

Effects of Age and Sex on the Single-Dose Pharmacokinetics and Pharmacodynamics of Apixaban

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Abstract

Background and Objectives The effects of age and sex on apixaban pharmacokinetics and pharmacodynamics were studied.

Methods This was an open-label, single-dose, 2×2 factorial study. Healthy young (aged 18–40 years) and elderly (aged ≥ 65 years) male and female subjects received a single oral 20 mg dose of apixaban. Blood and urine samples were collected for pharmacokinetic and pharmacodynamic (blood only) analyses. Subjects were monitored for adverse events throughout the study.

Results Seventy-nine subjects were enrolled into four groups: young males ($n = 20$), elderly males ($n = 20$), young females ($n = 20$) and elderly females ($n = 19$). Age did not affect the maximum observed plasma concentration

(C_{\max}). The mean area under the concentration–time curve from time zero extrapolated to infinite time (AUC_{∞}) was 32 % greater in elderly subjects than in young subjects. The mean C_{\max} and AUC_{∞} values were 18 and 15 % higher, respectively, in females than in males. The time course of the mean international normalized ratio (INR), modified prothrombin time (mPT) and anti-Xa activity tracked the apixaban concentration–time curve. All three pharmacodynamic measures exhibited a positive linear correlation with the plasma apixaban concentration. Differences in the mean INR, mPT and anti-Xa activity between age and sex groups were small (<15 % at the maximum mean values) and were generally related to pharmacokinetic differences. However, anti-Xa activity demonstrated less variability than the INR or mPT, and may have utility as a bioassay for apixaban. Apixaban was well tolerated, with no serious adverse events.

Conclusion There were no clinically meaningful age- or sex-related differences in the pharmacokinetics and pharmacodynamics of apixaban that would require dose modification on the basis of age or sex alone.

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Key Points

In this study, there were no clinically meaningful age- or sex-related differences in the pharmacokinetics and pharmacodynamics of apixaban.

Apixaban is safe and well tolerated in both elderly and young subjects of both sexes.

Dose adjustment of apixaban is not required on the basis of age or sex alone.

1 Background

Anticoagulation plays an important role in the prevention and treatment of venous and arterial thrombosis [1–3]. Conventional anticoagulants, such as unfractionated heparin, low molecular weight heparin (LMWH) and warfarin, have limitations that may contribute to suboptimal patient management [3–5]. In recent years, considerable effort has been directed towards the development of orally administered anticoagulants that have a rapid onset of action, predictable pharmacokinetics and pharmacodynamics, and a low propensity for food and drug interactions. Targets of these new oral anticoagulants include clotting factors such as factor Xa (FXa) and thrombin.

Apixaban is a novel oral, selective, direct-acting, reversible FXa inhibitor, which was approved in several countries for prevention of venous thromboembolism (VTE) in patients following elective knee or hip replacement surgery and for reduction in the risk of stroke or systemic embolism in patients with nonvalvular atrial fibrillation (NVAF), and recently for the treatment of venous thromboembolism. Apixaban has oral bioavailability of ~50 % and multiple pathways of elimination (including renal elimination, metabolism, biliary excretion and direct intestinal excretion), with renal clearance (CL_R) accounting for 27 % of total clearance [6–8]. The average elimination half-life ($t_{1/2}$) is approximately 12 h (range 8–15 h) [9].

Numerous studies have shown that age and sex have the potential to influence the pharmacokinetics and pharmacodynamics of some drugs, including anticoagulants [10–14]. Physiological changes associated with aging, such as increased gastric pH, reduced lean body mass, decreased liver size and hepatic blood flow, and a decreased glomerular filtration rate, can affect the absorption, distribution, metabolism and excretion of drugs [10]. The incidence and prevalence of many thromboembolic disorders, such as NVAF, increase with advancing age, as does the need for major orthopaedic surgery [1, 15]. Sex-related differences in drug pharmacokinetics have also been attributed to physiological differences between males and females [11, 14]. The primary objective of this study was to assess the effects of age and sex on the single-dose pharmacokinetics of apixaban. Secondary objectives were to assess the effects of age and sex on the single-dose pharmacodynamics of apixaban, along with the safety and tolerability profile.

2 Methods

2.1 Study Design

This was an open-label, single-dose, 2×2 factorial study in both young and elderly healthy subjects, who received a

single oral 20 mg dose of apixaban (clinical supply; Bristol-Myers Squibb, New Brunswick, NJ, USA). This was the highest dose evaluated in phase 2 studies [16, 17] and was expected to be well tolerated on the basis of previous experience [7]. This study was conducted at a single centre (Seaview Research, Miami, FL, USA), and the protocol and informed consent were reviewed and approved by an independent investigational review board (Plantation, FL, USA). The study was conducted in accordance with the codes and guidelines of the Declaration of Helsinki, Good Clinical Practice and all applicable laws and regulations.

Subjects were grouped by age (young and elderly) and sex. Given a null hypothesis of no age- or sex-specific differences in pharmacokinetics, data from 76 subjects (19 subjects per age/sex group) would provide 97 % power to conclude absence of effects with respect to the area under the plasma concentration–time curve (AUC) from time zero extrapolated to infinite time (AUC_{∞}) and the maximum observed plasma concentration (C_{max}) [i.e. with the 90 % confidence intervals (CIs) of the geometric mean ratios being within 80–125 %]. These calculations assumed that there is no age-by-sex interaction and that the AUC_{∞} and C_{max} are log-normally distributed with coefficients of variation of 25 %, as estimated from a previous study [7]. To allow for possible dropouts, it was planned that 80 subjects would be treated (20 subjects per age/sex group).

Subjects who signed the informed consent form were enrolled into the following four groups: young men (aged 18–40 years), elderly men (aged ≥ 65 years), young women (aged 18–40 years) and elderly women (aged ≥ 65 years). Subjects were admitted to the clinical facility 2 days prior to dosing (day –2) and remained until 96 h postdose (day 5). On day 1, after fasting overnight for at least 10 h, subjects received a single oral dose of 20 mg apixaban. Blood and urine samples were collected for pharmacokinetic and pharmacodynamic (blood only) analyses at intervals up to 96 h postdose. Physical examinations (including vital sign measurements), 12-lead electrocardiograms and clinical laboratory evaluations were performed at baseline and prior to study discharge.

2.2 Study Population

Healthy male and female subjects aged 18–40 years or ≥ 65 years and with a body mass index (BMI) of 18–32 kg/m² were eligible for the study. The exclusion criteria included evidence or history of a bleeding disorder; history of gastrointestinal disease or surgery that could interfere with the absorption of apixaban; and use of any hormonal contraceptive within 3 months before dosing, any prescription drug or over-the-counter acid controller within 4 weeks before dosing, drugs known to increase the potential for bleeding [such as aspirin, nonsteroidal anti-

inflammatory drugs (NSAIDs) or anticoagulants] within 2 weeks before dosing or any other drugs (including over-the-counter medications or dietary supplements) within 1 week before dosing.

2.3 Sample Collection and Assay

Venous blood samples (4.5 mL) were collected in sodium citrate (3.2 %) tubes at 0 h (predose) and at 1, 1.5, 2, 3, 4, 6, 8, 10, 12, 18, 24, 36, 48, 72 and 96 h postdose for the pharmacokinetic and pharmacodynamic analyses. Pharmacokinetic blood samples were centrifuged for 15 min at $1,500\times g$ at 4 °C, and the plasma was transferred to 4 mL cryogenic vials, which were frozen immediately in dry ice and stored for shipment at -20 °C. Following a predose void, subjects provided urine samples for the time intervals of 0–12, 12–24, 24–36, 36–48, 48–60, 60–72 and 72–96 h after dosing. For each collection interval, the urine volume was recorded and aliquots of the urine samples were collected and stored (frozen at -20 °C) until shipment to the analytical laboratory. Apixaban was quantitatively determined in plasma and urine samples by Intertek Pharmaceutical Services (El Dorado Hills, CA, USA), using validated high-performance liquid chromatographic–tandem mass spectrometric methods. The assay was performed on an AB Sciex 3000TM system using Analyst[®] 1.4 software. Plasma and urine samples were extracted using protein precipitation and solid-phase extraction, respectively. The lower limit of quantification was 1.0 ng/mL for both apixaban plasma and urine assays. The standard curves were fitted by a $1/x^2$ -weighted linear equation over the concentration range of 1.00–1,000 ng/mL. The between-run and within-run variability, expressed as coefficients of variation, for apixaban in quality-control plasma samples were ≤ 9.49 and ≤ 10.1 %, respectively, with deviations from the nominal concentration of no more than ± 16.2 %. The between-run and within-run variability for apixaban in quality-control urine samples were ≤ 3.65 and ≤ 3.58 %, respectively, with deviations from the nominal concentration of no more than ± 12.4 %. All of the runs for the study met the acceptance criteria.

The international normalized ratio (INR), modified prothrombin time (mPT) and anti-Xa activity were measured to assess apixaban pharmacodynamic activity. Two 4.5 mL venous blood samples per time point were collected in 3.2 % sodium citrate tubes and centrifuged for 10 min at $2,500\times g$ at 4 °C. The two plasma supernatants were pooled and then centrifuged again for 10 min at $2,500\times g$ at 4 °C to minimize the platelet content in the plasma. Aliquots of the plasma sample were transferred into separate cryovials for INR, mPT and anti-Xa activity analysis and were stored at or below -20 °C for shipment to the analytical laboratories.

The INR was measured by MDS (Toronto, ON, Canada), using an MLA 1600/1800 coagulation analyser and Hemoliance[®] RecombiPlasTin reagent (Beckman Coulter, Inc., Brea, CA, USA), having an international sensitivity index of ~ 1.0 (normal range 0.9–1.3), with results reported to one decimal point. The mPT was measured by a validated method at Covance Laboratories, Inc. (Chantilly, VA, USA), using an MLA 1600c coagulation analyser (assay range 7–600 s). For the mPT assay, the thromboplastin reagent (Dade Thromboplastin C Plus; Siemens Healthcare Diagnostics, Deerfield, IL, USA) was diluted 1:2.25 with 100 nmol/L calcium chloride to decrease the rate of the clotting reaction [18]. Analyses of anti-Xa activity were performed by the Clinical Discovery Technologies group at Bristol-Myers Squibb (Hopewell, NJ, USA). Anti-Xa activity was measured using an STA[®]-Rotachrom[®] heparin kit (Diagnostica Stago, Parsippany, NJ, USA) on an STA[®]-R analyser [19]. Apixaban anti-Xa activity was calculated as the inverse of colour generation measured at 405 nm and interpolated against a 3-point linear kit LMWH calibration curve [range 0–2 IU/mL (LMWH units)] and against a 9-point log-linear apixaban calibration curve [range 7.8–2,000 ng/mL (apixaban units)].

2.4 Pharmacokinetic and Pharmacodynamic Assessments

Single-dose pharmacokinetic parameters were calculated from the individual plasma apixaban concentration–time data, using noncompartmental methods, by Kinetica[®] (version 4.2; InnaPhase Corp., Philadelphia, PA, USA) within the eToolbox Software package (EP version 2.4; Thermo Electron Corporation, Philadelphia, PA, USA). The parameters included C_{\max} , the time of C_{\max} (t_{\max}), AUC_{∞} , AUC from time zero to the time of the last measurable concentration (AUC_{last}) and $t_{1/2}$. If the extrapolated AUC was greater than 20 % of the subject's AUC_{∞} , the subject was excluded from summaries of AUC_{∞} and $t_{1/2}$. CL_R was calculated as the total cumulative amount of drug recovered in the urine, divided by AUC_{last} over the same time interval. The apparent total clearance after oral administration (CL/F) was calculated as dose divided by AUC_{∞} . The apparent volume of distribution at steady state (V_{ss}/F) was calculated as $\text{dose} \times \text{AUMC}/AUC^2$, where AUMC is the area under the first-moment curve (the area under the first moment of the plasma concentration–time curve).

The mean pharmacodynamics–time profiles (INR, mPT and anti-Xa activity) for the age and sex groups were derived from individual subject data within each group, using the measurements collected from predose to 96 h postdose. Maximum values were determined for the INR,

mPT and anti-Xa activity from the mean data. The AUC from time zero to 24 h postdose (AUC_{24}) was calculated for the INR and mPT from individual subject data. Given that an exploratory anti-Xa activity method was used in this study and not optimized for measurement of apixaban, AUC for anti-Xa activity was not calculated.

2.5 Statistical Analysis

2.5.1 Pharmacokinetics

Summary statistics were presented by group for all apixaban parameters (C_{max} , AUC_{∞} , AUC_{last} , t_{max} , $t_{1/2}$, CL/F , V_{ss}/F and CL_R). Geometric means and coefficients of variation were provided for C_{max} , AUC_{∞} and AUC_{last} . The median and range were presented for t_{max} . Arithmetic means and standard deviations (SDs) were provided for $t_{1/2}$, CL/F , V_{ss}/F and CL_R .

To assess the effects of age and sex on the pharmacokinetics of apixaban, analysis of variance was performed on $\ln(AUC_{\infty})$ and $\ln(C_{max})$. The factors in the analyses were age, sex and the interaction of age and sex. Point estimates and 90 % CIs for means and for differences between means on a log scale were exponentiated to obtain estimates of geometric means and ratios of geometric means on the original scale. No adjustment was made for multiplicity. Absence of an age or sex effect on apixaban AUC_{∞} or C_{max} was concluded if the corresponding 90 % CI for the elderly to young ratio or for the female to male ratio of the population geometric mean was contained within an equivalence interval of 80–125 %. Regression analyses

were conducted to assess potential relationships between apixaban CL/F and CL_R and creatinine clearance (CL_{Cr}), apixaban exposure (AUC_{∞} and C_{max}) and CL_{Cr} , body weight and BMI.

2.5.2 Pharmacodynamics

Pharmacodynamic assessment–time profiles (means \pm SDs) were presented by age and sex groups for INR, mPT and anti-Xa activity values (expressed in both LMWH and apixaban units). Scatter plots of INR, mPT and anti-Xa activity values versus the plasma apixaban concentration were provided. The slope and intercept (90 % CI) were provided for INR, mPT and anti-Xa activity values (expressed in ng/mL and LMWH units) based on the model-based regression analysis. SAS/STAT[®] version 8.2 software was used to perform the statistical analyses of the pharmacokinetic and pharmacodynamic data.

2.6 Safety Assessments

Subjects were closely monitored for adverse events throughout the study. The incidence of adverse events was tabulated, and all adverse events were reviewed for their relation to apixaban and clinical importance. Blood and urine samples for clinical laboratory testing were collected under fasting conditions at screening, at baseline, on day 1, on day 2 and before study discharge. In addition, 12-lead electrocardiograms and physical examinations were performed at screening, at baseline and before study discharge.

Table 1 Demographic characteristics and physical measurements at baseline

Characteristic	Young ^a		Elderly ^b	
	Male (n = 20)	Female (n = 20)	Male (n = 20)	Female (n = 19)
Age, years				
Mean (SD)	31 (6)	34 (5)	71 (5)	69 (3)
Range	21–40	21–40	65–79	65–76
Race, n (%)				
White	15 (75)	18 (90)	19 (95)	16 (84)
Black	4 (20)	2 (10)	0	3 (16)
Hispanic	1 (5)	0	1 (5)	0
Weight, kg				
Mean (SD)	76.8 (12.4)	65.3 (8.9)	78.9 (12.0)	70.2 (10.8)
Range	60.0–97.0	53.7–86.0	55.0–114.0	54.0–89.0
BMI, kg/m ²				
Mean (SD)	26.1 (3.5)	25.4 (3.1)	28.3 (2.8)	28.4 (2.9)
Range	20.2–31.9	20.6–32.0	21.0–31.9	22.5–32.0
CL_{Cr} , mL/min ^c				
Mean (SD)	123.3 (23.0)	115.7 (15.0)	82.8 (20.8)	75.1 (12.4)
Range	87–179	92–152	62–144	60–104

BMI body mass index, CL_{Cr} creatinine clearance, SCr serum creatinine, SD standard deviation

^a 18–40 years of age

^b ≥ 65 years of age

^c CL_{Cr} is based on the Cockcroft–Gault calculation at screening: male $CL_{Cr} = [140 - \text{age (y)}] \times \text{weight (kg)} / [72 \times SCr \text{ (mg/dL)}]$; female $CL_{Cr} = 0.85 \times \text{male } CL_{Cr}$

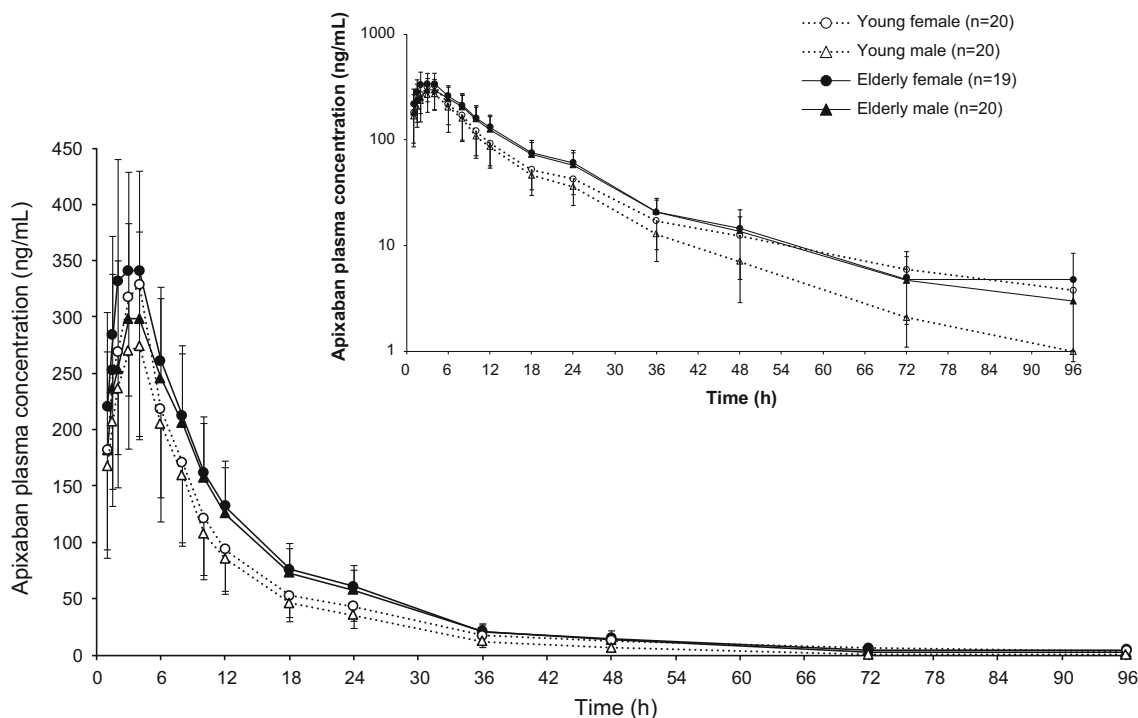


Fig. 1 Mean (\pm standard deviation) plasma concentration–time profiles of apixaban after single-dose administration (20 mg), by age and sex groups. The error bars represent ± 1 standard deviation

Table 2 Apixaban pharmacokinetic parameters by age group

	C_{max} , ng/mL, GM (CV%)	AUC_{∞} , ng-h/mL, GM (CV%)	AUC_{last} , ng-h/mL, GM (CV%)	t_{max} , h, median (min, max)	$t_{1/2}$, h, mean (SD)	CL_R , L/h, mean (SD)	CL/F , L/h, mean (SD)	V_{ss}/F , L, mean (SD)
Young ($n = 40$) ^a	315.4 (34)	3,424 (32)	3,360 (32)	3.0 (1.0, 4.0)	11.98 (5.15) ^b	0.63 (0.33)	6.10 (1.81)	85.2 (28.2)
Elderly ($n = 39$) ^c	336.9 (27)	4,536 (23)	4,451 (23)	3.0 (1.0, 4.0)	15.45 (7.39)	0.45 (0.13)	4.52 (0.98)	77.5 (27.0)
Ratio of adjusted GM, elderly to young (90 % CI)	1.07 (0.96–1.19)	1.32 (1.20–1.46)						

AUC_{∞} area under the plasma concentration–time curve from time zero extrapolated to infinite time, AUC_{last} area under the plasma concentration–time curve from time zero to the time of the last measurable concentration, CI confidence interval, CL/F apparent total clearance after oral administration, CL_R renal clearance, C_{max} maximum observed plasma concentration, CV coefficient of variation, GM geometric mean, max maximum, min minimum, SD standard deviation, $t_{1/2}$ plasma terminal half-life, t_{max} time of C_{max} , V_{ss}/F apparent volume of distribution at steady state

^a 18–40 years of age

^b One young female subject was excluded from the summary statistics (and related analyses) for AUC_{∞} and $t_{1/2}$ on the basis of predefined criteria

^c ≥ 65 years of age

3 Results

3.1 Study Population

A total of 79 subjects met the study criteria, were treated and completed the study. The clinical phase of the study was conducted from May to November 2005. The baseline characteristics of all subjects are summarized in Table 1. The mean age was 71 years for elderly males and 69 years

for elderly females, versus 31 and 34 years for young males and young females, respectively.

3.2 Pharmacokinetics

The mean plasma concentration–time profiles of apixaban for the four age and sex groups are shown in Fig. 1. Pharmacokinetic parameters and the results of statistical analyses of C_{max} and AUC_{∞} by age and sex are shown in

Table 3 Apixaban pharmacokinetic parameters by sex group

	C_{max} , ng/mL, GM (CV%)	AUC_{∞} , ng·h/mL, GM (CV%)	AUC_{last} , ng·h/mL, GM (CV%)	t_{max} , h, median (min, max)	$t_{1/2}$, h, mean (SD)	CL_R , L/h, mean (SD)	CL/F , L/h, mean (SD)	V_{ss}/F , L, mean (SD)
Female ($n = 39$)	353.4 (31)	4,235 (28) ^a	4,116 (29)	3.0 (1.0, 4.0)	14.89 (7.06) ^a	0.46 (0.18)	4.91 (1.41)	79.7 (28.8)
Male ($n = 40$)	301.0 (26)	3,680 (30)	3,626 (30)	3.0 (1.0, 4.0)	12.60 (5.92)	0.62 (0.31)	5.69 (1.79)	82.8 (26.9)
Ratio of adjusted GM, female to male (90 % CI)	1.18 (1.06–1.31)	1.15 (1.04–1.27)						

AUC_{∞} area under the plasma concentration–time curve from time zero extrapolated to infinite time, AUC_{last} area under the plasma concentration–time curve from time zero to the time of the last measurable concentration, CI confidence interval, CL/F apparent total clearance after oral administration, CL_R renal clearance, C_{max} maximum observed plasma concentration, CV coefficient of variation, GM geometric mean, max maximum, min minimum, SD standard deviation, $t_{1/2}$ plasma terminal half-life, t_{max} time of C_{max} , V_{ss}/F apparent volume of distribution at steady state

^a One young female subject was excluded from the summary statistics (and related analyses) for AUC_{∞} and $t_{1/2}$ on the basis of predefined criteria

Tables 2 and 3, respectively. Apixaban reached C_{max} within 3 h of administration, on average, regardless of age or sex. Age had no effect on apixaban C_{max} , whereas older age was associated with a 32 % greater apixaban AUC_{∞} , compared with younger age. In female subjects, the apixaban geometric mean C_{max} and AUC_{∞} values were 18 and 15 % higher, respectively, than the values in male subjects. The age by sex interaction was nonsignificant for C_{max} and AUC_{∞} ($p = 0.94$ and $p = 0.40$, respectively).

The apixaban CL_R tended to be lower in elderly subjects (0.45 L/h) than in young subjects (0.63 L/h) and lower in female subjects (0.46 L/h) than in male subjects (0.62 L/h); these differences in CL_R also appeared to be reflected in CL/F (young versus elderly 6.1 versus 4.5 L/h; male versus female 5.7 versus 4.9 L/h). The mean apixaban $t_{1/2}$ in young male subjects was approximately 10 h, whereas in young female and elderly subjects it ranged from 14 to 16 h. A weak increasing linear correlation was observed between apixaban CL/F and CL_{cr} ($R^2 = 0.2674$, $p < 0.0001$; Fig. 2a) and between CL_R and CL_{cr} ($R^2 = 0.1773$, $p < 0.0001$; Fig. 2b). Among the relationships between apixaban exposure (AUC_{∞} and C_{max}) and other factors (body weight and BMI) that were also explored, only apixaban C_{max} versus body weight showed a statistically significant ($p = 0.009$) but weak ($R^2 = 0.09$) negative linear correlation.

3.3 Pharmacodynamics

Data from 39 subjects (6 young males, 12 young females, 11 elderly males and 10 elderly females) were included in the summary and comparisons of apixaban pharmacodynamics. Pharmacodynamic data from the first 40 subjects enrolled in the study were excluded because of errors with sample collection and processing techniques (such as inadequate mixing of the sample, sustained application of the tourniquet and repeated venepuncture in the same location), which led to spuriously elevated clotting time

assessments regardless of age, sex or the plasma apixaban concentration. Sample collection and processing techniques have been shown to influence clotting time measurements [20, 21]. Following retraining of the clinical site on adequate mixing of blood with anticoagulant in the sample tube and timely sample transfer, etc., clotting time measurements showed greater consistency with prior experience [7, 9].

The time course of the mean INR, mPT and anti-Xa activity tracked the plasma concentration–time curve of apixaban (Fig. 3), with maximum values for all pharmacodynamic endpoints observed around the apixaban t_{max} (3 h). Consistent with the pharmacokinetic profile in elderly and young subjects, elderly subjects generally had higher mean INR, mPT and anti-Xa values than young subjects over the observed time course, but the differences in the maximum mean (SD) INR, anti-Xa activity and mPT between age groups were less than 15 %: INR, 1.55 (0.18) and 1.46 (0.19); anti-Xa activity, 275 (75) and 295 (91) ng/mL; mPT, 149 (25) and 130 (26) s in the elderly and young groups, respectively. Similarly, the differences in the mean (SD) AUC_{24} between age groups for these INR and mPT assessments were less than 20 %: INR, 31.5 (2.23) and 29.3 (2.09); mPT, 2,604 (378) and 2,137 (295) s·h in the elderly and young groups, respectively.

The mean INR, mPT and anti-Xa activity profiles in female subjects did not reflect the slightly higher apixaban exposure observed in that group relative to male subjects, with mean INR and mPT values being slightly lower in females than in males, and no discernible difference in anti-Xa. There were no substantial differences at maximal values; the differences between sex groups were less than 15 % at maximum mean (SD) values: INR, 1.47 (0.12) and 1.56 (0.16); mPT, 132 (23) and 150 (33) s; anti-Xa activity, 292 (96) and 274 (60) ng/mL in female and male subjects, respectively (Fig. 3). Furthermore, the differences in the mean (SD) AUC_{24} between the sexes for these INR and mPT assessments were less than 15 %: INR, 29.7 (1.58)

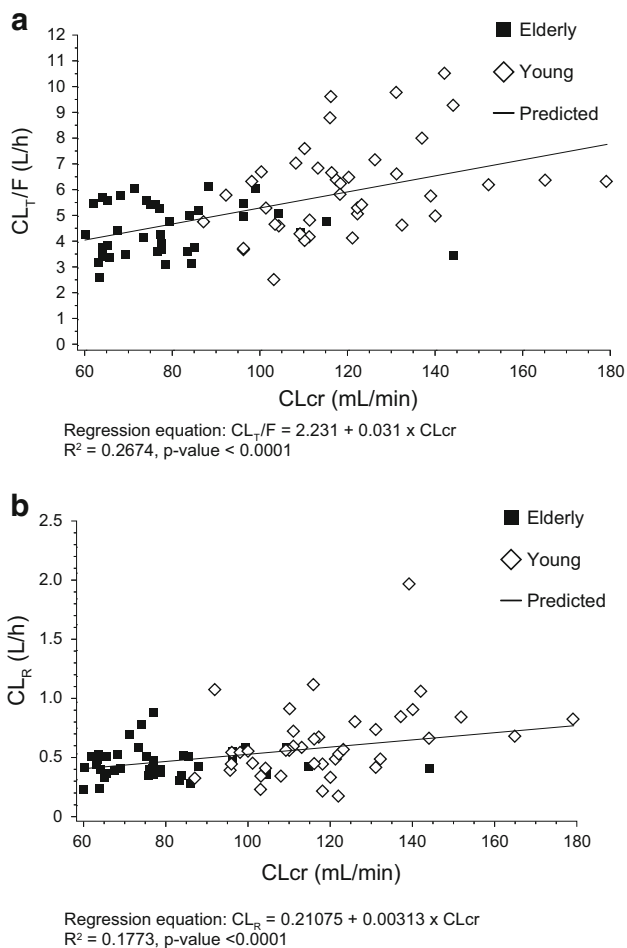


Fig. 2 Linear regression of **a** apparent total clearance after oral administration (CL_T/F) and **b** renal clearance (CL_R) of apixaban versus creatinine clearance (CL_{Cr}), by age groups. All subjects received apixaban 20 mg. For CL_R, one patient with a CL_R of 2.08 L/h was excluded from the statistical analysis

and 31.5 (2.91); mPT, 2,248 (296) and 2,570 (476) s·h in female and male subjects, respectively. These findings likely reflect limitations in the sensitivity of the respective pharmacodynamic assays and their ability to accurately detect small differences in apixaban exposure; however, the anti-Xa activity appeared to better reflect the observed apixaban concentration–time profiles of females and males.

There were no obvious differences in the plasma concentration–pharmacodynamic response relationships of apixaban (Fig. 4a, b) across age or sex. The slope and intercept (90 % CI) for the INR versus the apixaban concentration were 0.0021 (0.0017–0.0026) mL/ng and 1.09 (1.07–1.12), respectively; the slope and intercept (90 % CI) for the mPT versus the apixaban concentration were 0.31 (0.28–0.33) mL·s/ng and 61.7 (58.9–64.5) s, respectively. A positive linear relationship was observed between individual anti-Xa activity [expressed in apixaban units (ng/mL)] and the corresponding plasma apixaban

concentrations (Fig. 4c). The slope and intercept (90 % CI) for anti-Xa activity (expressed in ng/mL) versus the apixaban concentration were 0.85 (0.83–0.86) and 13.0 (8.7–17.2) ng/mL, respectively. When anti-Xa activity was expressed in LMWH units of IU/mL, based on the kit 3-point standard curve (0–2 IU/mL), a similar linear relationship was observed over a narrower plasma apixaban concentration range (approximately 0–200 ng/mL, as shown in Fig. 4d). The slope and intercept (90 % CI) for anti-Xa activity (expressed in IU/mL) versus the apixaban concentration were 0.0134 (0.0130–0.0138) IU/ng and 0.048 (0.023–0.072) IU/mL, respectively. The relationship between anti-Xa activity and the plasma apixaban concentration was less variable than that observed for the INR and mPT (Fig. 4a–d).

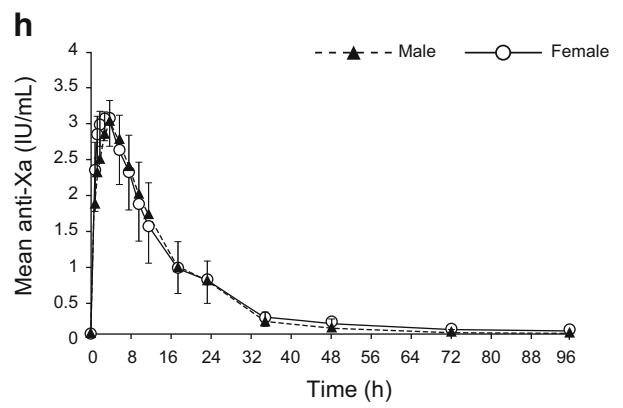
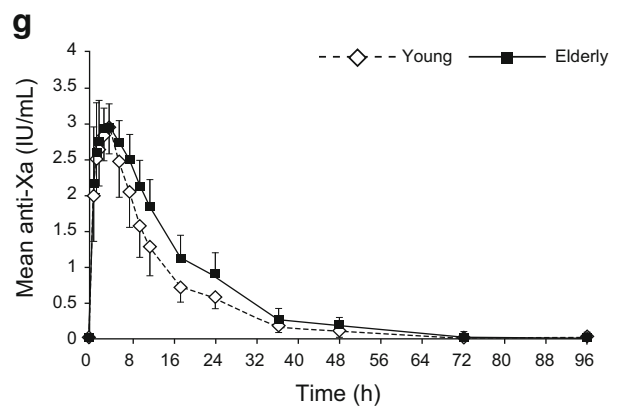
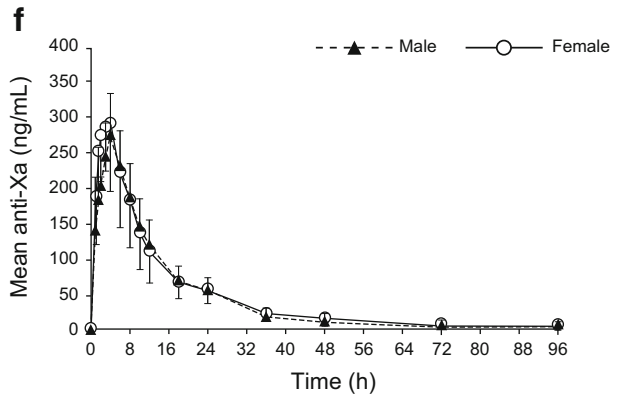
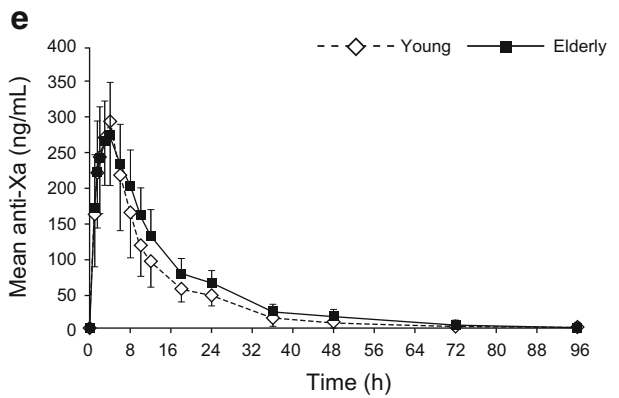
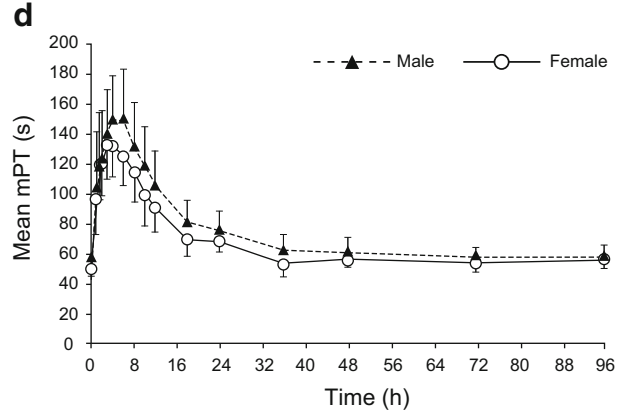
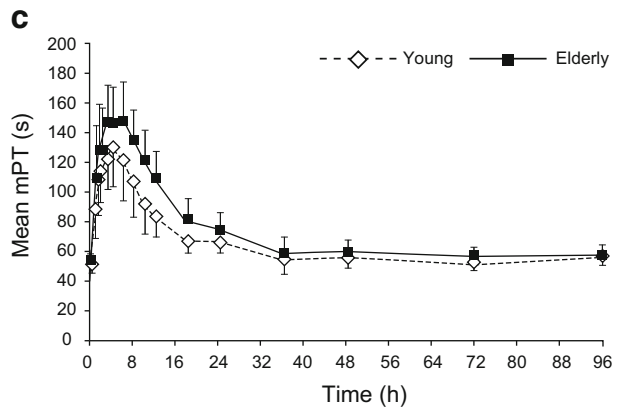
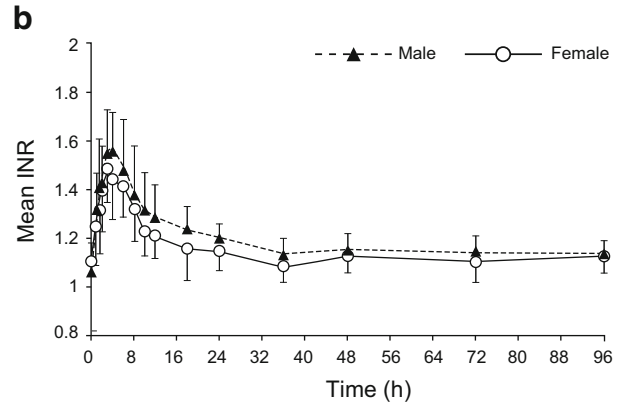
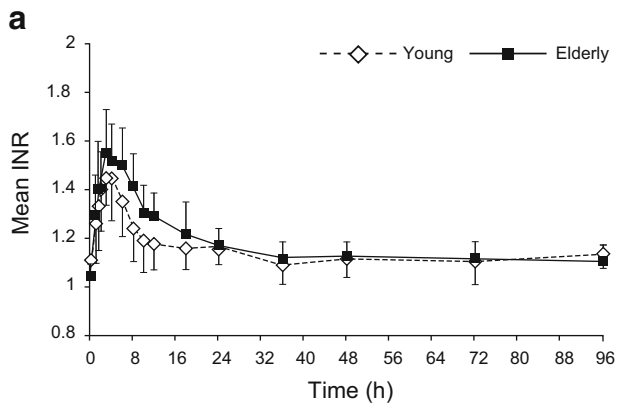
3.4 Safety and Tolerability

A single 20 mg dose of apixaban was well tolerated by all subjects in this study; there were no serious adverse events, significant bleeding-related adverse events or discontinuations due to adverse events. A total of 21 adverse events were reported by 16 subjects; all were considered to be of mild or moderate intensity. Seven subjects (including six females) reported minor bleeding-related adverse events, including three subjects with positive faecal occult blood (one young female, one elderly female and one elderly male), three subjects with ecchymosis (two young females and one elderly female) and one subject (an elderly female) with a haematoma secondary to venepuncture. All bleeding events were mild in intensity and generally resolved within 1–7 days. The remaining adverse events consisted of eczema, pruritus, headache, dizziness, iron deficiency anaemia, blood draw site pain, cholelithiasis with elevated transaminases, subcutaneous abscess, pharyngitis, cheilitis and constipation, each occurring in one subject except for headache (two subjects).

4 Discussion

Apixaban is approved for prevention of VTE following elective knee or hip replacement surgery and for stroke prevention in patients with NVAF; these patient populations tend to comprise elderly men and women [22–25]. Therefore, it is important to understand if age- or sex-related differences influence the pharmacokinetics and pharmacodynamics of agents used in the management of these patients.

In this study, a single oral 20 mg dose of apixaban was safe and well tolerated in all subjects, regardless of age or sex. A 20 mg dose was chosen for this study because the systemic exposure from a single 20 mg dose is



◀ **Fig. 3** **a, b** International normalized ratio (INR), **c, d** modified prothrombin time (mPT), **e, f** anti-Xa activity (expressed in ng/mL), and **g, h** anti-Xa activity (expressed in IU/mL) versus time, by age (young 18–40 years of age; elderly ≥65 years of age) and sex groups. The error bars represent ±1 standard deviation

representative of the steady-state exposure achieved with the highest dose (10 mg twice daily) used in the apixaban phase 3 clinical trials. Apixaban exposure was approximately 32 % higher in elderly subjects than in young subjects and 15 % greater in female subjects than in male subjects, indicating that age- and sex-related influences have a small to modest impact on apixaban exposure. Age- and sex-related influences on pharmacokinetics have been attributed to multiple factors [10], several of which were

explored in this study, including associations between apixaban exposure and renal function, body weight and BMI. Among these factors, the strongest trend was observed between apixaban exposure and renal function; therefore, the relationship between apixaban CL/F and renal function was explored further.

CL_R accounts for approximately 27 % of total clearance; therefore, a relationship between apixaban exposure and renal function would be expected. In this study, CL_R and CL/F tended to be lower in elderly subjects than in young subjects and lower in female subjects than in male subjects. There was a direct relationship between apixaban clearance and CL_{cr}, which was consistent with the observed inverse relationship between apixaban exposure

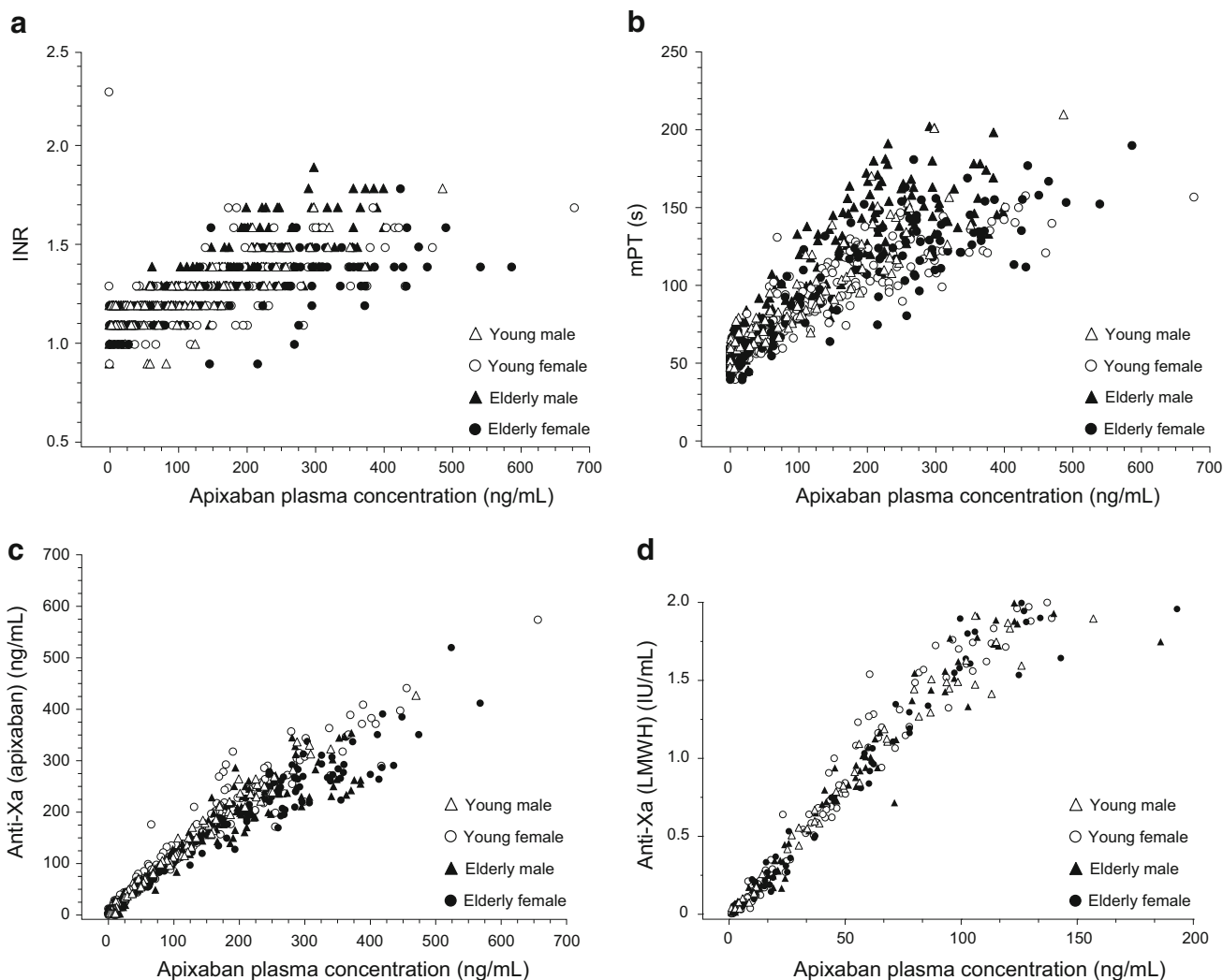


Fig. 4 Scatter plots of **a** the individual international normalized ratio (INR), **b** the individual modified prothrombin time (mPT), **c** individual anti-Xa activity levels (expressed in ng/mL of apixaban) and **d** individual anti-Xa activity levels [expressed in IU/mL of low molecular weight heparin (LMWH)] versus plasma apixaban

concentrations, by age (young: 18–40 years of age; elderly: ≥65 years of age) and sex groups. Data points that are above the upper limit of the anti-Xa activity assay range (2 IU/mL, corresponding to an apixaban concentration of ~200 ng/mL) are not included in the figure

and CL_{cr}. These trends suggest that differences in renal function may have contributed to the differences in apixaban CL_R therefore reflected in the CL/F and exposure observed between groups. While determining the effect of renal function on apixaban pharmacokinetics was not an aim of this study, the effect of renal impairment on apixaban pharmacokinetics has been evaluated in two other studies—one in subjects with mild, moderate and severe renal impairment, and another in subjects with end-stage renal disease on haemodialysis [26, 27]. The results from those two studies indicated that mild and moderate renal impairment had a modest effect on the apixaban AUC; the apixaban AUC was approximately 40 % greater in subjects with severe renal impairment or end-stage renal disease [26, 27]. Given that the range of CL_{cr} in the current study was 60–179 mL/min, consistent with the age range of the subjects, the difference in CL_R would partly contribute to the observed differences in exposure between groups. The apixaban *t*_{1/2} also appeared to be longer in elderly and female subjects (elderly versus young 15.45 versus 11.98 h; female versus male 14.89 versus 12.60 h).

As noted, since only weak relationships were observed between apixaban exposure and body weight and BMI, the relationship between apixaban clearance and these two demographic factors were not explored further in this study. A study evaluating the impact of body weight on apixaban pharmacokinetics has shown that apixaban exposure is approximately 30 higher and 22 % lower in individuals weighing <50 kg or >120 kg, respectively, compared with subjects weighing 65–85 kg [27]. Therefore, given the limited range of body weight in this study (54–114 kg), it is not surprising that a weak association between apixaban exposure and body weight and BMI was observed.

As expected, changes in the pharmacodynamic measures of INR, mPT and anti-Xa activity over time closely followed the apixaban concentration–time profile. All three measures exhibited a positive linear relationship with the plasma apixaban concentration. Similar to what has been previously reported, the relationship between the plasma apixaban concentration and the INR and mPT is weaker and more variable than the relationship between the plasma apixaban concentration and anti-Xa activity [18]. This is expected, since endogenous factors contributing to clotting play roles in INR and mPT measurement, while the anti-Xa activity assay used in this study relies on the addition of exogenous bovine FXa, thus making the anti-Xa test more dependent on an FXa inhibitor (apixaban) concentration and less on other endogenous factors.

Measurement of anti-Xa activity using an apixaban standard curve showed a positive linear correlation over the full range of plasma apixaban concentrations (from 0 to ~700 ng/mL) observed in this study. Anti-Xa activity

determined using the kit LMWH standards also showed a linear relationship, although over a narrower range of apixaban concentrations (~0 to 200 ng/mL). It should be noted that this was the first clinical study in which apixaban anti-Xa activity was measured using the STA[®]-Rotachrom[®] heparin kit. Given the use of exogenous bovine FXa in the Rotachrom[®] assay and the pharmacological characteristics of apixaban, the anti-Xa activity determined with this assay is expected to correlate well with apixaban concentrations. Therefore, any apparent discordance between anti-Xa activity and apixaban concentrations likely reflects assay limitations rather than a difference in the pharmacological effect of apixaban. On the basis of the results of this study, the STA[®]-Rotachrom[®] method was further optimized for use in clinical trials and was used to establish a direct linear correlation over a wider concentration range between apixaban anti-Xa activity (expressed in IU/mL) and the plasma apixaban concentration (expressed in ng/mL) [18, 19]. Therefore, anti-Xa activity may have utility as a bioassay for apixaban.

The small to modest effects of age and sex on apixaban pharmacokinetics and pharmacodynamics observed in this study are not likely to be clinically relevant, given the favourable benefit–risk profile of apixaban observed in phase 3 clinical trials. Elderly male and female patients were included in the apixaban phase 3 clinical trials for prevention of thromboembolism in knee and hip replacement surgery (mean age ~63 years; ~40 % males) and for stroke prevention in patients with AF (mean age ~69 years; 59–65 % males) [22–25, 29]. The 2.5 mg twice-daily regimen was used in all phase 3 studies of apixaban in VTE prevention, irrespective of patient age or sex, and the efficacy and safety outcomes from those trials confirmed that no dose adjustment is needed for age or sex in that population [30]. The safety and efficacy profiles observed for apixaban in the subpopulation analyses of the phase 3 ARISTOTLE and AVERROES studies in patients with NVAF also indicated that dose adjustment for age or sex alone is not necessary [24, 25]. The ARISTOTLE and AVERROES trials did have prespecified dose modification criteria that included age as a component. Subjects with at least two out of three risk factors for bleeding (age ≥80 years, body weight ≤60 kg and/or serum creatinine ≥1.5 mg/dL) received apixaban 2.5 mg twice daily rather than 5 mg twice daily, which maintained the desired safety and efficacy profiles in patients who met these criteria [24, 25]. The individual impact of these factors on apixaban exposure is modest and does not warrant dose modification. Nevertheless, when they exist in combination, the resulting increase in exposure may warrant a reduction in dose to mitigate the inherently higher risk of bleeding that may be present in some patient populations [26–29, 31].

5 Conclusion

This study demonstrated that age- and sex-related influences on apixaban pharmacokinetics were small to modest and do not appear to be clinically meaningful on the basis of age and sex alone. Corresponding minor differences were also present in pharmacodynamic markers of anticoagulation activity. Anti-Xa activity showed a direct linear relationship with plasma apixaban concentrations, regardless of age or sex groups, and appeared to be less variable than other pharmacodynamic assessments, such as PT/INR. Apixaban was well tolerated by all subjects in this study. No dose adjustment is recommended for apixaban on the basis of age or sex alone. However, in the presence of multiple factors that increase apixaban exposure, dose adjustment may be warranted, such as in AF patients with at least two of these three risk factors for bleeding: age ≥ 80 years, body weight ≤ 60 kg and/or serum creatinine ≥ 1.5 mg/dL.

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Conflict of interest Drs. Frost, Song, Wang, Barrett, Schuster and LaCreta are employees of Bristol-Myers Squibb. Dr. Shenker was an employee of Bristol-Myers Squibb at the time when this research was conducted, and he is currently an employee of GlaxoSmithKline. Dr. Harris is an employee of Seaview Research, Miami, FL, USA.

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