Oral bioavailability of dabigatran etexilate (Pradaxa[®]) after co-medication with verapamil in healthy subjects

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WHAT IS ALREADY KNOWN ABOUT THIS SUBJECT

- Dabigatran, the active principle of the oral prodrug dabigatran etexilate (DE), is a direct and reversible thrombin inhibitor with potent antithrombotic effects.
- DE and dabigatran are not metabolized by hepatic cytochrome P450 isoenzymes and do not affect the metabolism of other drugs that utilize the cytochrome P450 system.
- DE, but not dabigatran, is a substrate of the efflux transporter P-glycoprotein (P-gp) and drugs that affect P-gp may alter the bioavailability of dabigatran.

WHAT THIS STUDY ADDS

- The bioavailability of dabigatran is increased when co-administered with a P-gp inhibitor, such as verapamil. Several factors affected the interaction: (i) the dosing interval between the two drugs, such that the maximum increase in dabigatran exposure occurred when verapamil was given 1 h before DE, whereas there was virtually no effect on exposure when DE was given 2 h before verapamil, (ii) the formulation of verapamil, with the immediate release (IR) formulation having a greater effect than the extended release (ER) formulation and (iii) verapamil dosing, with a single dose having a greater effect on the PK of dabigatran than multiple dosing.
- An interaction between verapamil and DE might be minimized if DE is administered in the fasted state 2 h prior to verapamil.

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Parts of these results were presented in a late-breaking abstract at the 112th ASCPT meeting, Denver, 2–5 March 2011 (Poster No.LBIII-2).

Keywords

dabigatran etexilate, drug interaction, pharmacokinetics, Pradaxa[®], verapamil

Received

14 October 2011

Accepted

28 August 2012

Accepted Article

Published Online

5 September 2012

AIM

To investigate the effect of the P-glycoprotein inhibitor verapamil on the pharmacokinetics and pharmacodynamics of dabigatran etexilate (DE).

METHOD

In this two part multiple crossover trial in 40 healthy subjects, DE 150 mg was given alone or with verapamil at different doses, duration of treatment (single vs. multiple dosing), formulations, and timings (before, concurrently or after DE). Primary pharmacokinetic endpoints were determined from concentrations of total dabigatran (unconjugated plus conjugated). Pharmacodynamic endpoints were determined from clotting time.

RESULTS

The greatest effect was observed with single dose verapamil 120 mg immediate release given 1 h before single dose DE. Geometric mean area under the plasma concentration curve [AUC(0,∞)] and maximum analyte concentration in the plasma (C_{max}) were increased by 143% [90% confidence interval (CI) 91, 208] and 179% (90% CI 115, 262), respectively. The effect was reduced to a 71% and 91% increase in AUC and C_{max}, respectively, when DE was administered with verapamil 240 mg extended release. After multiple verapamil dosing, DE AUC(0,∞) and C_{max} increases were 54% and 63%, respectively. However, DE given 2 h before verapamil increased DE AUC(0,∞) and C_{max} by <20%. With regard to clotting prolongation, the dabigatran plasma concentration–effect relationship was generally not affected by the co-administration of verapamil. Concomitant administration of DE and verapamil did not reveal any unexpected safety findings.

CONCLUSION

Verapamil increased DE bioavailability, likely due to inhibition of P-glycoprotein. Our results suggest that an interaction between verapamil and DE can be minimized if DE is administered 2 h prior to verapamil.

Introduction

Dabigatran, the active principle of the prodrug dabigatran etexilate (DE; Pradaxa®, Boehringer Ingelheim Pharma GmbH & Co KG, Biberach, Germany), is an oral, direct thrombin inhibitor with potent antithrombotic effects [1–4]. It is currently approved in the USA, European Union, Canada and Japan for the prevention of stroke and systemic emboli in patients with non-valvular atrial fibrillation [5–7]. DE is also approved for the primary prevention of thromboembolism in adults after elective total hip or knee replacement surgery in more than 75 countries, including Europe [8] and Canada [6].

The oral prodrug DE is rapidly absorbed in the intestine and is converted by esterase catalyzed hydrolysis mainly in the liver, via two intermediates, BIBR 1087 SE and BIBR 951 BS, to the active moiety dabigatran. About 20% of dabigatran is conjugated with activated glucuronic acid to form a pharmacologically equipotent glucuronide. Dabigatran has a fast onset and offset [half-life ($t_{1/2}$) 12–17 h], dose proportional and linear pharmacokinetics (PK)/pharmacodynamics (PD), allowing for fixed dose treatment regimens [9–11]. No drug–food interactions have been observed with DE [10].

Dabigatran is not metabolized by hepatic cytochrome P450 isoenzymes and has a low potential for drug–drug interactions [10, 12]. However, studies have shown that drugs that inhibit the efflux transporter P-glycoprotein (P-gp), including verapamil, amiodarone and quinidine, raise dabigatran serum concentrations [12–14]. Preclinical and clinical data suggest that DE, but not dabigatran, is a substrate of P-gp [14, 15].

The present study was designed to investigate the effect of the P-gp inhibitor verapamil on the PK and PD of DE in order to provide guidance for the clinical use of this drug combination. Verapamil is a calcium antagonist and is widely used for the management of atrial fibrillation, angina pectoris and hypertension. To identify the cofactors determining the magnitude of this potential drug

interaction, verapamil was given at different doses, in different formulations and at different times relative to the DE dose.

The primary objective of this trial was to investigate whether, and to what extent, verapamil affected the PK parameters of DE. To identify cofactors important in any potential drug interaction, verapamil was given (i) at different dosages, 120 mg single dose, 240 mg day⁻¹ and 480 mg day⁻¹, (ii) in different formulations, immediate (IR) and extended release (ER) and (iii) in different intervals in relation to the dabigatran dose.

The secondary objectives were to determine the safety and tolerability of dabigatran and verapamil upon co-administration, to identify the effect of dabigatran on the PK of verapamil and to determine the PD of dabigatran, with and without co-administration of verapamil.

Methods

Study design

This was a two part multiple crossover study, with an open label, fixed sequence design in part 1 (multiple dose, steady-state verapamil) and an open label, randomized, five way crossover design in part 2 (single doses of verapamil). In both parts, following a screening period of up to 21 days, single oral doses of DE 150 mg were given alone or with verapamil (120–480 mg daily). Each part involved a separate group of 20 healthy subjects.

In study part 1 (multiple dose verapamil), subjects received five open label treatments (A to E) in a fixed sequence over a total of 23 days (Table 1).

In study part 2 (single doses of verapamil), subjects received five open label treatments (F to J) in a randomized five way crossover fashion [Williams design (16)] over a total of 25 days, with washout periods of at least 4 days between treatments (Table 1).

Table 1

Treatments

Study part 1 (multiple dose verapamil): fixed sequence		
A	Reference	DE 150 mg single dose*
B	Reference	V IR 120 mg twice daily for 3 days (days 1–3)
C	Test	V IR 120 mg twice daily for 4 days (days 4–7) with the day 4 morning dose 1 h before DE 150 mg
D	Test	V IR 120 mg twice daily for 1 day (day 8) with the morning dose 2 h after DE 150 mg
E	Test	V IR 120 mg three times daily for 1 day (day 9), then 120 mg four times daily (480 mg day ⁻¹) for 3 days (days 10–12) with the day 12 morning dose 1 h before DE 150 mg
Study part 2 (single dose of verapamil): crossover		
F	Reference	DE 150 mg single dose
G	Reference	V IR 120 mg single dose
H	Test	V IR 120 mg 1 h before DE 150 mg
I	Test	V IR 120 mg concomitantly with DE 150 mg
J	Test	V ER 240 mg concomitantly with DE 150 mg

*Treatment A was followed by a washout period of at least 4 days. DE, dabigatran etexilate; ER, extended release; IR, immediate release; V, verapamil.

Ethical approval was received from the local Independent Ethics Committee (Ethikkommission der Landesärztekammer Baden-Württemberg, Stuttgart, Germany) and by the German Competent Authority (BfArM, Bonn, Germany). The trial was conducted in compliance with the Declaration of Helsinki, in accordance with the International Conference on Harmonization Harmonized Tripartite Guideline for Good Clinical Practice and in accordance with applicable regulatory requirements. A protocol amendment was issued because the local independent ethics committee requested an extension of electrocardiogram (ECG) monitoring during verapamil multiple dosing and the exclusion of subjects with a PR interval > 170 ms.

Subjects

Eligible subjects were aged 18–55 years, had a body mass index (BMI) of 18.5–29.9 kg m⁻² and were judged to be healthy based on medical history, physical examination, vital signs, 12-lead ECG and clinical laboratory tests. Subjects were excluded if they had any of the following: gastrointestinal, hepatic, renal, respiratory, cardiovascular, metabolic, immunologic or hormonal disorders, risk of bleeding, use of drugs that might affect blood clotting or planned surgery within 4 weeks after the end of the trial. In accordance with the protocol amendment, ECG exclusion criteria were PR > 170 ms (study part 1) and atrioventricular block of any degree (study part 2). All eligible subjects signed written informed consent prior to enrolment.

Drug administration and clinical assessments

DE (Pradaxa®, Boehringer Ingelheim Pharma GmbH & Co. KG) was administered in capsules as the methanesulfonic acid salt. Verapamil was administered as commercially available IR or ER tablets (Isoptin®, Abbott GmbH & Co. KG, Wiesbaden-Delkenheim, Germany). Subjects took their study medication with ~240 ml water.

PK/PD profiles and safety variables in part 1 were assessed on six visits, including a screening visit, four treatment phase visits and an end of trial examination (Supplementary Table S1). Part 2 comprised seven visits: a screening visit, five treatment phase visits and an end of trial examination.

Vital signs and 12-lead ECG were measured at specified intervals throughout the trial. Adverse events (AEs) and concomitant medications were evaluated continuously and tolerability was assessed at the end of each treatment period. A full examination (including physical examination, vital signs, 12-lead ECG and clinical laboratory tests) was performed within 5 days following the last drug administration.

Study endpoints

The primary PK endpoints were area under the plasma concentration curve [AUC(0,∞)] and maximum analyte concentration in the plasma (C_{max}) of total dabigatran (unconjugated plus conjugated).

Other secondary endpoints included PK parameters for the enantiomers (S- and R-) of verapamil and

norverapamil. Additionally, dabigatran PK in urine [CL_R(0,24 h) ml min⁻¹] and the glucuronidation ratio [AUC(0,∞) dabigatran GLUC : AUC(0,∞) dabigatran] were assessed.

Secondary PD (blood coagulation) parameters derived from thrombin clotting time (TT) and ecarin clotting time (ECT) assessments were area under the effect–time curve [AUEC(0,24 h)] and the maximum effect ratio (ER_{max}). Safety and tolerability were also a secondary endpoint.

The main comparison was between DE 150 mg plus verapamil (120 mg IR or 240 mg ER) (test treatments C to E, H to J) compared with DE without verapamil (reference treatments A, F) and verapamil (120 mg IR or 240 mg ER) without DE (reference treatments B, G).

PK/PD determinations

On profiling days, blood samples were taken before and at specified intervals up to 24 h after the morning verapamil dose, and before and up to 34 h after DE (Supplementary Table S1). Blood samples of ~2.7 ml were collected into tubes containing potassium ethylenediaminetetraacetic acid (Biochemed, USA) for PK analysis and sodium citrate (Biochemed, USA) for PD analysis. The samples were centrifuged and the resulting plasma samples were separated and stored at –70°C (dabigatran PK analysis) or –20°C (verapamil PK analysis and all PD analyses).

Plasma concentrations of unconjugated dabigatran, total dabigatran (sum of unconjugated and conjugated dabigatran measured after alkaline cleavage of conjugates) were determined by a validated high performance liquid chromatography tandem mass spectrometry (HPLC-MS/MS) method at AAI Pharma Deutschland GmbH & Co. KG (Neu-Ulm, Germany).

For the determination of unconjugated dabigatran, 50 µl of plasma were aliquoted, diluted with 50 µl 0.2 M ammonium formate buffer (pH 3.5; Fluka, Deisenhofen, Germany), spiked with 40 µl internal standard spiking solution [100 ng ml⁻¹ (¹³C₆) dabigatran], mixed and centrifuged. For the determination of total dabigatran, 50 µl of plasma were aliquoted, spiked with 40 µl internal standard spiking solution and mixed with 20 µl 0.2 M sodium hydroxide (Merck, Darmstadt, Germany). After 2 h of incubation at 37°C, the samples were acidified with 30 µl 0.2 M hydrogen chloride (Merck, Darmstadt, Germany), mixed and then centrifuged. The analytes in the supernatant were extracted by column-switching (Waters OASIS HLB 60 × 1 mm, 60 µm particle size, supplied by GROM, Herrenberg, Germany) and were chromatographed on an analytical C18 reversed-phase HPLC column (Merck Purospher Star RP 18, 55 × 2 mm, 3 µm particle size, Merck, Darmstadt, Germany) with gradient elution. They were quantified by MS/MS with electrospray ionization in positive mode. The calibration curves for unconjugated and total dabigatran covered a range from 1.00 to 400 ng ml⁻¹ plasma in undiluted samples and were linear over the calibration range.

Inaccuracy was at maximum –3.81% and imprecision was less than 6.0% over the whole calibration range.

Plasma concentrations of the enantiomers of verapamil and its major metabolite, norverapamil, were determined by a validated chiral method using HPLC-MS/MS at SGS CephaC Europe (St Benoît, France). The analytes were separated on an enantioselective Chiralpak AD-H X analytical HPLC column [250 × 4.6 mm, 5 µm particle size; Chiral Technologies Europe (Daicel group), Illkirch, France] and quantified by MS/MS with electrospray ionization in positive mode. Verapamil enantiomers or their N-demethylated metabolites could be quantified in a range between 3.00 and 300 ng ml⁻¹. Inaccuracy was at maximum –6.27% and imprecision was less than 11.0% over the whole calibration range for all analytes measured. The sum of the S- and R-enantiomers is reported.

The plasma clotting times (ECT and TT) were assessed using a MC10PLUS coagulometer (MERLIN Medical®, Lemgo, Germany) and subsequent calculation of clotting time for each standard or subject sample under evaluation. Both assays have been used for measurement of dabigatran effects in plasma [within a range of 50 ng ml⁻¹ (lowest calibration level) to 500 ng ml⁻¹ dabigatran], when the effect of dabigatran on various coagulation assays was investigated [17]. The imprecision (CV%) of quality control samples (*n* = 13), was 3.6% and 1.8% for ECT and TT, respectively. The tests were conducted according to the standard operating procedures of the test facility, biochemA GmbH.

Briefly, ECT was assessed by adding *Echis carinatus* venom (ecarin; Pentapharm, Basel, Switzerland) to human plasma, prothrombin (F II), which is converted into meizothrombin, a thrombin analogue with lower fibrinogen-converting activity than thrombin. The time lag between the addition of ecarin and the clot formation was determined. TT was assessed by the addition of thrombin (STA Thrombin, Roche, Basel, Switzerland) to plasma. Pre-incubation of plasma for 2 min at 37°C was followed by addition of the thrombin reagent. The time lag between the addition of the thrombin reagent and clot formation was determined.

Safety

Safety was assessed by medical examination, pulse rate, blood pressure, 12-lead ECG, laboratory parameters and the occurrence and severity of AEs. The investigator assessed tolerability based on AEs and the laboratory evaluation. Tolerability was to be assessed by the investigator according to the categories 'good', 'satisfactory', 'not satisfactory' and 'bad'.

Pharmacokinetic and pharmacodynamic analysis

Pharmacokinetic parameters were estimated with non-compartmental methods using WinNonlin™ (Professional Network Version 5.01, Pharsight Corporation, Mountain View, CA, USA). The AUC spanning various time intervals

was calculated using the linear up/log down algorithm: if a drug concentration was equal to or higher than the preceding concentration, the linear trapezoidal method was used; if the drug concentration was smaller than the preceding concentration, the logarithmic method was used. The AUC from 0 to infinity [AUC(0,∞)] was calculated as $AUC(0,∞) = AUC(0,t_z) + C't_z/\lambda_z$ [where $C't_z$ is the concentration predicted by regression line for the time t_z (last time point with a plasma concentration above the quantification limit)]. The apparent terminal rate constant λ_z at steady-state was estimated from a regression of $\ln C$ vs. time over the terminal log-linear drug disposition portion of the concentration–time profiles.

The C_{max} and time to reach C_{max} (t_{max}) were directly determined from the concentration–time data for each subject. If the same C_{max} occurred at different time points, t_{max} was assigned to the first occurrence of C_{max} .

The terminal half-life was calculated as $\ln 2/\lambda_z$. The renal clearance was calculated using the following equation: $CL_R(0,24\text{ h}) = Ae_{t_1-t_2}/AUC_{t_1-t_2}$ with $Ae_{t_1-t_2}$ being the quantity of the drug that is excreted in urine over the time interval 0 to 24 h. The fraction of the dose excreted in the urine [$fe(0,24\text{ h})$] was calculated as $Ae(0,24\text{ h})/\text{dose}$.

The area under the effect–time curve [AUEC(0,24 h)] and the maximum effect ratio (ER_{max}) over baseline were calculated using the same methods as described for the primary endpoints, AUC and C_{max} .

Statistical analysis

Geometric means of the median intrasubject ratios of PK parameters and their two-sided 90% confidence intervals (CIs) were calculated for each pair of treatments that were compared. Analysis of variance was performed on log-transformed PK parameters. For study part 2, the model included effects for sequence, subject nested within sequence, period and treatment. The effect 'subject within sequence' was considered as random, whereas the other effects were considered as fixed. For tests on period and treatment effects, the denominator sum of squares was the sum of squares for error. A reduced model without sequence and period effects was applied for study part 1.

Taking into account an intra-individual variability (gCV) in AUC and C_{max} of 50%, 20 subjects were required in each study, with no adjustments for a dropout rate. In order to avoid any imbalances, at least eight subjects of each gender were enrolled in each study.

All other parameters were analyzed by descriptive statistics. The statistical software used was SAS® version 9.2.

Results

Study population

Study part 1 (multiple dose verapamil) included 12 male and eight female subjects, all of whom were Caucasian.

Their mean age was 38.3 ± 11.3 years and their mean BMI was $23.9 \pm 2.6 \text{ kg m}^{-2}$. Two subjects discontinued treatment due to AEs while receiving verapamil (see tolerability section below). Full PK data were available for one of these subjects since the AE occurred at the end of the dosing in the fifth period (treatment E). For the other subject, PK data were available up to treatment D (fourth period).

Study part 2 (single doses of verapamil) also included 12 male and eight female subjects, of whom all were Caucasian, except for one Black African-American subject. Their mean age was 40.1 ± 10.1 years and their mean BMI was $23.9 \pm 2.9 \text{ kg m}^{-2}$. All of these subjects completed the study and received all treatments.

Pharmacokinetic and pharmacodynamic analysis

The total dabigatran $\text{AUC}(0, \infty)$, the C_{max} and the test : reference ratios of these parameters in part 1 of the study (multiple doses of verapamil) are shown in Table 2 and Figure 1 and unconjugated dabigatran PK parameters are shown in Supplementary Table S2. In study part 1, taking a single dose of DE 150 mg 1 h after the morning dose of verapamil IR 120 mg twice daily on day 4 (treatment C) increased the geometric mean $\text{AUC}(0, \infty)$ and C_{max} of total dabigatran by 54% (90% CI 19, 99) and 63% (90% CI 22, 117), respectively, compared with DE alone (treatment A). The increase in unconjugated dabigatran was 52% for $\text{AUC}(0, \infty)$ and 45% for C_{max} (Supplementary Table S2). Doubling the daily dose of verapamil to 120 mg four times daily (treatment E) did not further increase $\text{AUC}(0, \infty)$ (total or unconjugated dabigatran). However, when DE was given 2 h before the morning dose of verapamil IR 120 mg twice daily (treatment D), the increase in total or unconjugated dabigatran $\text{AUC}(0, \infty)$ and C_{max} was less than 20%.

In part 2 of the study (single doses of verapamil), the change in the shape of the plasma concentration–time

curves for subjects receiving both verapamil and DE compared with DE alone was minimal (Figure 2). The time to peak was slightly later and the peak concentration was, on average, higher for treatments C and E, in which DE was given after pretreatment with multiple administration of 120 mg verapamil IR twice daily or four times daily, respectively. Almost no difference in the plasma concentration–time profile was observed comparing treatment A (reference) with treatment D, in which DE was given 2 h before the morning dose of 120 mg verapamil IR.

Figure 1

Overview of effects of verapamil on dabigatran exposure (study parts 1 and 2). $\text{AUC}(0, \infty)$, area under the concentration–time curve; CI, confidence interval; DE, dabigatran etexilate; ER, extended release; IR, immediate release; MD, multiple dose; SD, single dose; V, verapamil. The boundaries for bioequivalence (90% CI between 0.80 and 1.25) are shown as dashed lines. ■, Single dose study [$\text{AUC}(0, \infty)$ ratios]; □, Multiple dose study [$\text{AUC}(0, \infty)$ ratios]

Table 2

Effect of multiple doses of verapamil on total dabigatran exposure (study part 1)

Parameter	DE alone: treatment A gMean [gCV(%)]	DE 1 h after V IR 120 mg twice daily: treatment C gMean [gCV(%)]	Treatment C : A ratio, % (90% CI) gMean [gCV(%)]	DE 2 h before V IR 120 mg twice daily: treatment D gMean [gCV(%)]	Treatment D : A ratio, % (90% CI) gMean [gCV(%)]	DE 1 h after V IR 120 mg four times daily: treatment E gMean [gCV(%)]	Treatment E : A ratio, % (90% CI) gMean [gCV(%)]
$\text{AUC}(0, \infty)$ (ng ml ⁻¹ h)	854 (61.8)	1310 (54.7)	154 (119, 199)	1010 (74.7)	118 (91, 152)	1190 (74.1)	139 (107, 181)
C_{max} (ng ml ⁻¹)	99 (75.3)	162 (60.4)	163 (122, 217)	111 (87.3)	112 (84, 149)	132 (85.9)	134 (100, 180)
t_{max} (h)*	2.00 (1.50–3.02)	2.00 (1.00–3.00)		1.92 (1.92–3.00)		3.00 (1.00–3.02)	
$t_{1/2}$ (h)	8.38 (12.0)	8.08 (11.7)		8.91 (17.0)		8.92 (13.6)	
$\text{CL}_R(0, 24 \text{ h})$ (ml min ⁻¹)	71.2 (38.1)	64.0 (29.4)		65.1 (26.4)		60.3 (24.9)	
$f_e(0, 24 \text{ h})$ (%)	2.86 (71.1)	3.98 (69.1)		3.26 (62.7)		3.31 (71.6)	

*Median (range). $\text{AUC}(0, \infty)$, area under the concentration–time curve; CI, confidence interval; CL_R , renal clearance; C_{max} , maximum concentration; DE, dabigatran etexilate (150 mg); ER, extended release; f_e , fraction of dose excreted into urine; gCV, geometric coefficient of variation; gMean, geometric mean; IR, immediate release; t_{max} , time of peak concentration; $t_{1/2}$, half-life; V, verapamil.

Figure 2

Geometric mean plasma concentration–time profiles of total dabigatran after single oral administration of 150 mg dabigatran etexilate alone or with co-administration of 120 mg verapamil immediate release twice daily or four times daily (study part 1). DE, dabigatran etexilate; IR, immediate release; V, verapamil. —○—, Treatment A (150 mg DE alone); —△—, Treatment C (DE 1 h after V IR 120 mg twice daily); —△—, Treatment E (DE 1 h after V IR 120 mg four times daily); —●—, Treatment D (DE 2 h before V IR 120 mg twice daily)

Dabigatran did not meaningfully alter the PK of verapamil or the PK of the metabolite, norverapamil (Supplementary Table S3), in any of the test treatments vs. multiple dose verapamil alone (reference treatment B).

In study part 2, DE 150 mg given 1 h after a single dose of verapamil IR 120 mg (treatment H) increased the geometric mean $AUC(0,\infty)$ and C_{max} of dabigatran by 143% (90% CI 91, 208) and 179% (90% CI 115, 262), respectively, relative to DE alone (treatment F) (Table 3 and Figure 1). When DE was given concurrently with a single dose of 120 mg verapamil IR (treatment I), the geometric mean $AUC(0,\infty)$ and C_{max} were increased by 108% and 129%, respectively. When verapamil was administered as a 240 mg ER formulation concurrently with DE (treatment J), no greater effect on total dabigatran $AUC(0,\infty)$ and C_{max} was observed [geometric mean $AUC(0,\infty)$ and C_{max} increases of 71% and 91%, respectively]. Giving DE 1 h after a single dose of verapamil IR 120 mg (treatment H) led to a slight increase in verapamil exposure (Supplementary Table S3).

For dabigatran given after verapamil (treatments C and E), changes in PD parameters [TT, ECT, $AUEC(0,24\text{ h})$ and ER_{max}] were similar in magnitude to the PK changes observed with DE alone. The differences in mean TT and ECT ER_{max} and $AUEC(0,24\text{ h})$ between the reference treatment A and after dabigatran given 1 h after the morning dose of 120 mg verapamil IR twice daily (treatment C) or four times daily (treatment E) were clearly positive, i.e. coagulation times were prolonged (Table 4). No relevant or significant effects of verapamil on dabigatran PD were observed when verapamil was administered 2 h after dabigatran (treatment D) (Table 4). Thus, the dabigatran plasma concentration–effect relationship was generally not affected by the co-administration of verapamil. The

effects of daily doses of 240 mg or 480 mg verapamil were highly comparable.

The glucuronidation rate, as assessed by the ratio of the geometric means of the $AUC(0,\infty)$ data, ranged between 19.8% and 20.0% in the reference treatments and between 20.6% and 24.7% when verapamil was co-administered. There was no clinically meaningful change in renal clearance and half-lives were similar for all treatments. The percentage of dose excreted into urine over 24 h increased proportionally to the increased bioavailability (Tables 2 and 3 and Supplementary Table S4).

Safety evaluation

Tolerability was good and only two subjects discontinued study part 1 (multiple dose verapamil) due to an AE. One subject had non-serious asymptomatic ventricular premature beats during treatment D following the intake of 18 doses of verapamil 120 mg IR (and three doses of DE 150 mg). This persisted beyond the end of treatment, but was judged as unrelated to study treatment. The other subject had a non-serious second degree atrioventricular block during treatment E following the intake of 28 doses of verapamil 120 mg IR (and four doses of DE 150 mg). This resolved after 5 min and was judged as related to verapamil treatment.

Both study parts reported treatment-related AEs in 14 of 20 subjects in part 1 and in 15 of 20 subjects in part 2. The most common related AEs were nervous system and gastrointestinal disorders. All AEs were of mild to moderate intensity, there were no serious AEs and all resolved by the end of the study (with the exception of the one unrelated AE described above).

Vital signs and ECG, in particular heart rate and PR interval, as well as safety laboratory assessments did not indicate any relevant, consistent, treatment-related untoward reactions. Two subjects had clinically relevant individual ECG changes during study part 1, as described above. However, all subjects completed all trial assessments.

Discussion

The current study investigated the potential for PK and PD interactions between DE and verapamil, a probe inhibitor of P-gp. It was designed to evaluate the effects of verapamil on the PK and PD properties of dabigatran when administered at different doses and with varying times relative to DE and to determine whether immediate and sustained-release verapamil had different effects.

This two part multiple crossover study showed that the bioavailability of single dose dabigatran 150 mg is increased when co-administered with verapamil in healthy subjects. The maximum effect (~2.5-fold increase in exposure) was observed when DE was administered 1 h after a single dose of verapamil IR 120 mg. Concurrent administration only slightly diminished the effect. The increase was

Table 3

Effect of a single dose of verapamil on total dabigatran exposure (study part 2)

Parameter	DE alone: treatment F gMean [gCV(%)]	DE 1 h after V IR 120 mg: treatment H gMean [gCV(%)]	Treatment H : F ratio, % (90% CI) gMean [gCV(%)]	DE concomitant with V IR 120 mg: treatment I gMean [gCV(%)]	Treatment I : F ratio, % (90% CI) gMean [gCV(%)]	DE concomitant with V ER 240 mg: treatment J gMean [gCV(%)]	Treatment J : F ratio, % (90% CI) gMean [gCV(%)]
AUC(0,∞) (ng ml ⁻¹ h)	668 (93.3)	1620 (52.2)	243 (191, 308)	1390 (74.9)	208 (164, 264)	1140 (57.2)	171 (134, 217)
C _{max} (ng ml ⁻¹)	76 (108)	211 (51.8)	279 (215, 362)	173 (70.6)	229 (176, 297)	145 (58.9)	191 (147, 248)
t _{max} (h)*	2.00 (1.50–3.00)	2.00 (1.50–4.02)		2.00 (1.50–3.00)		2.00 (1.50–3.00)	
t _{1/2} (h)	8.36 (15.1)	7.82 (12.6)		7.96 (11.7)		7.98 (13.6)	
CL _R (0,24 h) (ml min ⁻¹)	66.3 (52.2)	71.2 (38.9)		70 (31.4)		66.1 (34.9)	
fe(0,24 h) (%)	2.12 (112)	5.54 (53.6)		4.65 (71.7)		3.69 (56.4)	

*Median (range). AUC(0,∞), area under the concentration–time curve; CI, confidence interval; C_{max}, maximum concentration; CL_R, renal clearance; DE, dabigatran etexilate (150 mg); ER, extended release; fe, fraction of dose excreted into urine; gCV, geometric coefficient of variation; gMean, geometric mean; IR, immediate release; t_{max}, time of peak concentration; t_{1/2}, half-life; V, verapamil.

Table 4

Pharmacodynamic effects of dabigatran on the coagulation parameters thrombin clotting time (TT) and ecarin clotting time (ECT) by treatment (study part 1)

Analyte	Parameter	Treatment effect* (90% CI) DE 1 h after V IR 120 mg twice daily: treatment C	DE 2 h before V IR 120 mg twice daily: treatment D	DE 1 h after V IR 120 mg four times daily: treatment E
TT	ER _{max}	1.3 (0.5, 2.2)	0.4 (–0.4, 1.3)	1.9 (0.9, 2.9)
	AUEC(0,24 h) (h)	14.1 (6.0, 22.1)	1.8 (–6.6, 10.3)	16.5 (6.3, 26.7)
ECT	ER _{max}	0.5 (0.3, 0.7)	0.1 (–0.1, 0.3)	0.5 (0.2, 0.8)
	AUEC(0,24 h) (h)	3.5 (1.9, 5.0)	1.3 (–0.5, 3.1)	4.5 (2.3, 6.7)

*Mean dabigatran + verapamil – mean dabigatran alone. AUEC(0,24 h), area under the effect time curve; CI, confidence interval; DE, dabigatran etexilate; ER, extended release; ER_{max}, maximum effect ratio; IR, immediate release; V, verapamil.

reduced to ~1.8-fold with the ER formulation of verapamil, despite the verapamil dose being doubled. Multiple dosing of verapamil IR 120 mg (twice daily over several days) reduced the increase in DE exposure to ~1.5-fold. Administering DE 2 h before verapamil did not significantly increase exposure to dabigatran and this was not changed by increasing the dose of verapamil. As t_{1/2} is not changed, the interaction is most likely related to the absorption of DE only.

P-gp is located throughout the body, including the gastrointestinal tract, where it can directly limit oral drug absorption [18, 19]. Previous studies have demonstrated that short term verapamil inhibits intestinal P-gp, whereas long term administration may induce P-gp expression [20, 21]. In the present study, the greater effect of a prior single dose of verapamil vs. multiple dose verapamil on the PK of dabigatran may reflect induction of P-gp expression in the gut after multiple dosing. The comparison of two previous studies indicates that the verapamil ER formulation has a lower inhibitory effect on P-gp than the IR formulation (although the studies involved two different subject popu-

lations, American and Japanese) [21, 22]. This is consistent with the present study, which showed that verapamil-ER had less of an effect on DE exposure than the IR formulation. The cause of this may be a lower concentration of verapamil available at the site and time of DE absorption due to the sustained release from the ER formulation. Based on the hypothesis of decreased efflux of DE into the gut in the presence of a P-gp inhibitor at the intestinal absorption site, administration of DE prior to verapamil should almost abolish the effect. This was confirmed in the current trial as dabigatran AUC and C_{max} were almost unaffected when DE was given 2 h before steady-state verapamil.

The PK of verapamil was unchanged with DE, except for a marginal increase in verapamil exposure when DE was given 1 h after a single dose of verapamil IR 120 mg. However, it is unlikely that the effect observed in the present study is clinically relevant because no ECG or blood pressure-related AEs were reported with this treatment.

As expected, the higher plasma levels of total dabigatran with verapamil co-administration were associated with

prolonged coagulation times. These changes were similar in magnitude to the PK changes, suggesting that verapamil did not interfere with the PD of dabigatran. Furthermore, neither glucuronidation nor the renal clearance of dabigatran were meaningfully affected by single or multiple verapamil co-medication.

The concomitant administration of DE and verapamil did not reveal any unexpected safety findings in the healthy male and female subjects. In both parts of the study, AEs related to study drug treatment were observed, none of which was serious. All AEs observed were of mild to moderate intensity, and all had resolved by the end of the study, with the exception of one case of non-serious asymptomatic ventricular premature beats, judged as unrelated to treatment.

Previous studies support the finding that the bioavailability of dabigatran can be increased when co-administered with P-gp inhibitors, including verapamil. The large, phase III, Randomized Evaluation of Long term anticoagulation therapy (RE-LY®) trial involved 18 113 patients with atrial fibrillation at an increased risk of stroke [1, 23]. Patients received two blinded doses of DE (150 mg or 110 mg twice daily) or open label warfarin [adjusted to international normalized ratio (INR) 2.0–3.0]. PK data were compared in patients taking DE with or without verapamil. Dabigatran concentrations at trough and 2 h post-dose were ~20% higher in patients receiving verapamil than in those without co-medication [13].

The present study is associated with potential limitations. Small numbers of healthy subjects were enrolled. Furthermore, although DE was administered at the therapeutic 150 mg dose, only single doses were given. Therefore, the drug concentrations achieved were not equivalent to those expected in patients who have reached steady-state concentrations.

The designs of the two parts of the trial were carefully chosen. In part 1 (multiple dose verapamil), a fixed sequence design was used to avoid an accumulation of washout and titration periods for verapamil. This fixed sequence may have introduced potential time-dependent effects. However, this was not expected to compromise the validity of the PK evaluation. In addition, the design included a sufficient washout period between the dabigatran reference and test treatments. However, the fixed sequence design may have led to an underestimation of the effect of the last treatment at the highest daily dose of 120 mg verapamil four times daily by comparison with the effect after 120 mg verapamil twice daily, as the process of induction may not yet have reached stable conditions. In part 2 (single doses of verapamil), the five treatments were administered in 10 sequences in such a way that all period main effects, on average, cancelled out (every treatment occurred twice in each of five periods). Furthermore, on average, any first order carry-over effects cancelled out (every treatment followed each of the others exactly twice). Most important for a PK trial, due to the washout

periods of at least 4 days between subsequent treatments, carry-over effects were not expected.

In conclusion, this trial confirms that the bioavailability of dabigatran is increased when co-administered with a P-gp inhibitor, such as verapamil. Several factors affected the interaction between DE and verapamil in the healthy subjects: (i) the dosing interval between the two drugs, with no effect when DE was given 2 h before verapamil, which suggests that the effect of verapamil is solely through inhibition of P-gp and extrusion of dabigatran at the site of intestinal absorption, (ii) the formulation of verapamil, with the IR formulation having a greater effect than the ER formulation and (iii) verapamil dosing, with a single dose having a greater effect on the PK of dabigatran than multiple dosing.

Although only a single dose of DE was tested (therapeutic regimen is twice daily), our results suggest that an interaction between verapamil and DE might be minimized if DE is administered 2 h prior to verapamil.

Competing Interests

The authors are all employees of Boehringer Ingelheim Pharma GmbH & Co. KG, Biberach, Germany, or Boehringer Ingelheim Pharmaceuticals, Ridgefield, CT, USA.

This trial was exclusively sponsored by Boehringer Ingelheim Pharma GmbH & Co. KG, Ingelheim, Germany, in order to support the submission of Pradaxa®.

Dietmar Gansser, Joachim Stangier, Karin Hoermann and Marion Schmid are acknowledged for dabigatran and verapamil bioanalysis, coagulation measurements and PK evaluation. Writing and editorial support was provided by PAREXEL MMS and was funded by Boehringer Ingelheim. The authors meet criteria for authorship as recommended by the International Committee of Medical Journal Editors (ICMJE) and were fully responsible for all content and editorial decisions, and were involved at all stages of manuscript development.

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Supporting Information

Additional Supporting Information may be found in the online version of this article at the publisher's web-site:

Table S1

Timings of PK/PD sampling in study part 1 (multiple doses of verapamil)

Table S2

Comparison of pharmacokinetic parameters of unconjugated dabigatran by treatment in part 1 (multiple doses of verapamil) (top) and part 2 (single doses of verapamil) (bottom)

Table S3

Pharmacokinetic parameters of sum of R-/S-verapamil and R-/S-norverapamil after administration of 120 mg verapamil IR twice daily or 120 mg four times daily with or without co-administration of 150 mg dabigatran etexilate in study part 1 (top) and study part 2 (bottom)

Table S4

Geometric mean glucuronidation ratio (ratio of conjugated : total dabigatran in study part 1 and part 2)