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Safety, pharmacokinetics and pharmacodynamics of multiple oral doses of apixaban, a factor Xa inhibitor, in healthy subjects

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WHAT IS ALREADY KNOWN ABOUT THIS SUBJECT

- Apixaban is a highly selective, potent, direct factor Xa inhibitor that is approved for stroke prevention in atrial fibrillation and thromboprophylaxis in patients who have undergone elective hip or knee replacement surgery and is under development for treatment of venous thromboembolism.
- Single doses of apixaban 0.5 mg to 50 mg in healthy subjects were well tolerated with a predictable pharmacokinetic/pharmacodynamic profile and a half-life of approximately 12 h.

WHAT THIS STUDY ADDS

- The present study provides the results from the first human multiple dose experience with apixaban.
- The results demonstrate that multiple oral doses of apixaban appear to be safe and well tolerated over a 10-fold dose range (2.5 mg twice daily to 25 mg twice daily) and its pharmacokinetics were dose proportional with low to moderate variability and concentration-related pharmacodynamic effects within the dose range examined.

AIM

Apixaban is an oral factor Xa inhibitor approved for stroke prevention in atrial fibrillation and thromboprophylaxis in patients who have undergone elective hip or knee replacement surgery and under development for treatment of venous thromboembolism. This study examined the safety, pharmacokinetics and pharmacodynamics of multiple dose apixaban.

METHOD

This double-blind, randomized, placebo-controlled, parallel group, multiple dose escalation study was conducted in six sequential dose panels – apixaban 2.5, 5, 10 and 25 mg twice daily and 10 and 25 mg once daily– with eight healthy subjects per panel. Within each panel, subjects were randomized (3:1) to oral apixaban or placebo for 7 days. Subjects underwent safety assessments and were monitored for adverse events (AEs). Blood samples were taken to measure apixaban plasma concentration, international normalized ratio (INR), activated partial thromboplastin time (aPTT) and modified prothrombin time (mPT).

RESULTS

Forty-eight subjects were randomized and treated (apixaban, $n = 36$; placebo, $n = 12$); one subject receiving 2.5 mg twice daily discontinued due to AEs (headache and nausea). No dose limiting AEs were observed. Apixaban maximum plasma concentration was achieved ~3 h post-dose. Exposure increased approximately in proportion to dose. Apixaban steady-state concentrations were reached by day 3, with an accumulation index of 1.3–1.9. Peak : trough ratios were lower for twice daily *vs.* once daily regimens. Clotting times showed dose-related increases tracking the plasma concentration–time profile.

CONCLUSION

Multiple oral doses of apixaban were safe and well tolerated over a 10-fold dose range, with pharmacokinetics with low variability and concentration-related increases in clotting time measures.

Introduction

Conventional anticoagulants used in clinical practice include heparin, low molecular weight heparins, fondaparinux and the vitamin K antagonists [1]. Although effective, heparin, low molecular weight heparins and fondaparinux require inconvenient subcutaneous administration. Vitamin K antagonists require frequent patient monitoring owing to variable pharmacodynamics, a narrow therapeutic window and multiple dietary and drug interactions [2, 3]. A medical demand for an anticoagulant that can be administered orally and that does not require patient monitoring has driven interest in small molecule inhibitors of coagulation cascade proteases, such as thrombin and factor Xa [4–8].

Apixaban (BMS-562247) is a highly selective (>30 000-fold selectivity over other coagulation proteases), potent (K_i = 0.08 nM), direct factor Xa inhibitor. Factor Xa, a trypsin-like serine protease, converts prothrombin to thrombin, the final enzyme in the coagulation cascade that is responsible for fibrin clot formation. Direct inhibition of factor Xa differs from direct inhibition of thrombin because it attenuates the generation, but not the activity, of thrombin, thereby preserving haemostatic function [9–12]. Preclinical experiments show that apixaban is able to bind both free and prothrombinase-bound factor Xa [13, 14]. In a rabbit model of venous thrombosis, apixaban exhibited potent antithrombotic effects at doses that preserved haemostasis [15]. Apixaban is approved in a number of countries for stroke prevention in atrial fibrillation [16, 17] and thromboprophylaxis in patients who have undergone elective hip or knee replacement surgery [18–20]. Apixaban is also under development for the treatment of venous thromboembolism [21] in adults and for the prevention and treatment of thromboembolism in children.

After single dose oral administration of apixaban 0.5 mg to 50 mg in healthy subjects, peak plasma concentration was reached approximately 3 h post-dose and the average elimination half-life was approximately 12 h [22]. The single dose pharmacokinetics of apixaban exhibited low variability, were dose proportional and correlated with pharmacodynamic effects. Single doses of apixaban resulted in prolongation of clotting measures consistent with its mechanism of action. Apixaban was safe and well tolerated following single doses up to 50 mg in healthy subjects, with no clinically relevant bleeding events observed.

The present study provides the results from the first multiple dose experience with apixaban in humans. The primary objective of this study was to evaluate the safety and tolerability of multiple oral doses of apixaban in healthy subjects. Secondary objectives included an assessment of the pharmacokinetics of apixaban and its effect on clotting measures: international normalized ratio (INR) and activated partial thromboplastin time (aPTT). The effect of apixaban on modified prothrombin time (mPT) [23] was included as a *post hoc* assessment.

Methods

Study design and subjects

This was a single centre, placebo-controlled, double-blind, parallel group, ascending multiple dose study conducted in healthy subjects. Eligibility criteria for male and female subjects included age 18–45 years and a body mass index in the normal range (18–30 kg m^{-2} inclusive). All subjects were in good health, as determined by medical history, physical examination, vital signs, electrocardiogram (ECG) assessment and clinical laboratory tests. Subjects with a previous medical history of coagulopathy or adverse reaction to anticoagulant or antiplatelet agents were excluded. Female subjects could not be nursing, pregnant or of child-bearing potential. Other exclusion criteria included a significant head injury within the previous 2 years, gastrointestinal disease within the previous 3 months, blood transfusion or donation of blood or plasma within 4 weeks before the study and an inability to tolerate venipuncture or venous access. Subjects were also excluded if they had a history of drug or alcohol abuse in the previous 6 months (as defined in the Diagnostic and Statistical Manual of Mental Disorders version IV [24]). Subjects were required to refrain from smoking for at least 3 weeks prior to enrolment. All subjects were required to give written informed consent before initiation of any study specific procedures. The protocol was approved by the New England Institutional Review Board (Wellesley, MA, USA) and complied with the Declaration of Helsinki, Good Clinical Practice guidelines and Therapeutic Products Programme.

Eight healthy subjects per dose panel were randomized in a 3:1 ratio to apixaban or matching placebo in a double-blind fashion. Six sequential apixaban dose panels were tested: 2.5, 5, 10 and 25 mg twice daily and 10 and 25 mg once daily. Study medication was administered orally for 7 days. All doses were administered with 250 ml of water. Morning doses were administered after at least a 10 h overnight fast and evening doses (twice daily group) after at least a 3 h fast. If a dose level was found to be safe and tolerated, then the succeeding panel of eight subjects received the next higher dose of apixaban or placebo. There was no intra-subject dose escalation.

Safety assessments

Safety assessments included routine laboratory tests, bleeding time measurement, ECG and vital sign monitoring, physical examination, clinical laboratory tests, and monitoring for adverse events (AEs). AEs were identified from information volunteered by the subjects and by the investigators' review of their vital signs, ECG and laboratory test results. Serial 12-lead ECGs were collected on day −1,

day 1 (first day of dosing) and day 7 before dosing and at 1, 3, 6 and 12 h post-dose (approximate corresponding times on day −1). Bleeding time was measured using the Simplate®-II R (Organon Teknika Corp., Durham, NC, USA) device at pre-dose and 4 h post-dose [near the time taken to achieve maximal plasma concentration (t_{max})] on days 1 and 7. Bleeding prolongation was defined as a bleeding time of ≥30 min both pre-dose and 4 h post-dose on the same day. Blood and urine samples for clinical laboratory analyses were collected from subjects in the fasted state on day −1, days 4 and 7, prior to study drug administration, and at study discharge on day 10. Subjects had faecal occult blood testing (Hemoccult sensa®; Beckman Coulter Inc., Brea, CA, USA) performed at screening and on days −2 to −1, days 3 to 4, days 6 to 7, and before study discharge on day 10. Samples were also collected on days 1, 2, 5 and 8, if possible. Samples testing positive for faecal occult blood were also tested with the HemoQuant® assay [25].

Pharmacokinetic analysis

On days 1 and 7, blood samples were collected for pharmacokinetic analyses at 0, 1, 2, 3, 4, 6, 9, 12 and 24 h after the morning dose for both twice daily and once daily dose panels. Additional samples were collected at 13, 14, 15, 16, 18 and 21 h after the morning dose for twice daily dose panels. Samples were also collected on days 9 and 10 (i.e. 48 and 72 h after the morning dose on day 7) for both twice daily and once daily dose panels. Trough samples were collected before the morning dose on days 3, 4 and 5 for both twice daily and once daily dose panels.

Serial blood samples (2.7 ml per sample) were collected into pre-chilled 3.2% sodium citrate tubes (Vacutainer®, light blue top; Becton-Dickinson, Franklin Lakes, NJ, USA). Blood samples were processed within 30 min of collection. Samples were kept on ice until centrifuged for 15 min at approximately 1500 *g* in a refrigerated centrifuge (~4°C). The separated plasma was immediately transferred to an appropriately labelled 4 ml cryogenic vial and stored at −20°C. Apixaban concentrations in plasma were measured, after solid-phase extraction, by a validated liquid chromatography/tandem mass spectrometry method using a stable label internal standard of apixaban (^{13}C-$^{2}H_3$-BMS-562247). Values for the between-run and within-run precision for analytical quality control samples were no greater than 8.1% coefficient of variation (CV), with deviations from the nominal concentrations of no more than ± 2.2%. The lower limit of quantitation in plasma was 1 ng ml^{-1}.

Apixaban pharmacokinetic parameters, including maximum observed plasma concentration (C_{max}), t_{max}, minimum observed plasma concentration (C_{min}), area under the plasma concentration–time curve in one dosing interval (AUC_{tau}) on days 1 and 7, and accumulation index (AI) and terminal half-life ($t_{1/2}$) on day 7, were determined using established non-compartmental methods [26] implemented in Kinetica within the eToolBox software package (Innaphase, PA). C_{min} was defined as the apixaban concentration 12 or 24 h after morning dosing for twice daily or once daily regimens, respectively.

Pharmacodynamic analysis

For INR and aPTT, samples were collected for both twice daily and once daily dose panels at 0, 3, 6, 9, 12, 15, 18, 21 and 24 h after the morning dose on days 1 and 7, before the morning dose on day 3 and before and 3 h after the morning dose on day 4. For the once daily dose panel, an additional sample was collected at 48 h after the morning dose on day 7 (day 9). For both twice daily and once daily dose panels, mPT was measured in samples collected at 0, 3, 6, 12 and 24 h after the morning dose on days 1 and 7, and at 3 h after the morning dose on day 4. mPT was also measured in samples collected 18 h after the morning dose on days 1 and 7 for the twice daily dose panels, and 48 h after the morning dose on day 7 (day 9) for the once daily dose panels.

Serial blood samples for INR, aPTT and mPT analyses were collected in a 3.2% sodium citrate tube. The INR and aPTT were assessed with STA-Neoplastin CI and STA-PTT Automate 5, respectively (Diagnostica Stago Inc., Parsippany, NJ, USA). The mPT assay is a novel modification of the traditional PT assay developed by the sponsor to provide greater sensitivity in measuring the effect of direct factor Xa inhibitors [23]. The mPT was measured at Covance Laboratory (Chantilly, VA, USA) using a modified thromboplastin reagent on an MLA-1800 coagulation analyser (Medical Laboratory Automation Inc., Mount Vernon, NY, USA). To determine mPT, a standard PT assay was modified by diluting the thromboplastin reagent (Thromboplastin C+, Dade Behring; now part of Siemens Healthcare Diagnostics, Erlangen, Germany) 1:2.25 with 100 nM calcium chloride. This slows down the clotting reaction and provides a broader dynamic range for measuring the effect of factor Xa inhibitors.

Statistical methods

Statistical analyses were carried out using SAS/STAT® version 8.2 (SAS Institute Inc., Cary, NC, USA). Summary statistics for C_{max}, C_{min}, AUC_{tau}, AI, $t_{1/2}$ and t_{max} were tabulated by dose group and study day (day 1 and day 7). For the twice daily dose panels, C_{max}, t_{max} and AUC_{tau} were calculated from 0 to 12 h after the morning dose. For the once daily and twice daily dosing regimens, $t_{1/2}$ was calculated on day 7 following administration of the last dose. Mean plasma apixaban concentration *vs.* time profiles were plotted for days 1 and 7 for all apixaban groups. Additional mean plasma apixaban concentration *vs.* time profiles were plotted for the 5 mg twice daily and 10 mg once daily dose panels from days 1 and 7, including trough concentrations on days 3, 4 and 5. For INR, aPTT and mPT, summary statistics were tabulated for absolute change from baseline for trough and maximum values by dose group on day 7. The baseline value for the clotting measures was the pre-dose

measurement on day 1 and their mean values were reported. The trough and maximum values for the clotting measures were defined as the measurements at 0 and 3 h, respectively. In addition, mean INR, aPTT and mPT *vs.* time profiles were plotted for days 1 and 7 where subjects who received placebo in any panel were pooled into a single placebo group. The relationship between these pharmacodynamic measures and apixaban plasma concentration was explored using scatter plots of all individual data available and characterized with regression analyses by a linear or a maximal effect (E_{max}) model.

Results

Subjects

A total of 48 healthy male subjects were included in the study and randomized to treatment (apixaban $n = 36$; placebo $n = 12$). Although enrolment was open to both male and female subjects, there were no female subjects who met all eligibility criteria. All randomized subjects received at least one dose of blinded study drug (apixaban or placebo). All subjects completed the study as scheduled, with the exception of one subject who withdrew on day 2 (from the 2.5 mg twice daily dose group) due to AEs of headache and nausea. Baseline demographics for all subjects are summarized in Table 1.

Safety and tolerability

There were no deaths, serious AEs, dose-limiting AEs or major bleeding-related events. One subject discontinued treatment due to AEs of headache and nausea after receiving one dose of apixaban 2.5 mg. These AEs were of moderate intensity and did not require treatment. Overall, 27 AEs were reported by 21 subjects, four of whom received placebo. The majority of AEs were of mild intensity, considered possibly related to drug by the investigator and resolved without dose interruption or treatment. The most frequently reported treatment-emergent AE was mild to moderate headache reported by a total of four subjects.

Three subjects had bleeding-related AEs. Haematochezia occurred in a subject following administration of apixaban 25 mg once daily on the first and second days of dosing. The event lasted 3 days and resolved without treatment and the subject completed the study without any interruption in study medication. Haematuria was reported by a subject 5 days after discharge from the study (day 15) following administration of apixaban 25 mg twice daily. All study urinalyses for this subject were negative for blood as were follow-up urinalyses performed subsequent to reporting of the event. Venous sampling site haemorrhage (bruising at the catheter site) was reported for one subject on day 7 after completing administration of apixaban 10 mg once daily for 7 days and the event resolved without treatment. This subject also had an AE of increased creatine kinase (CK) concentration (12 550 IU ml^{-1}) observed 11 days after discharge from the clinic. The event was treated with intravenous fluids and resolved by the end of follow-up. Upon questioning, the subject admitted to intense physical activity just prior to the event.

There were no clinically relevant changes in vital signs, physical examinations or ECG results. Three subjects had alanine aminotransferase (ALT) elevations: one AE recorded by a single patient in each of the 2.5 mg twice

Table 1

Baseline demographic characteristics and physical measurements by treatment group

Characteristic	Placebo pooled	Apixaban 2.5 mg twice daily	5 mg twice daily	10 mg once daily	10 mg twice daily	25 mg once daily	25 mg twice daily
Subjects, *n*	12	6	6	6	6	6	6
Age (years)							
Mean (SD)	34 (6)	27 (7)	31 (6)	25 (4)	30 (9)	28 (8)	27 (8)
Range	24–44	19–39	24–41	20–32	21–44	19–39	20–41
Gender, *n* (%)							
Male	12 (100)	6 (100)	6 (100)	6 (100)	6 (100)	6 (100)	6 (100)
Female	0	0	0	0	0	0	0
Race, *n* (%)							
White	7 (58)	3 (50)	4 (67)	4 (67)	5 (83)	4 (67)	2 (33)
Black	5 (42)	3 (50)	2 (33)	1 (17)	0	2 (33)	4 (67)
Asian	0	0	0	0	1 (17)	0	0
Other	0	0	0	1 (17)	0	0	0
Body weight (kg)							
Mean (SD)	78.9 (13.7)	78.3 (13.9)	72.3 (10.4)	79.7 (7.1)	70.5 (9.6)	82.2 (10.6)	82.3 (5.8)
Range	58.6–98.4	64.7–104.4	66.1–93.1	69.3–87.8	62.9–87.9	65.4–94.2	76.2–91.9
BMI (kg m^{-2})							
Mean (SD)	25.1 (3.9)	24.4 (2.1)	23.2 (3.2)	25.1 (2.3)	23.7 (3.1)	25.8 (2.8)	25.5 (1.2)
Range	19.6–29.5	21.2–27.5	20.8–29.3	22.9–28.8	20.5–29.0	22.3–29.6	23.5–27.0

BMI, body mass index; SD, standard deviation.

daily, 10 mg once daily (coinciding with the CK elevation noted above) and 25 mg twice daily apixaban dose groups. All ALT elevations were considered mild and none required treatment. All but one had resolved by follow-up evaluations and none exceeded three times the upper limit of normal. Except for the subject with elevated CK levels, there were no other notable laboratory results in these three subjects. No subject met the pre-defined dose-limiting criterion for bleeding time prolongation.

The bleeding time results were within the expected range for the Simplate®-II R device (2.3–9.5 min) except in five subjects whose bleeding times exceeded 9.5 min (range 9.8–13.5 min), two subjects with placebo and three with apixaban (one with 2.5 mg twice daily; two with 25 mg twice daily). None was associated with a bleeding-related AE. There did not appear to be a relationship between apixaban dose and bleeding time assessed at 4 h post-dose given that mean bleeding times (SD) were 8.50 (2.62) min and 7.38 (2.28) min for apixaban 2.5 mg twice daily and 25 mg twice daily, respectively. The mean bleeding times for the rest of the apixaban groups were less than 6 min.

Pharmacokinetics

All 36 subjects who received apixaban were included in pharmacokinetic analyses. However, owing to the discontinuation of one subject after receiving one dose of apixaban 2.5 mg, five subjects (instead of six) were included in the summary of day 7 pharmacokinetic parameters for the 2.5 mg twice daily apixaban dose panel. Pharmacokinetic parameters for apixaban on days 1 and 7 are summarized in Table 2 and the mean plasma apixaban concentration *vs.* time profiles are presented in Figure 1. Mean plasma apixaban concentration *vs.* time profiles, including trough concentrations on days 3, 4 and 5, are presented in Figure 2 for the apixaban 5 mg twice daily and 10 mg once daily dose panels. Median t_{max} occurred between 3 and 4 h after oral administration of apixaban. Within the dose range examined, exposure appeared to increase in a dose-proportional manner on both days 1 and 7 (Figure 1A, B). Following multiple daily doses of apixaban, steady-state concentrations were reached by day 3 (Figure 2), with an AI ranging from 1.3 to 1.9 for the once daily and twice daily dosing regimens (Table 2). A larger fluctuation between maximum and minimum concentrations was observed following administration of apixaban 10 mg once daily compared with that observed following administration of 5 mg twice daily (Figure 2): the average C_{max} : C_{min} ratio on day 7 following once daily administration was 8.1 compared with an average of 3.0 for twice daily administration. Between-subject variability was low for C_{max} (12–38% CV) and AUC_{tau} (7–30% CV), and was independent of dose. Following attainment of C_{max} on day 7, the mean $t_{1/2}$ of apixaban ranged from 8.1 to 15.3 h across dose panels.

Pharmacodynamics

Results from 47 subjects were included in the analyses of INR, aPTT and mPT (Table 3 and Figures 3 and 4) owing to the discontinuation of one subject. Mean change from baseline on day 7 is summarized in Table 3 for trough and maximum measurements. Mean INR, aPTT and mPT *vs.* time (Figure 3) and scatter plots for INR, aPTT and mPT *vs.* apixaban plasma concentration for individual patients (Figure 4) are also presented.

Table 2

Summary of apixaban pharmacokinetic parameters on days 1 and 7

Apixaban dose and regimen	C_{max} (ng ml^{-1}) GM (%CV)	C_{min}* (ng ml^{-1}) GM (%CV)	AUC_{tau}† (ng ml^{-1} h) GM (%CV)	t_{max} (h) Median (min, max)	AI GM (%CV)	$t_{1/2}$ (h) Mean (SD)
Day 1						
2.5 mg twice daily (n = 6)	51.0 (27)	14.2 (53)	353.3 (25)	3.5 (2.0,12.0)	–	–
5 mg twice daily (n = 6)	81.9 (18)	25.3 (20)	600.6 (20)	3.5 (3.0, 6.0)	–	–
10 mg twice daily (n = 6)	226.2 (38)	72.7 (27)	1608.3 (30)	4.0 (2.0, 4.0)	–	–
25 mg twice daily (n = 6)	425.3 (24)	129.0 (33)	3108.6 (25)	3.5 (2.0, 4.0)	–	–
10 mg once daily (n = 6)	178.4 (19)	14.5 (27)	1589.6 (20)	4.0 (3.0, 4.0)	–	–
25 mg once daily (n = 6)	310.0 (12)	36.5 (31)	2868.1 (7)	4.0 (2.0, 6.0)	–	–
Day 7						
2.5 mg twice daily (n = 5)	62.3 (37)	21.0 (17)	462.8 (35)	3.0 (3.0, 9.0)	1.3 (18)	8.1 (1.8)
5 mg twice daily (n = 6)	128.5 (10)	49.6 (20)	1051.9 (9)	4.0 (2.0, 4.0)	1.8 (22)	11.7 (3.3)
10 mg twice daily (n = 6)	329.8 (45)	103.8 (57)	2424.9 (47)	3.0 (2.0, 4.0)	1.5 (33)	10.9 (2.9)
25 mg twice daily (n = 6)	716.6 (21)	281.1 (38)	5850.3 (16)	3.5 (1.0, 4.0)	1.9 (17)	15.2 (7.2)
10 mg once daily (n = 6)	201.4 (15)	26.8 (43)	2015.7 (16)	3.5 (3.0, 4.0)	1.3 (23)	14.9 (7.2)
25 mg once daily (n = 6)	428.9 (20)	55.3 (33)	4248.3 (19)	3.0 (2.0, 4.0)	1.5 (17)	15.3 (4.3)

AI, accumulation index; AUC_{tau}, area under the plasma concentration–time curve in one dosing interval C_{max}, maximum observed plasma concentration; C_{min}, minimum observed plasma concentration; CV, coefficient of variation; GM, geometric mean; NA, not applicable; SD, standard deviation; $t_{1/2}$ plasma terminal half-life; t_{max}, time to maximum observed plasma concentration. *C_{min} defined as apixaban concentration 12 or 24 h after morning dosing for twice daily or once daily regimens, respectively. †t = 12 h for twice daily panels and 24 h for once daily panels.

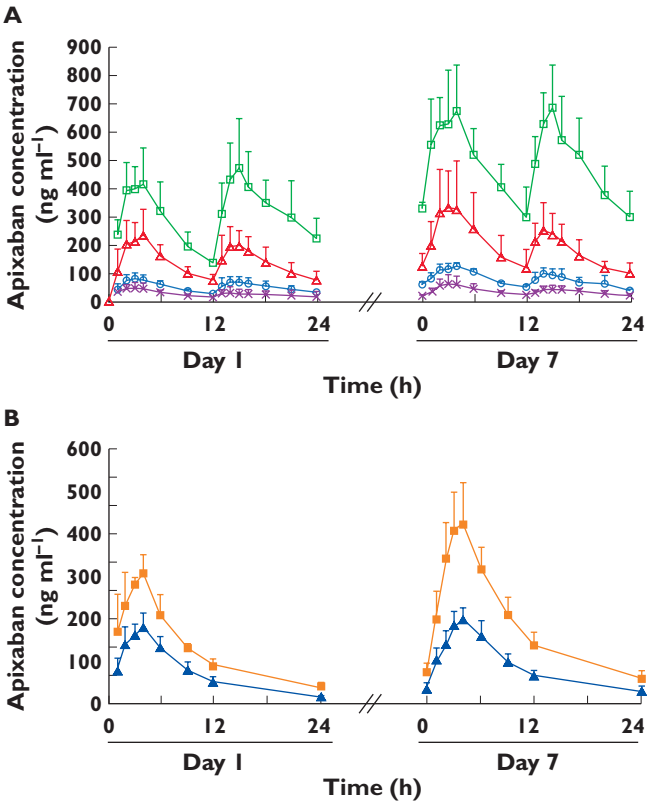

Figure 1

Mean (+ SD) plasma apixaban concentration *vs.* time profiles on days 1 and 7 for (A) twice daily and (B) once daily dosing regimens. ×, 2.5 mg twice daily; ○, 5 mg twice daily; △, 10 mg twice daily; □, 25 mg twice daily; ▲, 10 mg once daily; ■, 25 mg once daily

The ranges of mean baseline values across dose panels for INR, aPTT and mPT were 1.07–1.16, 30–38 s and 43–51 s, respectively. On day 1, a trend of small transient increases in INR, aPTT and mPT was observed for all apixaban dose groups with no evident change in placebo-treated subjects (Figure 3). Dose-related increases were generally observed in mean INR, aPTT and mPT. These were more prominent after apixaban steady-state was achieved (Figure 3), consistent with modest accumulation of apixaban to steady-state plasma concentrations. The time course for INR, aPTT and mPT appeared to follow the apixaban concentration–time profile, with no evident temporal lag. The maximum increases in clotting measures were observed at 3 h after dosing on both days 1 and 7 (Figure 3) for most dose panels. Mean trough values remained slightly higher than baseline at the end of the dosing interval for the 10 mg twice daily (INR) and 25 mg twice daily (INR and aPTT) doses (Table 3). Mean trough values for mPT remained greater than baseline values at the end of the dosing interval for all apixaban doses ≥5 mg twice daily.

The scatter plots for INR, aPTT and mPT *vs.* apixaban plasma concentration showed apixaban concentration-related increases for all three pharmacodynamic measures (Figure 4). The relationship with apixaban concentration appeared to be linear for INR and mPT (P value <0.0001 for the slope), while the relationship appeared to be non-linear and adequately described by an E_{max} model for aPTT (P value <0.0001 for the E_{max} estimate) (Figure 4). The scatter plots also showed that INR and aPTT do not appear to be reliable assessments of apixaban exposure due to high variability and relative insensitivity to the effects of apixaban; mPT had the largest dynamic range within the measured apixaban plasma concentration range (Figure 4).

Discussion

Multiple ascending doses of the oral, direct factor Xa inhibitor apixaban were safe and well tolerated in healthy subjects over a 10-fold dose range up to 25 mg twice daily. The predictable multiple dose pharmacokinetics, demonstrated by low variability, correlated with pharmacodynamics across all doses tested. These results are consistent with the single dose pharmacokinetic profile observed in the first study in humans [22].

The majority of AEs observed in this study were minor spontaneous events that resolved without intervention. The three bleeding-related events occurred in subjects who received apixaban doses ≥10 mg, and were considered to be minor and resolved without intervention. No dose-limiting AEs were observed and consequently the maximum tolerated dose for apixaban was not established.

Apixaban does not require biotransformation to the active form as does the oral anticoagulant dabigatran [27], which is administered as a pro-drug. Measurable concentrations of apixaban were observed shortly after oral administration, with maximum plasma concentrations reached approximately 3 h after oral administration. Apixaban generally exhibited dose-proportional increases in exposure after administration of the first dose as well as at steady-state exposure. Exposure parameters demonstrated moderate to low variability with %CVs typically below 40%. Peak : trough ratios were lower for the twice daily *vs.* the once daily dosing regimens, providing less fluctuation in drug exposure over the dosing interval. The $t_{1/2}$ of apixaban after 7 days of dosing was approximately 8–15 h, in agreement with that observed in the ascending single dose study [22]. As expected for a compound with a $t_{1/2}$ of approximately 12 h, apixaban achieved steady-state 2–3 days after administration of the first dose, with modest accumulation (AI 1.3–1.9). Based on these findings there was no evidence for auto-induction or auto-inhibition of apixaban-metabolizing enzymes following multiple dose administration. This observation is consistent with *in vitro* experiments demonstrating that apixaban does not induce or inhibit cytochrome P450 enzymes [28]. Although

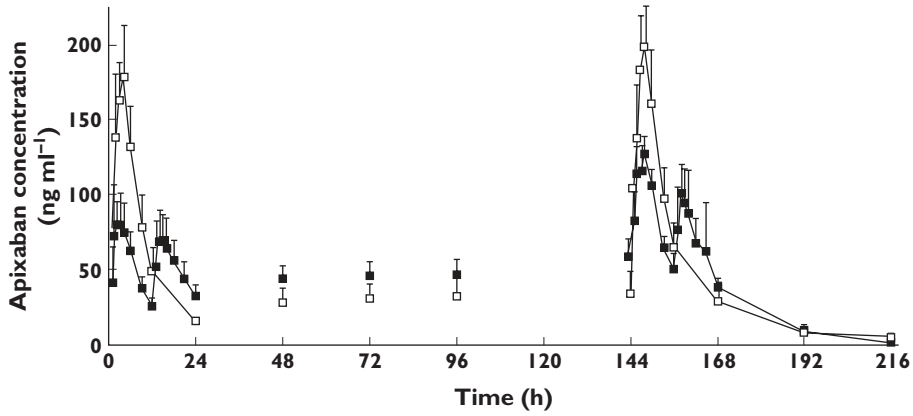

Figure 2

Mean (+ SD) apixaban plasma concentrations from day 1 to day 7 in subjects who received 5 mg twice daily or 10 mg once daily. ■, 5 mg twice daily; □, 10 mg once daily

Table 3

Mean change from baseline in clotting measures for trough and maximum measurements on day 7

Apixaban dose and regimen	INR Mean (SD) Trough*	Max†	aPTT (s) Mean (SD) Trough*	Max†	mPT (s) Mean (SD) Trough*	Max†
Placebo (n = 12)	–0.01 (0.056)	–0.00 (0.071)	0.25 (1.703)	–0.43 (2.955)	0.23 (8.317)	–2.18 (5.815)
2.5 mg twice daily (n = 5)	0.04 (0.033)	0.12 (0.016)	0.20 (2.351)	1.14 (0.305)	–6.06 (4.397)	9.06 (2.637)
5 mg twice daily (n = 6)	–0.00 (0.052)	0.09 (0.052)	1.63 (1.827)	2.87 (1.462)	17.50 (6.969)	25.90 (12.831)
10 mg twice daily (n = 6)	0.17 (0.058)	0.39 (0.129)	2.83 (1.496)	4.80 (3.062)	32.27 (8.464)	60.00 (20.333)
25 mg twice daily (n = 6)	0.23 (0.059)	0.63 (0.262)	4.80 (1.190)	7.38 (2.548)	55.28 (10.802)	116.75 (30.080)
10 mg once daily (n = 6)	0.05 (0.033)	0.20 (0.034)	1.50 (1.902)	3.88 (0.497)	17.47 (6.458)	55.25 (11.974)
25 mg once daily (n = 6)	0.04 (0.050)	0.47 (0.150)	1.78 (3.605)	7.60 (2.224)	12.70 (5.047)	82.70 (15.443)

INR normal range: 0.7–1.5; aPTT normal range: 24.0–35.9 s. aPTT, activated partial thromboplastin time; INR, international normalized ratio; mPT, modified prothrombin time; SD, standard deviation. *Trough is a measurement at 0 h on day 7. †Max is a measurement at 3 h on day 7.

there is no definitive evidence that the lower peak : trough ratio with twice daily dosing provides a benefit over once daily dosing for apixaban, a trend for lower rates of venous thromboembolism was found in a phase 2 study for prevention of venous thromboembolism in total knee replacement surgery [29] and the twice daily regimen was chosen for future study in this and other indications.

Unlike warfarin, apixaban is immediately active given its direct reversible effect on factor Xa. The full pharmacodynamic effects of warfarin are typically achieved approximately 6 days after the initiation of treatment and require a similar duration of time to return to baseline haemostasis [30]. The fast onset of apixaban action was evident in the changes in mPT, INR and aPTT observed shortly after the first dose, which thereafter followed the same time course as the apixaban plasma concentration, consistent with direct reversible inhibition of factor Xa. Based on the apixaban $t_{1/2}$ of approximately 12 h, the anticoagulant effect of apixaban would be greatly reduced 48 h (approximately four half-lives) after the last dose, with little if any effect remaining 72 h after the last dose (approximately six half-lives). While a direct relationship was observed between apixaban plasma concentration and INR (linear) and aPTT (E_{max}), the response was variable and the dynamic range was limited. These findings are consistent with the results from the apixaban single ascending dose study as well as *in vitro* experiments [22] and are not unique to apixaban. Studies including rivaroxaban and other factor Xa inhibitors have shown low sensitivity and high variability between assays and assay reagents for aPTT, PT and INR [31–33]. While a concentration–response relationship was observed for aPTT and INR within the setting of this controlled clinical study, the limited sensitivity and large variability limit their usefulness as clinical measures of apixaban pharmacodynamic response or exposure. Several studies have been conducted to identify a more suitable laboratory test for the new direct Xa inhibitors. In this study, mPT was found to have greater sensitivity to apixaban and exhibited a linear relationship with apixaban concentration with a wider dynamic range compared with INR and aPTT. The relationship between mPT and apixaban plasma concentration appeared to be somewhat less

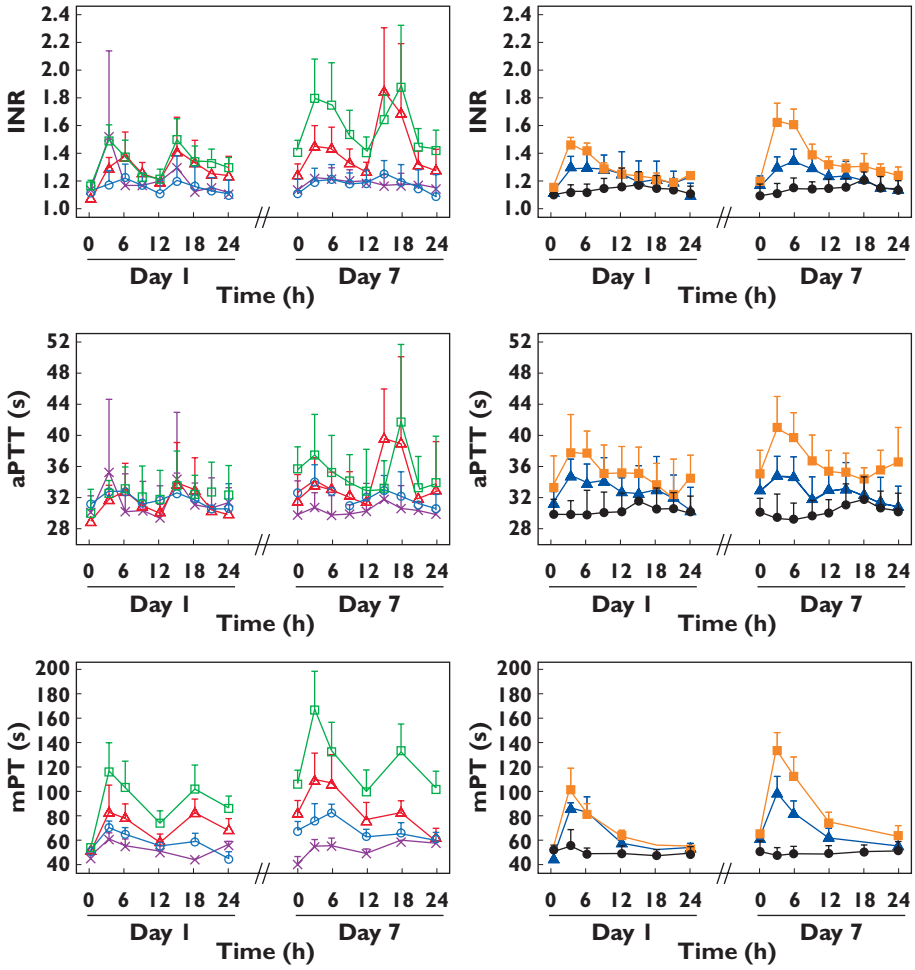

Figure 3

Mean international normalized ratio (INR), activated partial thromboplastin time (aPTT) and modified prothrombin time (mPT) values over time for day 1 to day 7. ×, 2.5 mg twice daily; ○, 5 mg twice daily; △, 10 mg twice daily; □, 25 mg twice daily; ▲, 10 mg once daily; ■, 20 mg once daily; ●, pooled placebo

variable than that for INR and aPTT, but more variable than that reported for an anti-factor Xa activity assay [23, 31]. A number of clinical trials in the apixaban development programme used the Diagnostica Stago Rotachrom® Heparin assay and demonstrated a much stronger correlation between anti-factor Xa activity and apixaban plasma concentration than observed for traditional clotting time assays and mPT. Thus, assays providing a more direct and specific measure of factor Xa activity are the preferred method for assessing direct factor Xa inhibitors such as apixaban [31–34].

In conclusion, apixaban pharmacokinetics were predictable, as demonstrated by low variability, and were dose proportional over a 10-fold dose range. The effect of apixaban on clotting time measures, such as INR, aPTT and mPT, was directly related to its plasma concentration, and consistent with its mechanism of action of direct reversible inhibition of factor Xa. All doses of apixaban were safe and well tolerated in healthy subjects, with no evidence of a clinically relevant increased risk of bleeding in this study. Taken together, the pharmacokinetic, pharmacodynamic and safety profiles of apixaban observed in this trial supported further clinical evaluation of apixaban as an anticoagulant and provided the foundation for dose selection in subsequent clinical trials.

Competing Interests

All authors have completed the Unified Competing Interest form at http://www.icmje.org/coi_disclosure.pdf and declare CF had support from Bristol-Myers Squibb (employee/stock/stock options), SN had support from Bristol-Myers Squibb (employee/stock/stock options), JW had support from Bristol-Myers Squibb (employee), Alan Schuster had support from Bristol-Myers Squibb (employee), WB had support from Pfizer Inc (employee/stock/stock options), RB had support from Pfizer Inc (employee/stock/stock options), ZY had support from Bristol-Myers Squibb (employee), Andrew Shenker had

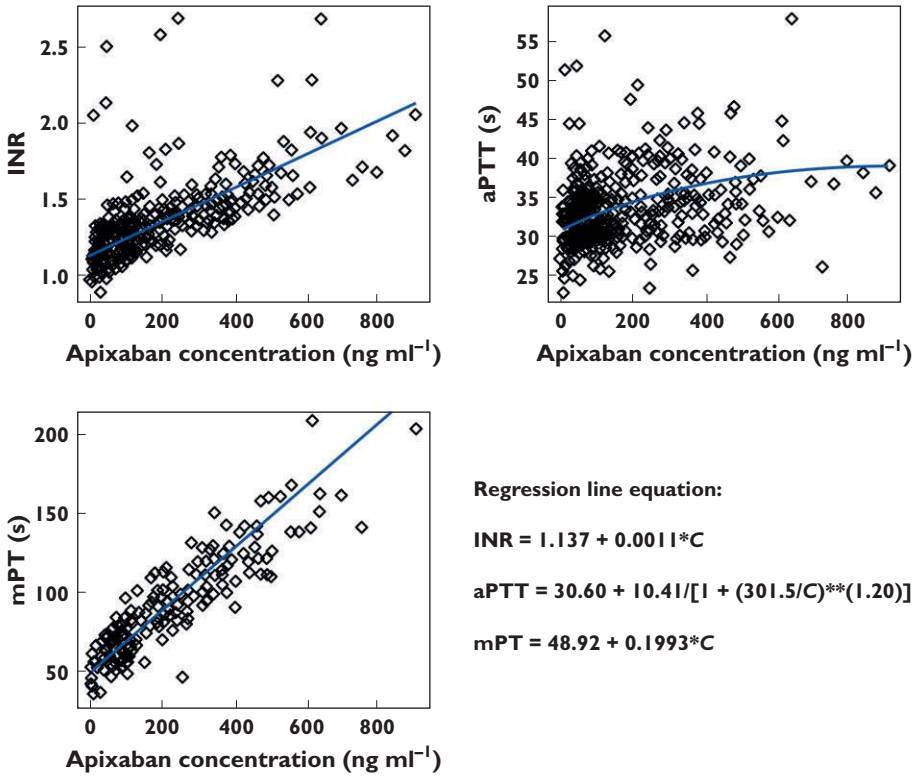

Figure 4

International normalized ratio (INR), activated partial thromboplastin time (aPTT) and modified prothrombin time (mPT) *vs.* apixaban plasma concentration for individual patients with regression lines. *C*, apixaban concentration (ng ml^{-1})

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