PHASE I STUDIES

Effect of ketoconazole on the pharmacokinetics of axitinib in healthy volunteers

Yazdi K. Pithavala · Warren Tong · Janessa Mount · Sadayappan V. Rahavendran ·
May Garrett · Brian Hee · Paulina Selaru · Nenad Sarapa · Karen J. Klamerus

Summary *Objective* Axitinib (AG-013736), an oral, potent, and selective inhibitor of vascular endothelial growth factor receptors 1, 2, and 3, is metabolized primarily by cytochrome P450 (CYP) 3A with minor contributions from CYP1A2, CYP2C19, and glucuronidation. Co-administration with CYP inhibitors may increase systemic exposure to axitinib and alter its safety profile. This study evaluated changes in axitinib plasma pharmacokinetic parameters and assessed safety and tolerability in healthy subjects, following axitinib co-administration with the potent CYP3A inhibitor ketoconazole. *Methods* In this randomized, single-blind, two-way crossover study, 32 healthy volunteers received placebo, followed by a single 5-mg oral dose of axitinib, administered either alone or on the fourth day of dosing with oral ketoconazole (400 mg/day for 7 days). *Results* Axitinib exposure was significantly increased in the presence of ketoconazole, with a geometric mean ratio for area under the plasma concentration–time curve from time zero to infinity of 2.06 (90% confidence interval [CI]: 1.84–2.30) and a geometric mean ratio for maximum plasma concentration (C_{max}) of 1.50 (90% CI: 1.33–1.70). For axitinib alone or with ketoconazole, C_{max} occurred 1.5 and 2.0 h after dosing, respectively. Adverse events were predominantly mild; the most commonly reported treatment-related adverse events were headache and nausea. *Conclusions* Axitinib plasma exposures and peak concentrations were increased following concurrent administration of axitinib and ketoconazole in healthy volunteers. Axitinib alone and in combination with ketoconazole was well tolerated. These findings provide an upper exposure for expected axitinib plasma concentrations in the presence of potent metabolic inhibition.

Keywords Axitinib · Ketoconazole · Pharmacokinetics · CYP3A · TaqMan® allelic discrimination · Simcyp® · Corrected QT · Drug interaction

Y. K. Pithavala (✉) · W. Tong · J. Mount · S. V. Rahavendran ·
M. Garrett · B. Hee · P. Selaru · N. Sarapa · K. J. Klamerus
Pfizer Oncology,
10555 Science Center Drive,
San Diego, CA 92121, USA
e-mail: yazdi.pithavala@pfizer.com

Present Address:
W. Tong
Allergan Inc.,
2525 Dupont Drive,
Irvine, CA 92612, USA

Present Address:
N. Sarapa
Hoffmann-La Roche Inc.,
340 Kingsland Street,
Nutley, NJ 07110, USA

Introduction

Axitinib (AG-013736) is an oral, potent, and selective inhibitor of vascular endothelial growth factor receptors 1, 2, and 3 that induces tumor regression [1]. Pre-clinical tumor growth inhibition studies identified an effective axitinib concentration of 0.28–0.85 nmol/L [1]. Axitinib plasma concentrations observed in clinical trials after dosing (5 mg twice daily [BID]) averaged 0.19±0.12 nmol/L [1]. Phase II trials evaluating this dose in approximately 300 patients have demonstrated antitumor activity combined with acceptable tolerability across multiple tumor types, including metastatic renal cell cancer, non-small cell lung cancer, melanoma, and thyroid cancer [2–6]. The pharmacokinetics of axitinib are generally linear [7]. Oral absorption occurs rapidly, with

peak plasma concentrations observed 1–4 h after dosing in the fed state. Axitinib has an effective plasma terminal half-life of 2–5 h and an oral absolute bioavailability of approximately 58% [7, 8].

In vitro studies with recombinant cytochrome P450 (CYP) enzymes and human liver microsomes showed that axitinib is primarily metabolized by oxidation via the CYP3A isoenzyme and to a lesser extent by CYP1A2, CYP2C19, and uridine diphosphate glucuronsyltransferase (UGT) 1A1 [8]. The major circulating metabolites in human plasma are an N-glucuronide product and a sulfoxide; the putative metabolic pathways for axitinib have been determined [9], and a schematic is provided in Fig. 1. In a human liver microsomal preparation, ketoconazole, a potent CYP3A inhibitor, blocked axitinib metabolism with a 50% inhibitory concentration (IC_{50}) of 0.4 μM. *In vitro*, ketoconazole has also been reported to inhibit UGT2B7-mediated glucuronidation of zidovudine and morphine and UGT1A1-mediated glucuronidation of SN-38, the irinotecan metabolite [10]. These findings suggest a mechanistic basis by which drugs that affect the activity of CYP3A and UGT1A1 isozymes could alter the pharmacokinetics of axitinib. This is further supported by the observation of reduced axitinib exposure in a patient who was also treated with phenytoin, a potent inducer of multiple CYP enzymes, during the first-in-human (FIH) phase I study of axitinib in patients with advanced solid tumors [7]. Concurrent administration of phenytoin reduced the area under the plasma concentration–time curve (AUC) from time zero to 24 h (AUC_{0-24}) and maximum plasma concentration (C_{max}) of axitinib approximately 10-fold. Study protocols currently prohibit use of drugs known to be potent inducers of the CYP3A and CYP1A2 isozymes during axitinib treatment.

Co-administration of a CYP3A inhibitor such as keto-conazole could have the opposite effect and lead to elevated plasma exposure to axitinib, potentially increasing the frequency and severity of adverse events. This randomized, single-blind, two-way crossover study evaluated the effects of repeated doses of ketoconazole plus a single axitinib dose on axitinib pharmacokinetic parameters in healthy volunteers. A separate study evaluated the effects of a potent CYP3A inducer on axitinib plasma exposure in healthy volunteers [8].
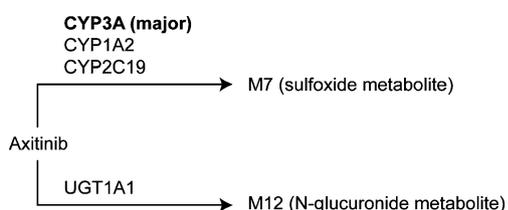
Fig. 1 Schematic of axitinib metabolism in humans

Methods

Subjects

Adults aged 18–55 years who were assessed as healthy based on a detailed medical examination, which included medical history, blood pressure (BP) and pulse-rate measurements, a 12-lead electrocardiogram (ECG) test, and clinical laboratory evaluations, were eligible to participate in the study. Subjects had a body mass index of 18–30 kg/m^2, a total body weight >50 kg, and normal ECG and resting BP measurement (<140/90 mm Hg). Volunteers agreed to abstain from strenuous physical activity and consumption of products containing grapefruit, caffeine, xanthine, or alcohol from 2–4 days prior to initiating the study until collection of the final pharmacokinetic samples. Exclusion criteria were conditions that might influence the absorption, distribution, metabolism, or excretion of axitinib or ketoconazole: recent history of drug abuse, high levels of alcohol consumption, or use of products containing tobacco or nicotine; donation of >500 ml of blood within 56 days before beginning study treatment; use of prescription or non-prescription drugs or supplements (other than acetaminophen) within 14 days before initiating treatment (28 days for herbal supplements and hormonal contraception methods or 6 months for depot medroxyprogesterone acetate); or treatment with an investigational agent or use of any known CYP450 enzyme-inducing or -inhibiting drug within 30 days of beginning study medication.

Study design

This was a randomized, two-way crossover study conducted in healthy volunteers. The primary objective was to characterize changes in axitinib plasma pharmacokinetic parameters when a single dose of axitinib was co-administered with repeated doses of ketoconazole to healthy subjects. Secondary objectives included assessing the safety and tolerability of axitinib administered with ketoconazole, as well as evaluating the effects of a single axitinib dose on the corrected QT (QTc) interval and BP when administered alone or in combination with ketoconazole.

This study was approved by the institutional review board or ethics committee of each participating center and was carried out in accordance with the ethical principles originating in or derived from the Declaration of Helsinki. Additionally, this trial complied with informed consent regulations and the International Conference on Harmonization Good Clinical Practice guidelines, as well as applicable local laws and regulatory requirements. Written informed consent was obtained prior to subjects entering the study.

Treatment

Placebo treatment was administered to all subjects on day –1 to characterize QT under the condition of no therapy. Using a computer-generated randomization schedule, subjects were assigned to receive either axitinib alone (treatment A) or combination treatment with ketoconazole plus axitinib (treatment B). Treatments were administered to subjects in one of two different sequences (A→B or B→A) separated by a 14-day wash-out period (Fig. 2). During treatment A, volunteers received a single, oral, 5-mg dose of axitinib on day 1. During treatment B, volunteers received oral doses of ketoconazole 400 mg/day in the morning on days 1–7, and a single 5-mg dose of axitinib administered with ketoconazole on day 4.

Axitinib and placebo treatment were administered in a single-blind manner, and ketoconazole treatment was not blinded. Subjects received axitinib or placebo in the morning after fasting overnight for at least 8 h; food and beverages were permitted 4 h after dosing. The axitinib tablet formulation used in this study was previously shown to produce higher exposures in the overnight fasted state in a phase I study conducted in patients with solid tumors [7] and was the basis for administration of axitinib in the fasted state in the current study. Ketoconazole was administered with breakfast once daily in the morning, except on day 4 when it was administered simultaneously with axitinib after an overnight fast of at least 8 h. During the first 4 h after dosing, subjects were required to refrain from lying down, except while BP, pulse rate, and ECG measurements were obtained.

Assessments

Blood samples (5 ml) for determination of axitinib pharmacokinetic profiles were collected on days 1–3 at 0 h (pre-dose) and 1, 1.5, 2, 3, 4, 6, 8, 12, 16, 24, 36, and 48 h post-dose during treatment A. During treatment B, samples were collected on days 4–8 at 0 h (pre-dose) and at 1, 1.5, 2, 3, 4, 6, 8, 12, 16, 24, 36, 48, 72, and 96 h post-dose. Blood pressure and pulse rate were recorded in triplicate at screening, and single measurements were obtained on days –2 and –1, days 1–3 during treatment A, on the day before commencing the second treatment, days 4–6 during treatment B, and at the end of the study. Standard 12-lead ECG tests were carried out at screening, days –2 and –1, day 1 during treatment A, days 3 and 4 during treatment B, and at the end of the study. Assessments of ECG, BP, and pulse rate were performed prior to the collection of blood samples at the timepoints indicated above plus an additional assessment 10 h post-dose.

Safety and tolerability were assessed by monitoring adverse events, vital signs, ECGs, and laboratory evaluations, as well as through physical examinations. Adverse events were graded according to the National Cancer Institute Common Terminology Criteria for Adverse Events, version 3.0 (ctep.cancer.gov/protocoldevelopment/ electronic_applications/docs/ctcaev3.pdf).

Bioanalytical methods

Axitinib concentrations in potassium (K_3) EDTA plasma were measured using a validated liquid chromatography/ tandem mass spectrometric assay (Charles River Laboratories, Worcester, MA) with a range of 0.1 to 25 ng/ml [7]. Due to axitinib light sensitivity, all sample processing and bioanalytical procedures were performed under red light or with appropriate protection from visible light. Plasma samples were added to a 96-well opaque microtiter plate and fortified with deuterium-labeled (d7) axitinib internal standard, followed by addition of 1.0 M sodium bicarbonate. The samples were extracted with ethyl acetate:hexanes (75:25, v/v), centrifuged, and the supernatants were evaporated to dryness under a gentle nitrogen stream in microtiter plates heated to 35°C. Sample residues were reconstituted in water/methanol/sodium bisulfite (75:25:0.5, v/v/v) and eluted from a C18 column (XTerra® MS C18, 30×2.1 mm, 3.5 μm; Waters Corporation, Milford, MA) using a multi-step gradient (mobile phase A = water, mobile

Fig. 2 Treatment overview
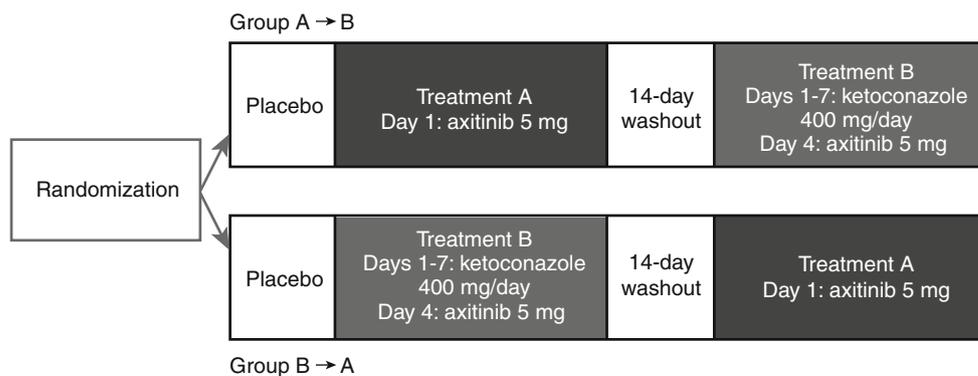
phase B = acetonitrile; 10–90% B in 3 min; 0.5 ml/min flow rate) onto an API 3000® mass spectrometer (AB Sciex, Ontario, Canada) with an electrospray ionization source. Detection was in positive ion mode with multiple reaction monitoring (mass transition [m/z] 387→355 and 394→360 for axitinib and axitinib-d7). Duplicate quality control (QC) samples at three concentration levels spanning the assay range (low, medium, and high) were included in each analytical run to measure assay performance. Inter-assay precision and accuracy were determined by QC samples from across all study sample runs. Precision was within 12% coefficient of variation (CV), while overall bias (expressed as percent relative error) was within 5.5%. No plasma metabolites were characterized in the study.

Genotyping of drug-metabolizing enzymes was carried out using polymerase chain reaction (PCR) to amplify DNA samples for all assays performed in this study (Pfizer-Ann Arbor, Worldwide Safety Sciences, Ann Arbor, MI). In particular, the following variants of UGT1A1 were assessed: UGT1A1*60, UGT1A1-3156 (-3156 G→A nucleotide change), UGT1A1 Promoter TA repeat (*28, *36, *37), UGT1A1*6 and UGT1A1*27. Genotyping for the CYP2C19 *2, *3, and *17 alleles was also conducted. The TaqMan® Allelic Discrimination procedure was used for detection of all alleles, with the exception of the CYP3A4*2 (MassEXTEND™; Sequenom, San Diego, CA) and UGT1A1*28 (sequencing) genotyping assays.

TaqMan® allelic discrimination assay

TaqMan® allelic discrimination employs the 5′-nuclease activity of AmpliTaq Gold® DNA polymerase to allow direct detection of PCR products by the release of a fluorescent reporter. Two TaqMan® probes (Applied Biosystems, Carlsbad, CA) were used in this assay, one probe for each allele. Each probe consists of an oligonucleotide with a 5′ reporter (TET™ [tetrachloro-6-carboxyfluorescein]/VIC® or FAM® [6-carboxyfluoroscein] dye and a 3′-quencher dye [TAMRA™ (6-carboxytetramethylrhodamine)] or a non-fluorescent quencher). An intact probe results in quenching of the reporter dye fluorescence.

During the annealing phase of PCR, forward and reverse primers hybridize to the flanking region of the polymorphic site. In addition, the TaqMan® probes hybridize to the target polymorphic site within the PCR product. The reporter dye is cleaved by the Taq Gold® enzyme, resulting in an increase in the reporter dye fluorescence. By measuring the intensities of TET™/VIC® (allele 1 or homozygous wild type) and FAM™ (allele 2 or homozygous variant) signal, the specific genotype of an allele is discriminated.

Individual reaction plates with 20 ng of genomic DNA per reaction were used. Master mixes were prepared for each assay performed, and controls for both no template and each allele were included for each plate. After master mix (10X PCR buffer with MgCl$_2$, forward and reverse primers, TaqMan® probes, deoxynucleotides, and AmpliTaq Gold® DNA polymerase) had been added to the controls and unknown DNA samples, plates were sealed and PCR cycling (GeneAmp® PCR System 9700, Applied Biosystems) performed. The sealed 96-well plates were then transferred to the ABI 7700® (Applied Biosystems) and fluorescence data collected.

Simcyp® simulations

A Simcyp®-based simulation (Simcyp® Population-based ADME Simulator, version 6.0 [Simcyp Ltd, Sheffield, UK] on Microsoft Windows® XP [Redmond, WA]) was performed to assess changes in axitinib plasma concentrations with co-administration of ketoconazole (inhibitor) in healthy volunteers. Simcyp® uses the relationship between the inhibitor concentration at the active site in vivo and the inhibition constant (K_i) determined in vitro to predict the effects of drug–drug interactions involving CYP enzymes. Competitive inhibition, induction, and/or mechanism-based inhibition can be investigated using this software [11].

A Simcyp® model was developed for axitinib using in vitro and clinical pharmacokinetic parameters for axitinib (input parameters are shown in Table 1). The required Simcyp® model for ketoconazole was available within Simcyp® as an inhibitor file. Due to the inability to determine glucuronidation clearance from in vitro tissues accurately, the following approach was used. Systemic clearance (CL$_{sys}$) for axitinib was determined from a previous study in healthy volunteers who received intravenous axitinib (1 mg). In vivo CL$_{sys}$ was then converted to intrinsic clearance (CL$_{int}$) using amount of microsomal protein per gram of liver and liver weight to yield a CL$_{int}$ of 500 μl/min/mg microsomal protein. Contribution of CYP3A and glucuronidation to total CL$_{int}$ was determined through sensitivity analyses to be 50% (CYP3A4=250 μl/min/mg microsomal protein) and 30% (non–CYP-mediated clearance=150 μl/min/mg), respectively, when simulations with ketoconazole were conducted. The remaining 20% of clearance was attributed to non-metabolic pathways (4.2 L/h).

Physiologic variability (healthy Northern European Caucasians: height, weight, age, etc.) and variation in phenotype were calculated using parameters within the Simcyp® software. Dose, dose interval, and duration of administration of axitinib and ketoconazole were set according to the clinical protocol. Simcyp® predicts the mean value as well as the distribution in the population for the effect by applying a Monte Carlo approach. Population simulations were performed using 100 Northern European Caucasians aged 20–50 years (1:1 male/female) in 10 trials, with each trial containing 10 subjects. To prevent bias, the Simcyp® end-user did not have access to the clinical drug–drug interaction results.

Table 1 *In vitro* input parameters used in Simcyp® model

Input parameter	Value	Data source
Molecular weight	386.47	
Log P	3.1	*In vitro* determination
B/P ratio	0.79	*In vitro* determination
fu plasma	0.02	*In vitro* plasma protein binding
fa	1	Assumption
ka (1/h)	0.7	Estimated as $\sim 1/T_{max}$; T_{max} from clinical data
Vss (L/kg)	1	Clinical data
fu microsomal	1	Simcyp® *in silico* calculator
Q gut (L/h)	10	Based upon Caco-2 cell permeability data
CL_{int} (ul/min/mg – microsomal protein)	250	Experimental; for P_{450} contribution
Additional undefined HLM CL_{int}	150	Experimental; for non-P_{450} (glucuronidation) contribution

P partition coefficient, *B/P ratio* blood to plasma ratio for axitinib, *fu plasma* fraction of drug unbound in plasma, *fa* fraction absorbed from gastrointestinal tract, *ka* first-order rate of absorption, *Vss* volume of distribution at steady state, *fu microsomal* fraction of drug unbound in microsomal fraction, Q_{gut} intestinal blood flow, CL_{int} intrinsic clearance, *HLM* human liver microsomes

Statistical and pharmacokinetic analyses

Based on an IC_{50} of 0.4 μM for ketoconazole-mediated inhibition of axitinib metabolism observed in human liver microsomal preparations, ketoconazole 400 mg was predicted to result in a 1.8-fold increase in axitinib geometric mean AUC from time zero to infinity ($AUC_{0-\infty}$) and a 1.5-fold increase in geometric mean C_{max}.

The standard deviation (SD) of the difference between (natural log) $AUC_{0-\infty}$ of axitinib plus ketoconazole and axitinib alone was estimated to be 0.42, based on variability in axitinib pharmacokinetics observed in the FIH phase I study in patients with solid tumors [7]. The sample size was calculated as 24 subjects (12 per sequence) to provide 80% power to ensure that the lower limit of the 90% confidence interval (CI) for the AUC ratio was above 1.6 (a 60% increase), if ketoconazole treatment increased axitinib exposure by 100%.

Data analyses included all subjects who received at least one dose of study medication (placebo, axitinib, or ketoconazole). In addition, evaluable pharmacokinetic data were required for analyses of pharmacokinetic parameters, and at least one baseline and post-baseline BP measurement were needed for BP analyses.

Pharmacokinetic evaluation of axitinib was performed with WinNonlin®, version 4.01 (Pharsight, Mountain View, CA) using a non-compartmental approach. Estimated axitinib pharmacokinetic parameters included AUC, C_{max}, and half-life ($t_{1/2}$). The primary analysis for the study was based on axitinib $AUC_{0-\infty}$. This value was log-transformed and analyzed using a mixed-effects model with treatment, period, and sequence as fixed effects and subject (nested within sequence) as the random effect. The 90% CI for the difference in the means of the log-transformed $AUC_{0-\infty}$ was

calculated. The antilogs of the confidence limits obtained constituted the 90% CI for the ratio of the geometric mean of ketoconazole plus axitinib compared with axitinib alone. Secondary analyses were performed using axitinib AUC from time zero to the time of last quantifiable concentration (AUC_{last}) and C_{max} in a similar fashion.

Results

Subject characteristics and disposition

A total of 35 subjects were enrolled, 20 of whom were randomized to group A→B and 15 of whom were randomized to group B→A. Baseline characteristics were comparable between subjects in the two groups (Table 2). The majority of participants were male (91.4%) and the median age was 32 years (range: 19–54 years). Thirty-two subjects received at least one dose of axitinib; 28 subjects (80% of the 35 enrolled subjects) completed the study. The remaining seven subjects discontinued at the investigator's discretion, i.e., serum chemistry abnormalities before receiving axitinib ($n=2$); positive cotinine test after the washout period ($n=1$); difficulty swallowing ($n=1$), withdrawal of consent ($n=2$), or terminated due to an adverse event assessed as unrelated to treatment ($n=1$, grade 1 increased serum creatinine).

Pharmacokinetics

In all, 32 subjects were included in the pharmacokinetic analysis, which included data for 31 subjects treated with axitinib alone and 28 subjects treated with axitinib plus ketoconazole.

Table 2 Subject characteristics at baseline

	Group A→B (n=20)	Group B→A (n=15)	Total (N=35)
Sex, n (%)			
Male	18 (90.0)	14 (93.3)	32 (91.4)
Female	2 (10.0)	1 (6.7)	3 (8.6)
Age, median (min, max), years	33.5 (19, 54)	32.0 (21, 53)	32.0 (19, 54)
Race, n (%)			
Black	2 (10.0)	2 (13.3)	4 (11.4)
Caucasian	10 (50.0)	9 (60.0)	19 (54.3)
Hispanic/Latino	7 (35.0)	3 (20.0)	10 (28.6)
Other	1 (5.0)	1 (6.7)	2 (5.7)
Systolic BP at baseline, median (min, max), mm Hg	114.0 (96, 135)	112.0 (98,135)	114.0 (96, 135)
Diastolic BP at baseline, median (min, max), mm Hg	67.5 (58, 86)	72.0 (62, 83)	70.0 (58, 86)

BP blood pressure

All pre-dose axitinib concentrations were below the lower limit of quantification (0.100 ng/ml), demonstrating that the washout period between treatments A and B was sufficient. QC assay performance had an average accuracy within 6% and average precision was within 13% CV. As expected, median plasma concentrations of axitinib were higher following combination treatment with ketoconazole and axitinib compared with axitinib treatment alone (Fig. 3). Similarly, axitinib exposure was significantly increased in the presence of ketoconazole, with a geometric mean ratio for $AUC_{0-\infty}$ of 2.06 (90% CI: 1.84–2.30) and a geometric mean ratio for C_{max} of 1.50 (90% CI: 1.33–1.70) (Table 3). The mean plasma $t_{1/2}$ of axitinib increased following co-administration with ketoconazole to 13.1 h (SD: 8.85), compared with 9.44 h (SD: 9.98) following

axitinib treatment alone. Ketoconazole treatment did not appear to have any effect on axitinib median time to reach C_{max} (T_{max}). Values for T_{max} were 1.50 h (range: 1.00–3.00) for axitinib alone and 2.00 h (range: 1.00–4.13) for axitinib plus ketoconazole.

Genotypic analysis of axitinib drug-metabolizing enzymes

Since axitinib metabolism has minor contributions from two genetically polymorphic enzymes, CYP2C19 and UGT1A1, genotyping for these enzymes was included here and in most axitinib studies conducted in healthy volunteers. The intent of the genotyping is to conduct a pooled meta-analysis of the influence of polymorphic enzymes on inter-subject variability in axitinib drug exposure. Due to a

Fig. 3 Median axitinib concentration-time profiles following treatment with axitinib alone (n=31) or axitinib plus ketoconazole (n=29), linear scale **a** and log scale **b**
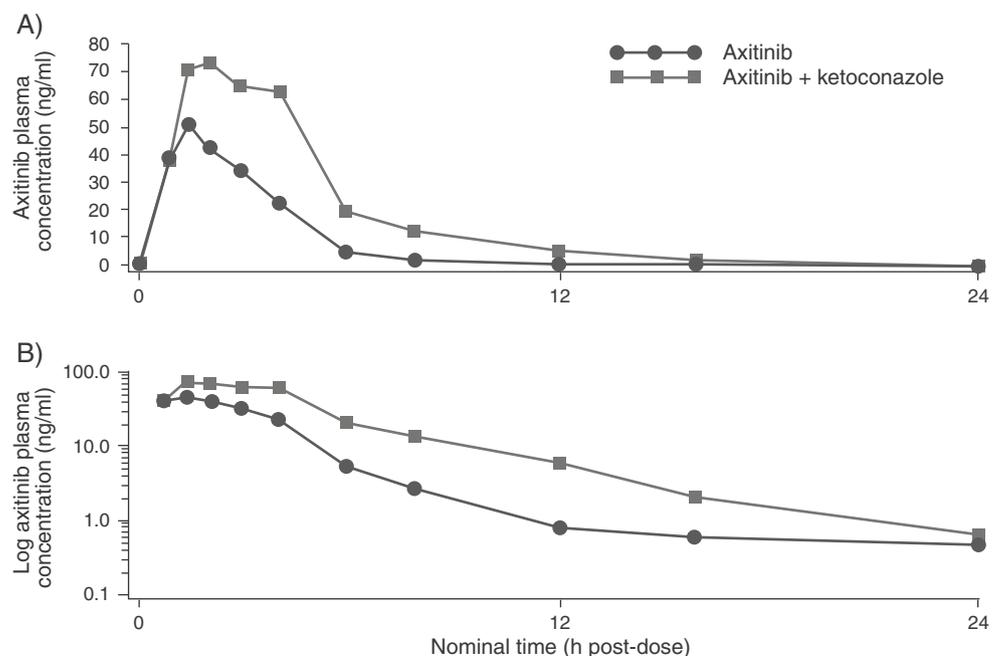
Table 3 Summary of axitinib PK parameters (evaluable population; $n=32$)

	Geometric LS mean (95% CI)		Statistical comparison	
	Axitinib ($n=31$)	Axitinib + ketoconazole ($n=28$)	Geometric LS mean ratio	90% CI
$AUC_{0-\infty}$ (ng·h/ml)	196.7 (162.0–238.8)	404.8 (332.3–493.2)	2.06	1.84–2.30
AUC_{last} (ng·h/ml)	193.8 (159.9–234.9)	401.9 (330.1–489.3)	2.07	1.86–2.31
C_{max} (ng/ml)	51.0 (43.9–59.3)	76.7 (65.6–89.7)	1.50	1.33–1.70

$AUC_{0-\infty}$ area under the plasma concentration–time curve from time zero to infinity, AUC_{last} area under the plasma concentration–time curve from time zero to the time of last quantifiable concentration, *CI* confidence interval, C_{max}, maximum plasma concentration, *LS*, least squares

lack of statistical power, it was not possible to assess the effect of genotype on axitinib pharmacokinetics from this study alone.

Simcyp® simulation

The Simcyp® simulation was conducted using a design that mirrored the clinical ketoconazole drug interaction study design. The Simcyp® simulations predicted a 2.08-fold increase in AUC and a 1.41-fold increase in C_{max} for axitinib in the presence of ketoconazole. The overall predicted shift in axitinib plasma concentration by Simcyp® was similar to that observed clinically in the presence of ketoconazole (2.06-fold increase in $AUC_{0-\infty}$; 1.50-fold increase in C_{max}).

Pharmacodynamics

Mean time-matched changes from baseline in BP were minimal and followed similar trends for axitinib treatment alone and in combination with ketoconazole (Fig. 4). Mean values for systolic and diastolic BP were within normal ranges. Findings from the QTc analysis will be reported elsewhere.

Safety and tolerability

Adverse events were generally mild and resolved without treatment, as expected in this population of healthy volunteers. One subject (2.9% of all subjects) in group A→B experienced grade 2 nausea and vomiting during treatment with axitinib plus ketoconazole. No action was required, and the adverse event resolved before the end of the study. All other treatment-emergent adverse events, regardless of causality, were grade 1. No grade 3 or 4 laboratory abnormalities were reported. Treatment-related adverse events were reported in eight volunteers (40.0%) in group A→B and in one volunteer (6.7%) in group B→A. Treatment-related adverse events primarily affected the nervous system (e.g., dizziness or headache) or gastrointestinal system (e.g., nausea) (Table 4). Most treatment-related adverse events occurred during treatment with ketoconazole alone or with axitinib plus ketoconazole.

Fig. 4 Mean change from baseline in **a** systolic and **b** diastolic blood pressure (*BP*)
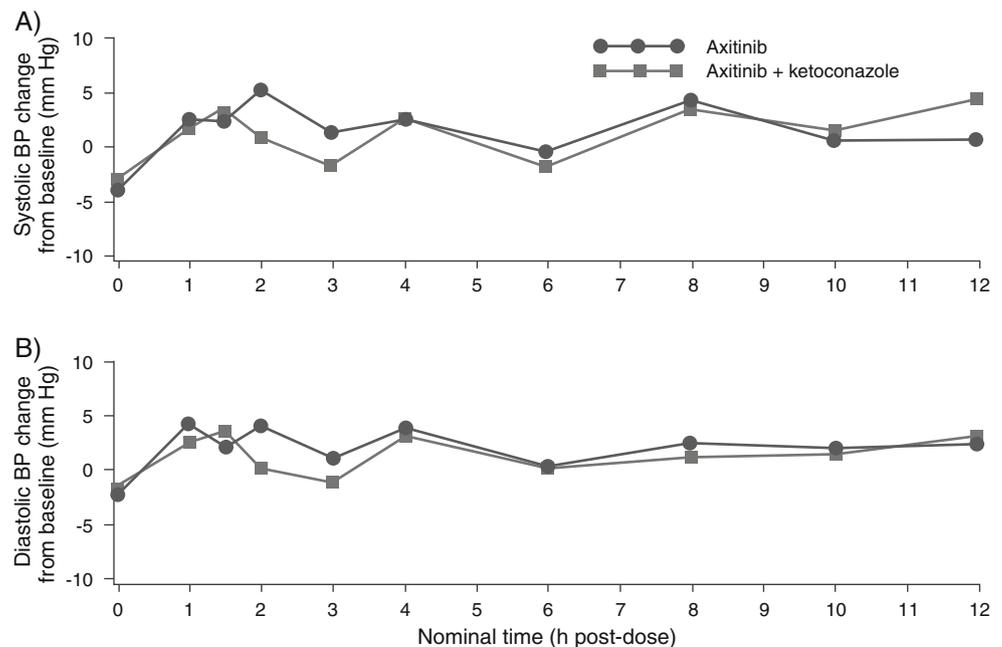
Table 4 Treatment-related adverse events (AEs) for prescribed treatment at time of AE[a]

AE, n (%)	Axitinib (n=32)	Ketoconazole (n=29)	Axitinib+ketoconazole (n=28)	
	Grade 1[b]	Grade 1[b]	Grade 1	Grade 2
Any AE	2 (6.3)	3 (10.3)	5 (17.9)	1 (3.6)
Headache NOS	1 (3.1)	3 (10.3)	3 (10.7)	0
Nausea	0	2 (6.9)	2 (7.1)	1 (3.6)
Dizziness	0	2 (6.9)	1 (3.6)	0
Loose stools	1 (3.1)	0	1 (3.6)	0
Dysgeusia	1 (3.1)	0	0	0
Blurred vision	0	1 (3.4)	0	0
Gastritis NOS	0	1 (3.4)	0	0
Vomiting NOS	0	0	0	1 (3.6)

NOS not otherwise specified

[a] No treatment-related AEs were reported during prescribed treatment with placebo

[b] No AEs grade ≥2 were reported

Discussion

Data from this study represent maximized axitinib exposures obtained when axitinib is co-administered with ketoconazole, a drug that inhibits CYP3A activity. Concomitant treatment with ketoconazole significantly increased axitinib exposure, resulting in a ~2-fold increase in geometric mean axitinib $AUC_{0-\infty}$ and a ~1.5-fold increase in geometric mean C_{max}. These findings support the exclusion criteria in ongoing axitinib studies for patients to avoid concurrent treatment with potent inhibitors of CYP3A. These data are also useful in quantitatively predicting the magnitude of change in axitinib exposure (i.e., more than 2-fold) expected when potent CYP3A inhibitors other than ketoconazole are administered together with axitinib.

Based on data from human liver microsomal preparations (unpublished data), ketoconazole was predicted to result in a 1.8-fold increase in axitinib geometric mean $AUC_{0-\infty}$. Similarly, the Simcyp® prediction was a 2.08-fold increase in axitinib plasma concentrations in the presence of ketoconazole. The clinical result (2.06-fold increase) was similar to these pre-clinical predictions and confirms the validity of this simulation.

Although this study was not sufficiently powered to evaluate the effect of genotypic variation in drug-metabolizing enzymes on axitinib pharmacokinetics, data from this trial were included in a separate population pharmacokinetic analysis that evaluated the potential effect of the *UGTA1A1*28* genotype on axitinib pharmacokinetics in 119 healthy volunteers [12]. In the population pharmacokinetics model, the *UGT1A1*28* genotype was not identified as a significant covariate for oral clearance (CL/F). However, the model predicted that subjects who are homozygous or heterozygous for this mutation are predisposed to have reductions in CL/F of 10% and 5%, respectively, compared with individuals homozygous for the wild-type gene. At the highest simulated dose (10 mg

BID), the upper 90% bound for the predicted AUC distribution in homozygous variant subjects was lower than the plasma exposure associated with unacceptable toxicity in the FIH phase I dose-escalation study in patients [7]. Based on these findings, axitinib dose modification in patients based on *UGT1A1*28* genotype is not warranted at this time.

Administration of a single dose of axitinib in combination with repeated ketoconazole treatment was well tolerated. Most treatment-related adverse events affected the nervous or gastrointestinal systems, with headache and nausea reported most frequently during combination treatment (n=10.7% each). With the exception of one patient who experienced grade 2 nausea and vomiting, all other treatment-related adverse events were mild in severity (grade 1). No clinically significant effects on diastolic or systolic BP were observed for axitinib plus ketoconazole treatment compared with axitinib treatment alone. Treatment-related adverse events reported for axitinib treatment alone were similar to those reported in other clinical trials with axitinib [2–6].

In conclusion, axitinib treatment was well tolerated in this study, both alone and in combination with ketoconazole. However, as predicted, axitinib and ketoconazole co-administration significantly increased axitinib plasma exposure and peak concentrations in healthy volunteers compared with axitinib alone. Concomitant treatment with potent CYP3A4 inhibitors and axitinib should be avoided since the combination may increase the frequency or severity of adverse events.

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