ORIGINAL RESEARCH ARTICLE

Effect of Daridorexant on the Pharmacokinetics of Midazolam, and on the Pharmacokinetics and Pharmacodynamics of Warfarin in Healthy Male Subjects

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Abstract

Background and Objectives Daridorexant, a dual orexin receptor antagonist was recently approved for the treatment of insomnia at doses up to 50 mg once per night. This study investigated the effect of single-dose and multiple-dose daridorexant 50 mg at steady state on the pharmacokinetics (PK) of the cytochrome P450 (CYP) 3A4-sensitive substrate midazolam, and the effect of single-dose daridorexant 50 mg on the PK and pharmacodynamics (PD) of the CYP2C9-sensitive substrate warfarin.

Methods In this prospective, single-center, open-label, fixed-sequence, phase I, drug–drug interaction study, 18 healthy male subjects sequentially received Treatment A, B, and C in three periods. Treatment A consisted of a single oral concomitant administration of midazolam 2 mg and warfarin 25 mg on day 1 of the first period. Treatment B consisted of one oral administration of daridorexant 50 mg followed 1 h later by a single oral dose of midazolam 2 mg concomitantly with a single oral dose of warfarin 25 mg on day 1 and a once-daily oral administration of daridorexant 50 mg for 6 days of the second period. Treatment C consisted of a single oral administration of daridorexant 50 mg at steady state followed 1 h later by a single oral administration of midazolam 2 mg on day 1 of the third period. Blood samples were assessed for midazolam and S-warfarin PK, and PD (international normalized ratio and factor VII). Noncompartmental PK parameters and PD variables were evaluated with geometric mean ratios and 90% confidence intervals of Treatment B/A versus C/A for midazolam, and treatment B/A for warfarin. Safety and tolerability of each treatment were also assessed.

Results Midazolam maximum plasma concentration (C_{max}) and area under the plasma concentration–time curve from 0 to 24 h (AUC_{0-24}) were 1.13- and 1.42-fold higher, respectively, after single-dose administration of daridorexant 50 mg compared to administration of midazolam alone, while C_{max} and AUC_{0-24} were 1.12- and 1.35-fold higher, respectively, after administration of daridorexant 50 mg once daily at steady state. Terminal half-life and time to maximum plasma concentration were comparable between treatments. Daridorexant had no influence on the PK and PD of warfarin. All treatments were safe and well tolerated.

Conclusions Daridorexant at 50 mg is classified as a weak CYP3A4 inhibitor after single- and multiple-dose administration once daily at steady state. Daridorexant 50 mg did not induce CYP3A4 activity or inhibit CYP2C9 activity.

Clinical Trial Registration This trial (NCT05480488) was registered on 29 July, 2022.

Key Points

This drug–drug interaction study investigated the effect of daridorexant, a dual orexin receptor antagonist, on cytochrome P450 (CYP) 3A4 and CYP2C9 activities with the sensitive substrates midazolam and warfarin in healthy male subjects.

Daridorexant at 50 mg is a weak inhibitor of CYP3A4 and did not induce CYP3A4 activity at steady state.

Daridorexant at 50 mg did not inhibit CYP2C9.

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1 Introduction

Daridorexant is a selective and potent dual orexin receptor antagonist, acting equipotently on both orexin receptor type 1 and type 2 in wake-promoting brain regions [1]. It antagonizes the activation of orexin receptors by the orexin neuropeptides and consequently decreases the wake drive, allowing sleep to occur [1]. In 2022, daridorexant (Quviviq™) [at 25 mg or 50 mg once per night, taken before going to bed] was granted approval by the US Food and Drug Administration for the treatment of adult patients with insomnia characterized by difficulties with sleep onset and/or sleep maintenance [2, 3] and by the European Medicines Agency at 50 mg for the treatment of adult patients with insomnia characterized by symptoms present for at least 3 months and a considerable impact on daytime functioning [4], among other countries. The pharmacokinetic (PK) profile of daridorexant at the clinical dose range of 25–50 mg is characterized by a quick absorption, with a time to maximum plasma concentration (t_{max}) of 1–2 h, a terminal half-life ($t_{1/2}$) of approximately 8 h, a clearance of 5.0 L/h, and a volume of distribution at steady state of 31 L [5–7]. The PK parameters of daridorexant after single-dose and multiple-dose administration are similar and no relevant accumulation was observed [7].

Cytochrome P450 (CYP) isoenzymes CYP3A4 and CYP2C9 are known to be involved in the metabolism of a wide variety of xenobiotic compounds, including many therapeutic drugs [8]. In vitro, daridorexant has been shown to be an inhibitor of CYP2C9 and CYP3A4, the latter of which was time dependent [6, 9]. In addition, daridorexant has been shown to be an activator of the nuclear pregnane X receptor, leading to an upregulation of expression of CYP3A4, CYP2B6, and CYP2C9 messenger RNA in human hepatocytes [9]. Thus, the perpetrator potential of daridorexant on CYP3A4 activity was investigated in a clinical drug–drug interaction (DDI) study in healthy subjects with the CYP3A4-sensitive substrate midazolam at single and multiple daridorexant doses of 25 mg once daily (o.d.) [6]. The results demonstrated that midazolam exposures in the absence and presence of daridorexant were within bioequivalence limits, indicating that daridorexant at 25 mg o.d. is neither an inhibitor nor an inducer of CYP3A4 [6]. Consequently, these results obtained with a 25-mg dose did not trigger a modification of the dosing recommendation for drugs metabolized by CYP3A4 or by enzymes inducible by a pregnane X receptor-dependent mechanism. To investigate whether this absence of dosing recommendation of victim drugs could be extrapolated to the highest approved therapeutic dose of daridorexant, this study investigated the inhibitory/inductive effect on CYP3A4 of single-dose and multiple-dose daridorexant 50 mg o.d. by their effects on the PK of midazolam. As the inhibitory effect of daridorexant on CYP2C9 had not been studied clinically before, this study further aimed to investigate the effect of daridorexant 50 mg on the PK and pharmacodynamics (PD) of the CYP2C9-sensitive substrate warfarin. Warfarin is an anticoagulant and acts by inhibiting vitamin K-dependent coagulation factors II, VII, IX, and X, while the activity levels of these factors directly reflect the extent of anticoagulation [10]. Warfarin is a narrow therapeutic index drug. Therefore, the degree of anticoagulation induced by this drug needs to be carefully monitored during treatment by measuring the international normalized ratio (INR) and levels of factor VII activity [11–13].

This single-center, open-label, fixed-sequence DDI study investigated the effect of single- and multiple-dose daridorexant 50 mg at steady state on the PK of a single dose of midazolam 2 mg, and the effect of a single dose of daridorexant 50 mg on the PK and PD of a single dose of warfarin 25 mg in healthy male subjects. In addition, safety and tolerability of concomitant administration of midazolam and warfarin, and of daridorexant when administered with midazolam and warfarin, or when administered at steady state with midazolam were assessed.

2 Methods

2.1 Study Design

This was a prospective, single-center, open-label, fixed-sequence, phase I study. Screening of subjects took place between 21 and 3 days before first study treatment administration. Subjects sequentially received Treatment A, B, and C in three fixed-sequence periods as shown in Fig. 1. Treatment A consisted of a single oral concomitant administration of midazolam 2 mg and warfarin 25 mg on day 1 of the first period. Treatment B consisted of one oral administration of daridorexant 50 mg followed 1 h later by a single oral dose of midazolam 2 mg concomitantly with a single oral dose of warfarin 25 mg on day 1 and an oral administration of daridorexant 50 mg o.d. for 6 days of the second period. Treatment C consisted of a single oral administration of daridorexant 50 mg at steady state followed 1 h later by a single oral administration of midazolam 2 mg on day 1 of the third period. Treatment periods A and B were separated by an in-between period of at least 7 days based on the elimination half-life of warfarin (mean: 40 h) [14]. The half-life of R-warfarin ranges from 37 to 89 h, while the half-life of S-warfarin ranges from 21 to 43 h [15]. The observation period of 6 days for Treatment A in addition with the in-between period of at least 7 days between the end of period A and start of period B was considered sufficient to avoid any carry over of S-warfarin from Treatment
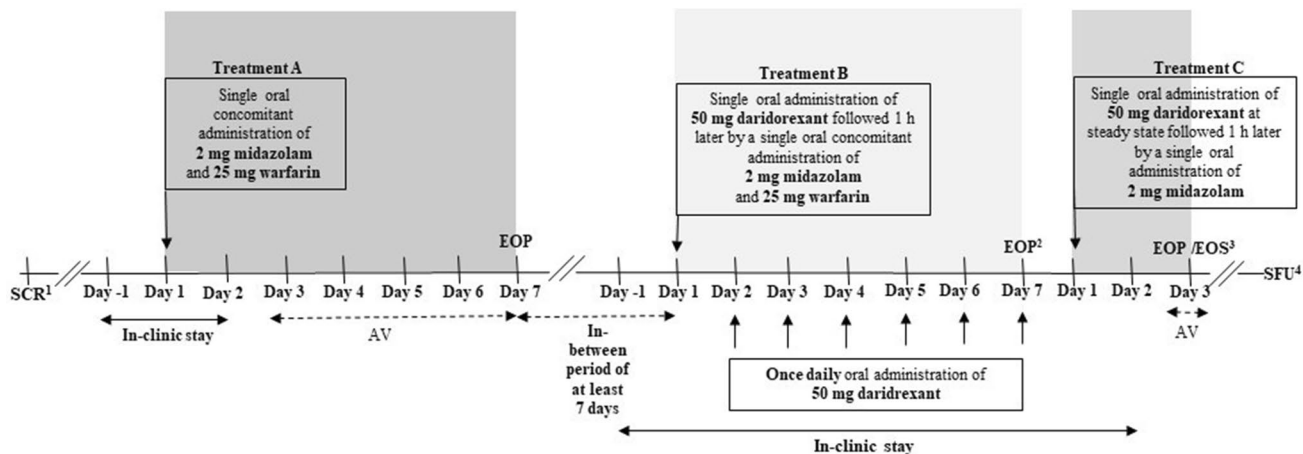
Fig. 1 Study design. [1]Screening took place 3–21 days before the first study treatment administration. [2]No in-between period between Treatments B and C. [3]End-of-study (EOS) took place 2 days after last administration of daridorexant. [4]Safety follow-up (SFU) telephone call was performed 5–7 days after EOS. *AV* ambulatory visit(s), *EOP* end-of-period, *h* hour, *SCR* screening

period A to Treatment period B. There was no in-between period between Treatment periods B and C. All study treatments were administered to the subjects in the morning in the fasted state. Depending on the study treatment received, administrations were followed by a 24-h period to assess the PK profile of midazolam and/or a 144-h period to assess the PK and PD profiles of warfarin. Subjects stayed at the study site from the evening of day − 1 until the evening of day 2 of Treatment A and from the evening of day − 1 of Treatment B until the morning of day 2 of Treatment C (Fig. 1). Subjects had to refrain from strenuous physical exercise and work, and were not allowed to consume alcohol, nicotine, or food and beverages that could influence CYP3A activity (e.g., grapefruit, and/or Seville orange or xanthine-containing beverages) or prothrombin time in subjects treated with warfarin (cranberry, excessive quantities of liver, broccoli, brussels sprouts, green leafy vegetables). Concomitant medication was not allowed unless required for the treatment of an adverse event (AE).

2.2 Subjects

Healthy non-smoking male subjects aged between 18 and 45 years who had a body mass index of 18.0–30.0 kg/m^2 at screening were eligible. Subjects were judged to be healthy based on their medical history, physical examination, electrocardiogram, vital signs, and clinical laboratory tests. Subjects were excluded from the study if they had any contraindications to daridorexant, midazolam, and/or warfarin, any history of hemorrhagic disease, any bleeding complications, any history of narcolepsy or cataplexy, an activated partial thromboplastin time > 40 s and/or an INR >1.15 at screening, and if they had a platelet count < 150 or > 400 × 10^9/L at screening.

2.3 Blood Sampling and Bioanalytical Methods

Blood samples for the determination of midazolam were collected at the following timepoints: pre-dose, 10 min, 20 min, 30 min, 1 h, 2 h, 4 h, 6 h, 8 h, 12 h, 16 h, and 24 h post-dose in Treatments A, B, and C. Blood samples for the determination of S-warfarin were collected at pre-dose, 2 h, 4 h, 8 h, 12 h, 24 h, 36 h, 48 h, 72 h, 96 h, 120 h, and 144 h post-dose in Treatments A and B. For both analytes, blood samples were collected in ethylenediaminetetraacetic acid tubes and afterwards centrifuged, and the obtained plasma was stored at − 20 °C ± 5 °C. Plasma concentrations of midazolam and S-warfarin were determined using a validated liquid chromatography with tandem mass spectrometry assay. Midazolam-d4 was used as internal standard (Midazolam-D4 maleate/ Midazolam-d4, Lot No FE03311701; Sigma Aldrich, Buchs, Switzerland) and the lower limit of quantification was 0.1 ng/mL. The chromatographic system consisted of an analytical column (ReproSil-Gold C18, 10 × 2 mm, 5 μm; Dr. Maisch HPLC GmbH, Ammerbuch, Germany). The mobile phase consisted of ammonium formate 5 mM in water for Phase A and acetonitrile for Phase B. Mass spectrometric detection was performed with a triple-stage quadrupole mass spectrometer (TSQ Vantage; Thermo Fisher Scientific, San Jose, CA, USA) operating in an electrospray ionization mode. Midazolam quality-control samples resulted in an inter-batch precision between 3.3 and 3.6% and inter-batch accuracy between 99.1 and 103.7%.

Warfarin-d5 was used as an internal standard (Warfarin-d5, Lot No 4-YMK-34-3; Toronto Research Chemicals, Toronto, ON, Canada) and the lower limit of quantification was 12.5 ng/mL. The chromatographic system consisted of an analytical column (Astec CHIROBIOTIC V2, 150 mm × 2.1 mm, 5 μm). The mobile phase consisted of 0.1% acetic

acid/acetonitrile 70/30. Mass spectrometric detection was performed with a high-performance liquid chromatography system (Perkin Elmer Series 200 Micropump) and an MS/MS system (Applied Biosystems/MDS SIEX API 4000). S-warfarin quality-control samples resulted in an inter-batch precision between 4.9 and 7.7% and an inter-batch accuracy between 94.7% and 102.4%.

Blood samples for the determination of factor VII and INR were collected in tubes containing citrate over a period of 144 h after warfarin administration in Treatments A and B. These samples were put on ice immediately after collection and sent as soon as possible to the local laboratory for analysis. International normalized ratio and factor VII activity in citrated plasma were determined using two commercial in vitro assays, Cobas®, PT Rec, prothrombin time and cobas®, FVII, and factor VII (both Roche Diagnostics GmbH, Mannheim, Germany).

2.4 PK and PD Analysis

The PK and PD analyses (except for the maximum effect on factor VII activity VII_{max}) were determined by noncompartmental methods using Phoenix WinNonlin 8.3.3.33 (Pharsight Inc., Princeton, NJ, USA). Maximum plasma concentration (C_{max}) and t_{max} were directly obtained from the individual plasma concentrations of midazolam and S-warfarin. The area under the plasma concentration–time curve (AUC) from 0 to time t of the last measured concentration (AUC_{0-t}) was calculated by using the linear trapezoidal rule. The AUC from time 0 to infinity ($AUC_{0-\infty}$) was calculated by $AUC_{0-t} + Ct/\lambda z$, where C_t is the last concentration above the lower limit of quantification and λz is the terminal elimination rate constant determined by log-linear regression analysis of the measured plasma concentrations of the terminal elimination phase. The $t_{1/2}$ of midazolam and S-warfarin was calculated as ln $2/\lambda z$. As the concentrations of midazolam were still measurable 24 h post-dose, $AUC_{0-\infty}$ was calculated by $AUC_{0-24} + C_{24}/ \lambda z$, where C_{24} is the plasma concentration measured at 24 h.

For PD variables, VII_{max} was obtained as the value of maximum decrease of factor VII activity, expressed as a percentage change from baseline. The measured individual values of INR and factor VII activity were used to directly obtain the maximum effect on INR (INR_{max}), VII_{max}, and time to reach maximum effect, AUC_{0-144} for INR, and AUC_{0-144} for factor VII activity were calculated according to the linear trapezoidal rule using the measured INR and factor VII values from zero to 144 h.

2.5 Safety and Tolerability Analysis

Subjects were monitored for safety and tolerability throughout the study. Assessments were based on the recording of AEs, physical examination, vital signs, body weight, 12-lead electrocardiogram, and clinical laboratory tests.

2.6 Statistical Analysis

Midazolam PK parameters show higher variability than those of warfarin [16, 17]. To ensure the suitability of the sample size of the study for both drugs, the precision estimate was therefore performed based on midazolam PK parameters from a previous study [16]. Based on a mixed-effects model analysis, the within-subject standard deviation on the log scale of 0.29 for C_{max} and 0.32 for $AUC_{0-\infty}$ was calculated from a previous study [16]. With a sample size of 16 evaluable subjects, the lower and upper bounds of the 90% confidence interval (CI) for the true ratio test versus reference would be approximately (0.88–1.13) for C_{max} and (0.87–1.15) for $AUC_{0-\infty}$ if the estimated ratio was 1. Therefore, 18 subjects were enrolled to have at least 16 evaluable subjects.

Differences in PK parameters (C_{max}, $AUC_{0-\infty}$, AUC_{0-24}, and $t_{1/2}$) of midazolam between Treatments B and C (tests) and Treatment A (reference) and of S-warfarin between Treatment B (test) and Treatment A (reference) were explored using the geometric mean ratio and the 90% CI after back-transformation of linear mixed-effects model estimates. The linear mixed-effects model included the log-transformed value of PK parameters as dependent variables, treatment as a fixed effect, and subject as a random effect. Differences for t_{max} of midazolam between Treatments B and C (tests) and Treatment A (reference) and of S-warfarin between Treatment B (test) and Treatment A (reference) were explored using the nonparametric Hodges–Lehmann estimates of the median of differences (test-reference) and their 90% CIs [18].

Differences in PD variables (INR_{max}, AUC_{0-144} for INR, VII_{max}, and AUC_{0-144} for factor VII activity) of warfarin between Treatment B (test) and Treatment A (reference) were explored using the GMR and the 90% CI after back-transformation of the linear mixed-effects model estimates. The linear mixed-effects model included the log-transformed value of PD variables as dependent variables, treatment as a fixed effect, and subject as a random effect. Differences for time to reach maximum effect on factor VII activity and INR of warfarin between Treatment B (test) and Treatment A (reference) were explored using the nonparametric Hodges–Lehman estimates of the median of differences (test-reference) and their 90% CIs [18]. Descriptive statistics were used for baseline demographics, safety, and tolerability.

3 Results

3.1 Subjects

In this study, 18 healthy male subjects were enrolled and their mean (range) values for age and body mass index were 28.5 (19–44) years and 25.1 (18.7–29.7) kg/m^2. All subjects completed the study as per protocol. Out of the 18 subjects, 16 were White, one was Asian, and one was of mixed race (Table 1).

3.2 Pharmacokinetics

The mean plasma concentration–time profiles of midazolam in the absence (Treatment A) and presence of daridorexant (Treatments B and C) are shown in Fig. 2a, b. Administration of midazolam with a single dose of daridorexant 50 mg (Treatment B) increased geometric mean C_{max}, $t_{1/2}$, and AUC_{0-24} of midazolam by 1.13-fold, 1.08-fold, and 1.42-fold, respectively, compared to administration of midazolam alone (Treatment A) [Table 2]. The median difference for t_{max} was 0.25 h (Table 2). After o.d. administration of daridorexant 50 mg at steady state followed by midazolam 2 mg administration 1 h later (Treatment C), midazolam geometric mean C_{max}, $t_{1/2}$, and AUC_{0-24} increased by 1.12-fold, 1.14-fold, and 1.35-fold, respectively, compared to Treatment A (Table 2). The median difference for t_{max} was 0.25 h (Table 2).

The mean plasma concentration–time profiles of S-warfarin alone (Treatment A) and during administration of 50 mg daridorexant (Treatment B) were superimposable (Fig. 3). The PK parameters of S-warfarin were similar in the absence and presence of daridorexant (Table 3). The statistical analysis confirmed the absence of a relevant interaction with GMRs for S-warfarin of 0.97 (90% CI 0.91–1.04) for C_{max}, 1.15 (90% CI 1.09–1.22) for $t_{1/2}$, and 1.02 (90% CI 0.98–1.07) for $AUC_{0-\infty}$. The median difference for t_{max} was 1.00 h (90% CI 0.04–1.06) [Table 3].

3.3 Pharmacodynamics

The arithmetic mean versus time profiles of INR and factor VII after administration of warfarin alone (Treatment A) and after administration with daridorexant and warfarin (Treatment B) are shown in Figs. 4 and 5. A single dose of warfarin 25 mg led to an increase in INR that was similar in the absence and presence of daridorexant 50 mg (Fig. 4). After administration of warfarin 25 mg alone (Treatment A), the maximum increase in INR was reached after 47.8 h, while after administration with daridorexant 50 mg and warfarin 25 mg (Treatment B), the maximum INR was observed after 36.0 h, resulting in a median difference of − 6.01 h (Table 4). Derived PD variables of AUC_{0-144} for INR and INR_{max} were comparable between Treatments A and B

Table 1 Demographic characteristics of study subjects

Characteristics	Statistics	Enrolled subjects $N = 18$
Sex		
Male	n (%)	18 (100)
Race		
Asian	n (%)	1 (5.6)
White	n (%)	16 (88.9)
Other: mixed	n (%)	1 (5.6)
Age [years]	Mean (SD)	28.5 (8.2)
	Range	19, 44
Weight [kg]	Mean (SD)	80.7 (13.5)
	Range	53.3, 105.1
Height [cm]	Mean (SD)	179 (8.8)
	Range	167, 202
BMI [kg/m^2]	Mean (SD)	25.1 (3.1)
	Range	18.7, 29.7

Arithmetic mean is presented

BMI body mass index, *N* number of subjects in the study, *n* number of subjects in the considered situation, *SD* standard deviation, % percentage of subjects

with GMRs and corresponding 90% CIs within the bioequivalence limit of 0.80–1.25 (Table 4).

Factor VII activity similarly decreased after administration of warfarin 25 mg alone (Treatment A) or after administration with daridorexant 50 mg and warfarin 25 mg (Treatment B) [Fig. 5]. AUC_{VII} activity and VII_{max} did not differ between treatments with GMR values of 1.14 (90% CI 1.10–1.19) and 0.90 (90% CI 0.86–0.95), respectively (Table 4). The median difference for the time to reach maximum effect between treatments was again − 6.01 h (Table 4). At 144 h after study treatment administration, which was the final sampling time, mean IN, and factor VII returned to baseline levels (Figs. 4 and 5).

3.4 Safety and Tolerability

No serious AEs or AEs leading to premature discontinuation were reported in this study. Out of 18 subjects, one subject (5.6%) reported at least one AE in Treatment A and 16 subjects (88.9%) in Treatment B (Table 5). No subject reported any AEs in Treatment C (Table 5). The most frequently reported AE was somnolence (16/18 subjects [88.9%]) when midazolam and warfarin were administered concomitantly with o.d. dosing of daridorexant 50 mg (Treatment B). All AEs (except contusion and arthropod bite) were considered related to study treatment and resolved without sequelae. All AEs were either of mild (17 events) or moderate (six events) intensity. There were no clinically relevant changes in vital signs, electrocardiogram, and clinical laboratory variables throughout the study. All study treatments were safe and well tolerated.
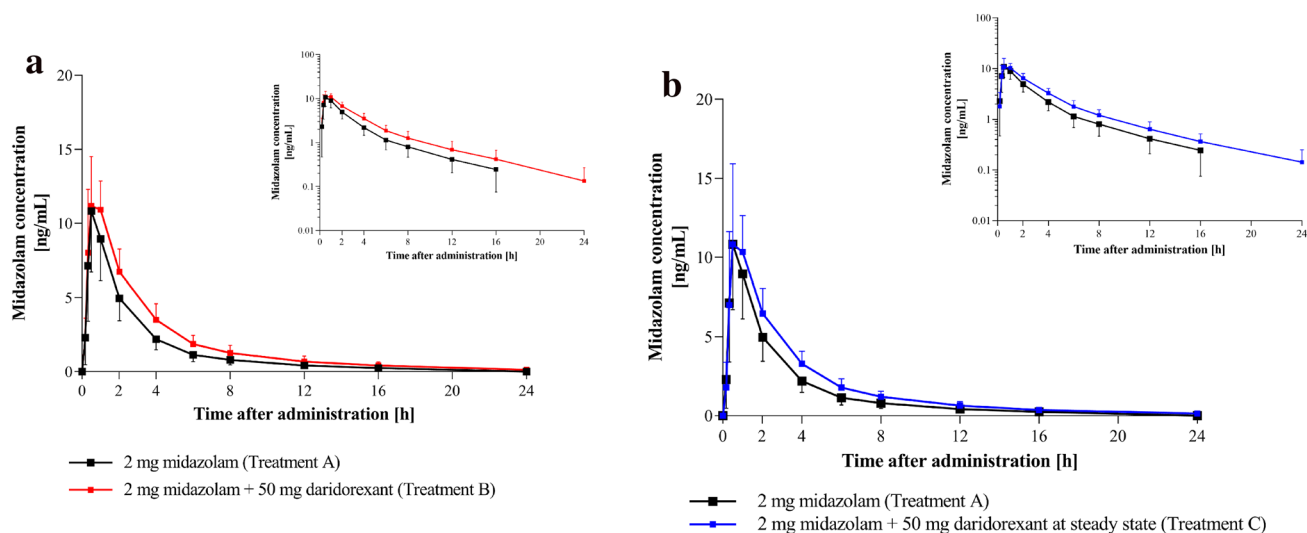
Fig. 2 Arithmetic mean (±standard deviation) plasma concentration–time profiles of midazolam after (**a**) administration of midazolam 2 mg alone (Treatment A) and after administration with daridorexant 50 mg (Treatment B), and after (**b**) administration of midazolam 2 mg alone (Treatment A) and after administration with daridorexant 50 mg at steady state (Treatment C) on linear (lower) and semilogarithmic (upper) scales, $N = 18$. N number of subjects with available value

Table 2 Effect of daridorexant on the pharmacokinetic parameters of midazolam ($n = 18$)

Parameter (unit)	Midazolam		Midazolam	
Statistics	Treatment A	Treatment B	Treatment A	Treatment C
C_{max} [ng/mL]				
Geometric mean (95% CI)	10.8 (9.2–12.7)	12.2 (10.9–13.6)	10.8 (9.2–12.7)	12.1 (10.4–14.4)
GMR (90% CI)	1.13 (0.98–1.29)		1.12 (0.96–1.29)	
t_{max} [h]				
Median (range)	0.5 (0.3–1.0)	1.0 (0.4–1.0)	0.5 (0.3–1.0)	1.0 (0.3–1.0)
Median of differences (90% CI)	0.25 (0.00–0.50)		0.25 (0.0–0.25)	
AUC_{0-24} [ng·h/mL]				
Geometric mean (95% CI)	30.3 (26.0–35.3)	42.9 (37.9–48.6)	30.3 (26.0–35.3)	40.8 (36.9–45.1)
GMR (90% CI)	1.42 (1.28–1.56)		1.35 (1.23–1.47)	
$t_{1/2}$ [h]				
Geometric mean (95% CI)	4.7 (4.0–5.6)	5.0 (4.5–5.7)	4.7 (3.9–5.6)	5.3 (4.6–6.0)
GMR (90% CI)	1.08 (1.00–1.17)		1.14 (1.05–1.23)	

Treatment A = midazolam (2 mg) + warfarin (25 mg), Treatment B = single-dose daridorexant (50 mg) + midazolam (2 mg) + warfarin (25 mg), Treatment C = daridorexant (50 mg) at steady state + midazolam (2 mg)

AUC$_{0-24}$ area under the plasma concentration–time curve from zero to 24 h, *CI* confidence interval, *C$_{max}$* maximum plasma concentration, *GMR* geometric mean ratio, *n* number of subjects with available value, *t$_{1/2}$* terminal half-life, *t$_{max}$* time to reach maximum plasma concentration

4 Discussion

The present DDI study investigated the perpetrator potential of daridorexant at the highest approved therapeutic dose of 50 mg on midazolam PK and on warfarin PK and PD in healthy male subjects. A cocktail approach was chosen for this study in order to simultaneously evaluate the effect of daridorexant on CYP3A4 and CYP2C9 activities, and to minimize the number of healthy subjects exposed to drugs who do not experience therapeutic benefit. This simultaneous investigation was possible because of the documented lack of mutual PK interactions between the two probe substrates midazolam and warfarin [19, 20]. With midazolam 2 mg and warfarin 25 mg as interacting treatments, three treatment periods were integrated in the study design, to first, investigate the influence of single and multiple doses of daridorexant 50 mg at steady state
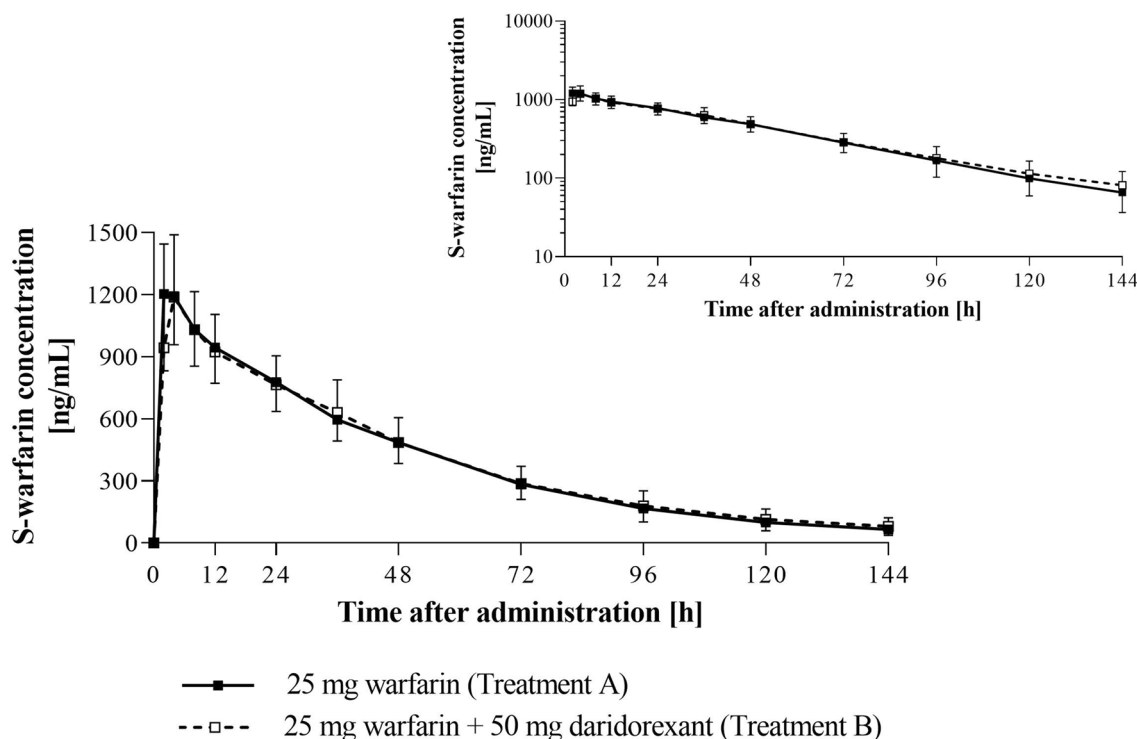
Fig. 3 Arithmetic mean (±standard deviation) plasma concentration–time profiles of S-warfarin after administration of warfarin 25 mg alone (Treatment A) and after administration with daridorexant 50 mg (Treatment B) on linear (lower) and semilogarithmic (upper) scales, $N = 18$. *h* hours, *N* number of subjects with available value

Table 3 Effect of daridorexant on the pharmacokinetic parameters of S-warfarin ($n = 18$)

Parameter [unit]	S-Warfarin	
Statistics	Treatment A	Treatment B
C_{max} [ng/mL]		
Geometric mean (95% CI)	1302 (1165–1455)	1268 (1115–1443)
GMR (90% CI)	0.97 (0.91–1.04)	
t_{max} [h]		
Median (range)	2.0 (2.0–4.0)	4.0 (2.0–8.0)
Median of differences (90% CI)	1.00 (0.04–1.06)	
$AUC_{0-\infty}$ [ng·h/mL]		
Geometric mean (95% CI)	59,159 (53,847–64,994)	60,600 (53,595–68,520)
GMR (90% CI)	1.02 (0.98–1.07)	
$t_{1/2}$ [h]		
Geometric mean (95% CI)	33.0 (30.7–35.4)	38.0 (34.1–42.3)
GMR (90% CI)	1.15 (1.09–1.22)	

Treatment A = midazolam (2 mg) + warfarin (25 mg), Treatment B = daridorexant (50 mg) + midazolam (2 mg) + warfarin (25 mg)

$AUC_{0-\infty}$ area under the plasma concentration–time curve from zero to infinity, *CI* confidence interval, C_{max} maximum plasma concentration, *GMR* geometric mean ratio, *n* number of subjects with available value, $t_{1/2}$ terminal half-life, t_{max} time to reach maximum plasma concentration

on the PK of midazolam. Second, the effects of a single dose of daridorexant 50 mg on the PK and PD of warfarin were explored. This three-period study design enabled the simultaneous investigation of potential inhibitory and inductive effects of daridorexant on CYP3A4 and CYP2C9 activities in one study. Moreover, the repeated dosing of

Fig. 4 Arithmetic mean (±standard deviation) international normalized ratio (INR) time profiles after administration of warfarin 25 mg alone (Treatment A) and after administration with daridorexant 50 mg (Treatment B), $N = 18$. *h* hours, *N* number of subjects with available value
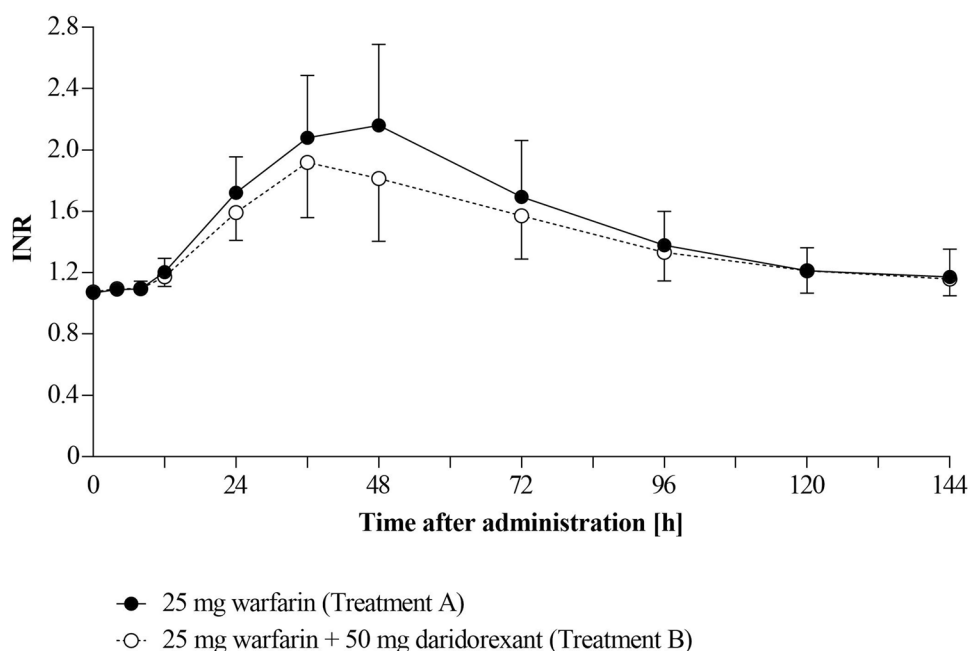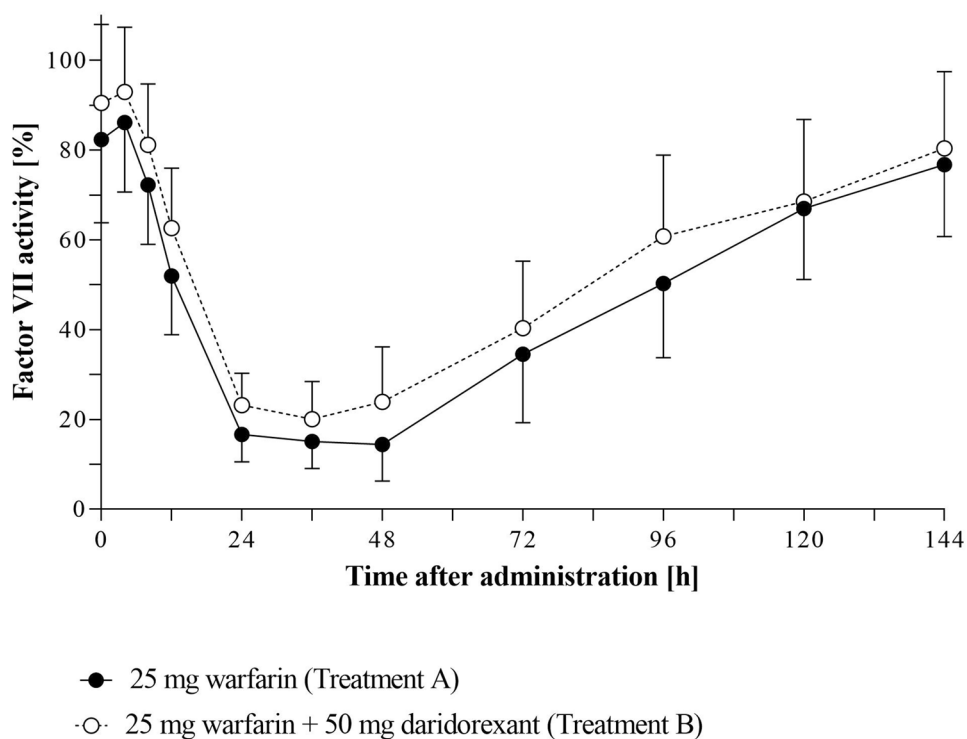

- 25 mg warfarin (Treatment A)
- 25 mg warfarin + 50 mg daridorexant (Treatment B)

Fig. 5 Arithmetic mean (standard deviation) factor VII activity-time profiles after administration of warfarin 25 mg alone (Treatment A) and after administration with daridorexant 50 mg (Treatment B), $N = 18$. *h* hours, *N* number of subjects with available value

- 25 mg warfarin (Treatment A)
- 25 mg warfarin + 50 mg daridorexant (Treatment B)

daridorexant during the investigation of CYP2C9 and CYP3A4 inhibition allowed the build-up of steady-state conditions and potential enzyme induction for Treatment period C.

The PK parameters of midazolam 2 mg and warfarin 25 mg in the absence of daridorexant were within the range of the 95% CI of the geometric means of C_{max} and AUC from previously reported studies [6, 21–25] and no outlier subjects were detected for PK parameters or PD variables. The midazolam PK parameters C_{max}, $t_{1/2}$, and AUC_{0-24} were slightly increased (1.13-fold, 1.08-fold, and 1.42-fold), while t_{max} was comparable, when midazolam was administered with a single dose of daridorexant 50 mg compared to administration of midazolam alone. After o.d. administration of daridorexant 50 mg at steady state, midazolam PK parameters were comparable to midazolam PK parameters when administered

Table 4 Effect of daridorexant on pharmacodynamic variables ($n = 18$)

Parameter [unit]	Pharmacodynamic set	
Statistics	Treatment A	Treatment B
AUC_{INR} [h]		
Geometric mean (95% CI)	220 (204–236)	206 (193–220)
GMR (90% CI)	0.94 (0.91–0.96)	
INR_{max}		
Geometric mean (95% CI)	2.2 (1.9–2.4)	1.9 (1.7–2.1)
GMR (90% CI)	0.89 (0.85–0.93)	
t_{Emax} on INR [h]		
Median (range)	47.8 (24.0–48.2)	36.0 (36.0–48.0)
Median of differences (90% CI)	− 6.01 (− 6.09 to − 0.05)	
AUC_{VII} [%·h]		
Geometric mean (95% CI)	6256 (5557–7043)	7163 (6340–8093)
GMR (90% CI)	1.14 (1.10–1.19)	
VII_{max} [% inhibition]		
Geometric mean (95% CI)	83.9 (80.1–87.8)	75.6 (69.4–82.4)
GMR (90% CI)	0.90 (0.86–0.95)	
t_{Emax} on factor VII [h]		
Median (range)	48.0 (24.0–48.2)	36.0 (24.0–48.0)
90% CI of the median	− 6.01 (− 12.0 to − 0.02)	

Treatment A = midazolam (2 mg) + warfarin (25 mg), Treatment B = daridorexant (50 mg) + midazolam (2 mg) + warfarin (25 mg)

AUC_{INR} area under the plasma concentration–time curve from zero to time 144 h for the INR, *AUC_{VII}* area under the plasma concentration–time curve from zero to time 144 h for factor VII activity, *CI* confidence interval, *GMR* geometric mean ratio, *INR* international normalized ratio, *INR_{max}* maximum effect on INR, *n* number of subjects with available value, *t_{Emax}* time to reach maximum effect, *VII_{max}* maximum effect on factor VII activity

with a single dose of daridorexant, but slightly increased (1.12-fold, 1.14-fold, and 1.35-fold for C_{max}, $t_{1/2}$, and AUC$_{0–24}$) compared to administration of midazolam alone. These results demonstrated that CYP3A4 is not induced by daridorexant 50 mg administered o.d. Given the increased observed AUC$_{0–24}$ GMR data of midazolam in Treatments B and C compared with Treatment A, these results indicated that daridorexant is a weak inhibitor of CYP3A4 at 50 mg, both after a single dose and at steady state. These conclusions are drawn based on the Food and Drug Administration and European Medicines Agency classification criteria, whereby weak inhibitors result in a ≥ 1.25-fold increase to a < 2-fold increase in AUC values [26, 27].

Warfarin and midazolam were administered 1 h after single-dose administration of daridorexant when potential CYP2C9 and CYP3A4 inhibition was expected to be maximal, and any possible inhibition of CYP2C9 was maintained during the elimination phase of warfarin by continued administration of daridorexant in Treatment B. Warfarin is clinically used as a racemic mixture of two isomers, R-warfarin and S-warfarin, which have different pathways of metabolic clearance. The pharmacologically more active S-warfarin is metabolized mainly by CYP2C9 [11, 28] and

was therefore selected as a substrate to investigate a potential CYP2C9 DDI with daridorexant in this study. The PK of S-warfarin were unchanged in the absence and presence of daridorexant 50 mg with GMRs and corresponding 90% CIs within the bioequivalence limit of 0.80–1.25. Consequently, CYP2C9 was not inhibited by daridorexant 50 mg. In agreement with the lack of an effect of single-dose daridorexant 50 mg on S-warfarin pharmacokinetics, administration of daridorexant had no relevant effect on the warfarin-induced increase in INR and the decrease of factor VII plasma concentrations. Median time differences to attain INR_{max} and VII_{max} were −6.01 h for both variables, while the other PD variables were unchanged following administration of daridorexant 50 mg and warfarin 25 mg compared to administration of warfarin 25 mg alone. The activity versus time profiles remained comparable and were characterized by broad peaks (Figs. 4 and 5), which may explain the apparent median time differences to attain INR_{max} and VII_{max}. PD variables should be included in DDI studies whenever possible, as there may be a discrepancy between PK and PD. For instance, the INR in healthy subjects was enhanced by the intake of cranberry juice without affecting warfarin PK [29]. In this study, no plasma concentrations of daridorexant

Table 5 Summary of treatment-emergent AEs by treatment and preferred term

Preferred term	Enrolled subjects								
	Treatment A, $N_T = 18$			Treatment B, $N_T = 18$			Treatment C, $N_T = 18$		
	nAEs	n	(%)	nAEs	n	(%)	nAEs	n	(%)
Any preferred term									
Number of subjects with at least one AE		1	(5.6)		16	(88.9)		0	
Number of different AEs	2			5			0		
Total number of AEs	2			21			0		
Somnolence	0	0		16	16	(88.9)	0	0	
Arthropod bite	0	0		2	2	(11.1)	0	0	
Headache	1	1	(5.6)	1	1	(5.6)	0	0	
Abnormal dreams	0	0		1	1	(5.6)	0	0	
Contusion	1	1	(5.6)	0	0		0	0	
Hyperhidrosis	0	0		1	1	(5.6)	0	0	

Treatment A = midazolam (2 mg) + warfarin (25 mg), Treatment B = daridorexant (50 mg) + midazolam (2 mg) + warfarin (25 mg), Treatment C = daridorexant (50 mg) at steady state + midazolam (2 mg)

AE adverse event, *N* number of subjects in the population, *n* number of subjects experiencing the event, *nAEs* number of adverse events, N_T number of subjects in the study having received the related treatment (denominator of percentages), % percentage of subjects

were assessed as the PK of the compound had been extensively studied in former phase I studies and compliance was ensured as all treatments were administered under close medical monitoring at the study site [30]. Only male subjects were included to have a more homogenous population and to limit variability in PK parameters and PD variables.

5 Conclusions

In summary, daridorexant at 50 mg is classified as a weak inhibitor of CYP3A4. CYP3A4 is not induced by daridorexant 50 mg administered o.d. Daridorexant has no influence on the PK and PD of warfarin, and thus, CYP2C9 is not inhibited by daridorexant 50 mg. All treatments were safe and well tolerated.

Declarations

Ethics Approval The study was conducted in full conformity with the principles of the Declaration of Helsinki. The protocol was approved by the German Health Authority (BfArM) and by the local independent Ethics Committee (Ethik Kommission der Bayerischen Landesärztekammer).

Consent to Participate All subjects provided written informed consent prior to any study procedure.

Consent for Publication Not applicable.

Availability of Data and Material The datasets generated and/or analyzed during the study are available from the corresponding author on reasonable request.

Code Availability Not applicable.

Authors' Contributions IZ wrote the manuscript, IZ, JD, and MG designed the study, PK and JD analyzed the study, and CR was the principal investigator of the study. All authors revised the manuscript critically for important intellectual content and approved the version to be submitted.

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