL_R). For rifampicin, plasma C_{max}, t_{max} and AUC_{τ} (AUC during one interval at steady state) were also assessed. Pharmacokinetic analysis of darexaban was planned as part of the study protocol; however, plasma darexaban concentrations were too low to determine PK parameters. As the terminal elimination phase could not be characterized in 13 subjects in the darexaban plus rifampicin group, AUC_{∞} values for darexaban glucuronide could not be calculated reliably for these subjects. Therefore, statistical testing was performed on both the protocol-planned analysis of AUC_{∞} and an additional analysis using AUC_{last} (which was considered more reliable than AUC_{∞}). Pharmacokinetic sampling and analyses were also performed for another metabolite of darexaban (AS2486616); however, this is a minor metabolite and results are not reported here.

Safety assessments

Safety was assessed for all patients who received at least one dose of study drug. The investigator provided his assessment of the causal relationship (not related, possible or probable) for each AE to the study drug. Adverse events were reported by treatment as follows: treatment-emergent AE (TEAE) darexaban alone (after administration of darexaban on day 1, but prior to the first dose of rifampicin on day 4), TEAE rifampicin alone (after administration of the first dose of rifampicin alone on day 4, but prior to the administration of the combination of rifampicin and darexaban on day 11), or TEAE rifampicin plus darexaban (after the administration of the combination dose of rifampicin plus darexaban on day 11, but no later than the EOS visit). Laboratory assessments included haematology, biochemistry, urinalysis, haemostasis and transaminase levels. Vital signs and 12-lead ECGs were measured at screening, baseline, day 14 and EOS. Physical examinations were performed at screening and EOS. Safety data were analysed descriptively, with no formal statistical testing.

Statistical methods

The sample size of the study (n = 26) was calculated to ensure that the treatment effect, defined as GMR of ketoconazole + darexaban/darexaban for C_{max} and AUC_{∞}, could be described with acceptable precision, with 95% coverage probability. Acceptable precision was defined as having the 90% CI for the GMR with a multiplicative distance from the GMR to the limits of 15% (i.e. 90% CI ranging from GMR/1.15 to GMR × 1.15).

Results

Subject disposition and study drug exposure

This study was conducted from January 2010 to March 2010 in a Phase I clinical trial unit at SGS, Paris, France. In total, 26 subjects were enrolled and received treatment, of whom one prematurely discontinued treatment on day 4 after the first rifampicin dose owing to two mild AEs (oral paraesthesia and urticaria on forearms), neither of which were considered related to treatment with darexaban. All 26 subjects received a single dose of darexaban on day 1; as one subject discontinued treatment on day 4, the mean number of days of treatment with rifampicin was 10.6. All 25 remaining subjects received their doses of rifampicin and darexaban as scheduled. All subjects were male, ranging in age from 20 to 51 years, with a mean age of 32.4 years; body mass index ranged from 20.2 to 29.8 kg m^{-2}, with a mean of 24.5 kg m^{-2}.

Plasma pharmacokinetics of darexaban glucuronide

The mean plasma concentration *vs*. time profiles for darexaban glucuronide (linear and semi-log scales) are presented in Figure 1, and plasma PK parameters are shown in Table 1. When darexaban was dosed with rifampicin, darexaban glucuronide plasma concentrations were initially higher compared with darexaban alone, until approximately 12 h postdose (Figure 1); however, total exposure was not affected. Although darexaban glucuronide C_{max} was increased 1.5-fold, AUC_{last} and $t_{1/2}$ were similar for both treatments, i.e. darexaban alone and darexaban plus rifampicin (Table 1). The effect of rifampicin on the C_{max} and AUC_{last} for individual subjects is illustrated in Figure 2. Of the 25 subjects, 23 subjects (92%) showed an increase in darexaban glucuronide C_{max} in the presence of rifampicin,

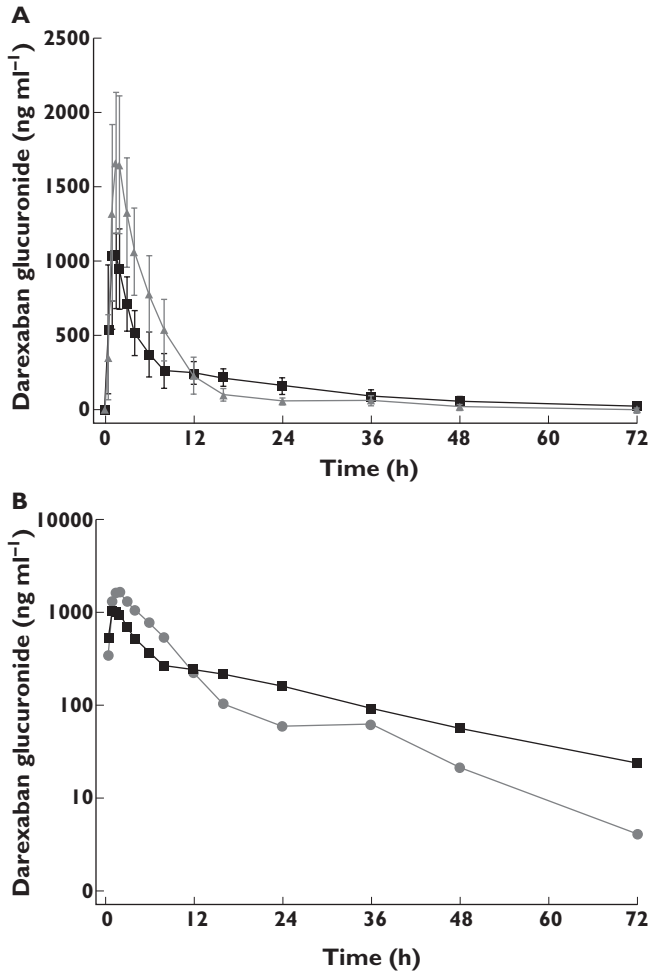

Figure 1

Mean darexaban glucuronide plasma concentration *vs.* time profiles. (A) Linear scale; ■, darexaban; ▲, darexaban + rifampicin. (B) Log scale; ■, darexaban; ●, darexaban + rifampicin

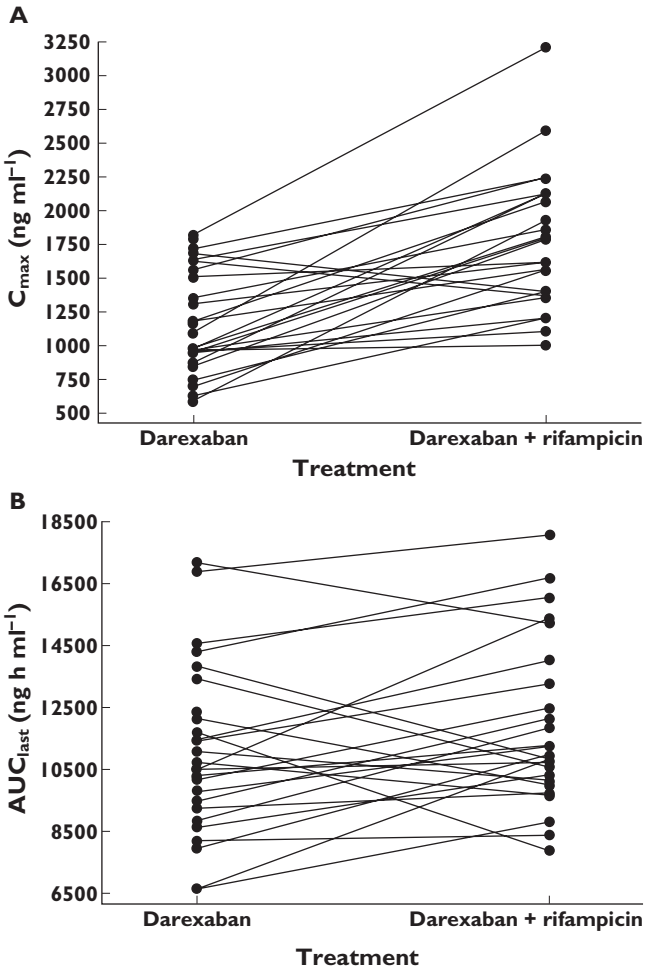

Figure 2

Individual darexaban glucuronide plasma PK parameters *vs.* treatment. (A) C_{max}, the maximal plasma drug concentration. (B) AUC_{last}, the plasma concentration–time curve from time zero extrapolated to infinity

Table 1

Plasma pharmacokinetic parameters for darexaban glucuronide

Treatment		t_{max}* (h)	C_{max} (ng ml^{-1})	AUC_{last} (ng h ml^{-1})	AUC_{∞}† (ng h ml^{-1})	$t_{1/2}$ (h)
Darexaban 60 mg	*n*	26	26	26	26	26
	Mean (SD; CV%)	1.00 (1.0; 6.0)	1176 (382; 33)	11,083 (2746; 25)	11,866 (3122; 26)	17.1 (7.0; 41)
Darexaban 60 mg + rifampicin 600 mg once daily	*n*	25	25	25	12	12
	Mean (SD; CV%)	1.50 (1.0; 3.0)	1774 (506; 29)	11,860 (2684; 23)	12,583 (2745; 22)	16.7 (7.5; 45)
Darexaban + rifampicin *vs.* darexaban alone	Ratio (90% CI)	NA	1.54 (1.37, 1.73)	1.08 (1.00, 1.16)	1.02 (0.93, 1.13)	NA

Abbreviations: AUC_{last}, area under the concentration–time curve from time zero to time of last quantifiable plasma concentration; AUC_{∞}, AUC_{last} extrapolated to infinity; C_{max}, maximal plasma concentration; CI, confidence interval; CV, coefficient of variation; NA, not applicable; $t_{1/2}$, elimination half-life; and t_{max}, time to maximal concentration. *For t_{max}, median (minimum; maximum) is presented. †As the terminal elimination phase could not be characterized in a significant subset of subjects, AUC_{last} is considered a more reliable analysis than AUC_{∞}.

with a maximal increase of 226% compared with darexaban alone, whereas two subjects (8%) showed a decrease with a maximum of 17%. In addition, 18 of the 25 subjects (72%) showed an increase in darexaban glucuronide AUC_{last} in the presence of rifampicin, with a maximal increase of 62%, and seven of the 25 subjects (28%) showed a decrease with a maximum of 33%. The lack of a significant DDI on total exposure (AUC) was confirmed

statistically, becuase the 90% CIs of the darexaban plus rifampicin *vs*. darexaban-alone ratios for the AUC_{last} of darexaban glucuronide were within the predefined 80–125% limits. Median t_{max} was comparable for both treatments; 1 h for treatment with darexaban alone and 1.5 h for treatment with darexaban plus rifampicin (Table 1), indicating rapid absorption. Overall, there was no apparent effect on total darexaban glucuronide exposure.

Plasma pharmacokinetics of darexaban

In keeping with previous findings [15], darexaban concentrations were very low (<1% of darexaban glucuronide concentrations) in all subjects, owing to rapid conversion to darexaban glucuronide. For the treatment with darexaban alone, plasma darexaban concentrations above LLOQ were observed in 18 subjects for a maximum of 6 h. In the presence of rifampicin, plasma darexaban concentrations above LLOQ occurred in only four subjects for a maximum of 1.5 h. Consequently, PK parameters could not be determined, and the magnitude of the effect of rifampicin on darexaban exposure could not be estimated reliably.

Plasma pharmacokinetics of rifampicin

Pharmacokinetic profiles of rifampicin were observed in subjects on day 11, confirming the presence of rifampicin at the time of darexaban dosing. Mean ± SD values for the combination treatment with darexaban and rifampicin were as follows: C_{max}, 8192 ± 3115 ng ml^{-1} and t_{max}, 1.6 ± 0.76 h. All trough levels were below the LLOQ, as expected based on the $t_{1/2}$ of rifampicin.

Urine pharmacokinetics of darexaban and darexaban glucuronide

For darexaban glucuronide, the proportion of the dose excreted in urine as darexaban glucuronide, as well as CL_R, was similar between the darexaban-alone and darexaban plus rifampicin treatment groups (Table 2). For darexaban, CL_R could be determined in only three subjects for treatment with darexaban alone and in none of the subjects for treatment with darexaban plus rifampicin. The first urine collection interval was 0–6 h, and in most subjects, plasma concentrations had dropped below the LLOQ within 6 h of dosing.

Safety

Overall, darexaban administered alone or in combination with rifampicin was safe and well tolerated. No subjects treated with darexaban alone experienced a TEAE. Seven subjects treated with rifampicin alone experienced TEAEs (abdominal pain, $n = 2$; diarrhoea, $n = 2$; mouth ulceration, $n = 1$; oral paraesthesia, $n = 1$; headache, $n = 1$; and urticaria, $n = 1$), and one subject receiving rifampicin in combination with darexaban experienced an AE (viral conjunctivitis). All TEAEs were reported as mild or moderate in severity. The only AEs reported as study drug related were those reported for the seven (27%) subjects treated with rifampicin alone. There were no deaths or serious AEs. One subject discontinued on day 4, due to mild oral paraesthesia and mild urticaria, which was probably related to treatment with rifampicin. There were no clinically significant drug-related changes in haematology, biochemistry and urinalysis. There were no clinically significant or notable changes in vital signs or ECGs.

Discussion

Rifampicin is a strong inducer of pregnane X receptor-regulated drug-metabolizing enzymes and transporter proteins, including CYP3A4 and P-gp [24]. As such, rifampicin is routinely used as a prototypic investigative mechanistic tool to examine the role of those disposition pathways in the absorption, metabolism and clearance of new molecular entities [23]. In addition, rifampicin also induces some UGT isozymes, including UGT1A9 [25], a feature that could be of relevance in the interpretation of the present data. In addition to the well-recognized chronic effects in terms of enzyme/transporter induction, it was more recently realized that rifampicin also displays acute inhibitory effects on hepatic uptake transporters (organic anion transporter family, OATPs) [26]. These acute OATP inhibitor characteristics of rifampicin could also be of

Table 2

Urine pharmacokinetic parameters for darexaban glucuronide

Treatment		Darexaban glucuronide				
		Ae_{last} (mg)	$\%Ae_{last}$ (%)	Ae_{∞} (mg)	$\%Ae_{\infty}$ (%)	CL_R (l h^{-1})
Darexaban 60 mg	*n*	26	26	26	26	26
	Mean (SD; CV%)	25.0 (5.7; 23)	30.4 (6.9; 23)	26.6 (6.2; 23)	32.3 (7.5; 23)	2.29 (0.45; 20)
Darexaban 60 mg + rifampicin 600 mg once daily	*n*	25	25	25	25	25
	Mean (SD; CV%)	29.5 (6.5; 22)	35.8 (7.9; 22)	29.7 (6.5; 22)	36.1 (7.9; 22)	2.44 (0.47; 20)

Abbreviations: Ae_{last}, cumulative amount of drug excreted in urine up to the last quantifiable sample; $\%Ae_{last}$, percentage cumulative amount of drug excreted in urine up to the last quantifiable sample; Ae_{∞}, Ae_{last} extrapolated to infinity; $\%Ae_{\infty}$, $\%Ae_{last}$ extrapolated to infinity; CL_R, renal clearance; and CV, coefficient of variation.

relevance for the interpretation of the present data, because darexaban glucuronide was shown to be an OATP1B3 substrate with a K_m value of 29.8 µmol l^{-1} [15].

The design of this study was appropriate to allow maximal induction of CYP3A4 and P-gp at the time of darexaban dosing on day 11. A single dose of darexaban was administered, because there were no differences in the pharmacokinetics of darexaban and darexaban glucuronide between single and multiple dosing [27]. All subjects showed relevant exposure to rifampicin, as demonstrated by the PK profiles of rifampicin on day 11, with concentrations being in line with previously published data [28]. Although darexaban and darexaban glucuronide are both active and are equipotent, it is darexaban glucuronide that is responsible for the majority of the antithrombotic activity, because conversion of darexaban to darexaban glucuronide is rapid and extensive, and the systemic exposure of darexaban is generally negligible [27]. The study results show that this pattern is unaltered by rifampicin co-administration. Whilst the AUC_{last} of darexaban glucuronide remained unchanged, the C_{max} of darexaban glucuronide was modestly increased (1.5-fold) after rifampicin co-administration compared with darexaban alone. It has previously been suggested for another FXa inhibitor (apixaban) that the AUC is the best predictor for bleeding events in a population with venous thromboembolism [29], with C_{max} offering lower predictivity. For darexaban, historical data (Figure 3; [27]) show that the slope of the PK–pharmacodynamic (PD) relationship for darexaban glucuronide is only modest, and therefore, the change in International normalized ratio at the plasma concentrations observed in this study is expected to be small. The historical data as displayed in Figure 3 were consistently observed across several other studies, including studies in patients. In addition, PK–PD modelling of the bleeding data did not clearly indicate a specific PK or PD parameter to be most predictive for the occurrence of bleeding events (data on file). Taken together, the moderate and transient increase in C_{max} of darexaban glucuronide may, therefore, not be of great clinical relevance in terms of bleeding risk. Overall, these study findings suggest that co-administration of darexaban with rifampicin does not lead to clinically relevant changes in the total exposure of the active moieties darexaban glucuronide and darexaban.

There may be several explanations for the modest increase in the C_{max} of darexaban glucuronide observed in this study. As darexaban is metabolized to darexaban glucuronide mainly via UGT1A9 in the liver and UGT1A10 in the intestine [14], rifampicin-mediated UGT1A9 induction may increase the rate of darexaban conversion to darexaban glucuronide. However, the increase in C_{max} is only modest, which may be explained by the fact that the rate of conversion from darexaban into darexaban glucuronide is already very high in the absence of rifampicin. Induction of UGTs will therefore not change the conversion rate to a significant extent. It is also possible that darexaban glucuronide hepatic uptake/distribution may be affected due to the acute inhibitory effects of rifampicin on OATP1B3, although the present study design, with PK assessment after 10 days of rifampicin repeat dosing, does not allow a final conclusion to be made in that regard. However, none of these mechanisms altered the total exposure (AUC) to darexaban glucuronide; therefore, substantial rifampicin-mediated net effects on the extent of absorption (bioavailability) or overall clearance of darexaban glucuronide can be excluded. The lack of a significant DDI with respect to the total darexaban glucuronide exposure (i.e. AUC_{last}) was confirmed statistically, because the limits of the CI of the geometric mean ratio of darexaban plus rifampicin *vs.* darexaban alone were within the predefined limits of 80–125%. Based on this outcome, it can be further inferred that CYP3A4-mediated metabolic pathways and systemic P-gp-based clearance mechanisms do not play a significant role in the overall disposition of darexaban and

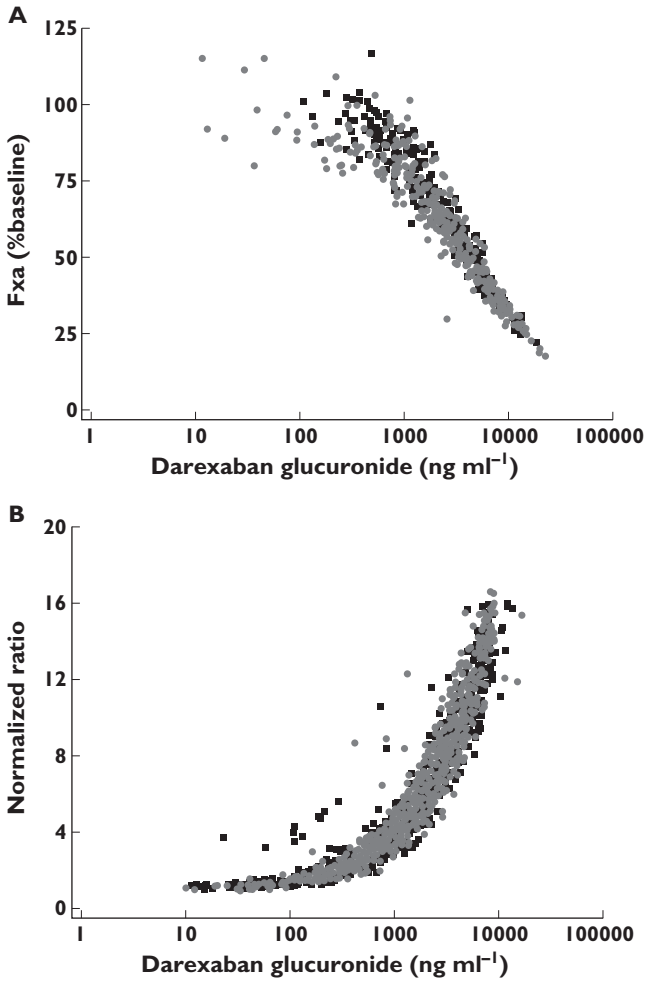

Figure 3

Mean darexaban glucuronide plasma concentration *vs.* time profiles. (A) Factor Xa (Fxa) inhibition. (B) ■, first dose; ●, last dose.

darexaban glucuronide. This finding is consistent with the outcome of a complementary mechanistic DDI study using ketoconazole as a potent CYP3A4/P-gp inhibitor [30], where the exposure of darexaban glucuronide remained unchanged following once-daily treatment with ketoconazole 400 mg.

Based on AE reporting, clinical laboratory evaluations, ECGs and vital signs, darexaban was generally safe and well tolerated, whether administered alone as a single 60 mg dose or in combination with rifampicin.

As anticoagulants are used across a range of conditions and patient populations, it is often necessary to co-administer them with other agents. It is, therefore, very important to assess the potential for DDIs with any new anticoagulant agent. It has been shown previously that concomitant administration of rifampicin reduces exposure to dabigatran, decreasing the AUC and C_{max} by 66 and 67%, respectively [31]. The US label for dabigatran states that concomitant use with P-gp inducers should be avoided [31]. A study of rivaroxaban has demonstrated that co-administration with rifampicin leads to an approximate 50% decrease in mean rivaroxaban AUC, with parallel decreases in its PD effects [21]. The product information for rivaroxaban states that strong CYP3A4 inducers should be co-administered with caution. Likewise, for apixaban, rifampicin decreases the AUC by 54% and the C_{max} by 42%, and the product information for apixaban mentions that strong inducers of both CYP3A4 and P-gp should be co-administered with caution. In line with these observations, all three compounds also display significant DDIs with potent inhibitors of P-gp and CYP3A4 (only rivaroxaban, apixaban), which in each case results either in contraindications for the co-administration of certain P-gp/CYP3A4 inhibitors or in recommendations for dose adjustments of the antithrombotic [21, 22, 32]. Furthermore, other oral anticoagulants in advanced clinical development, such as edoxaban [33] and betrixaban [34], are reported as sensitive P-gp substrates, with edoxaban [35], like rivaroxaban and apixaban, being dependent on CYP (in particular CYP3A4) metabolic pathways for elimination. In summary, it seems that all of the newer oral anticoagulants are drugs where P-gp and/or CYP3A4 play significant roles in drug disposition.

As many drugs used in cardiology for the control of heart rate and rhythm in patients with atrial fibrillation are known to be P-gp and/or CYP3A4 inhibitors, for example dronedarone [36], quinidine, amiodarone, verapamil [37] and diltiazem [38], an oral anticoagulant that does not display P-gp/CYP-based DDI liabilities would be advantageous in the clinical management of patients with atrial fibrillation, especially as a recent study suggests that nearly half of the patients with atrial fibrillation who receive oral anticoagulation are also being treated with P-gp-altering drugs [19]. The DDI potential of dabigatran, rivaroxaban and apixaban has recently been reviewed from a clinical perspective [39].

In summary, this study shows that potent CYP3A4/P-gp inducers do not have a clinically relevant impact on darexaban/darexaban glucuronide total exposure. Taken together with the findings from a related DDI study of darexaban with ketoconazole [30], it can be concluded that darexaban has a low liability for P-gp/CYP-based DDIs, which appears to be a unique characteristic among oral anticoagulants currently available or in advanced clinical development.

Competing Interests

Dorien Groenendaal, Alberto Garcia-Hernandez, Marten Heeringa, Roelof Mol, Charlotte Eltink and Hartmut Onkels are all employees of Astellas Pharma Global Development Europe. Takeshi Kadokura is an employee of Astellas Pharma Japan. Gregory Strabach is an employee of SGS Paris, which conducts clinical research on a contract basis for Astellas and other pharmaceutical companies.

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