ORIGINAL RESEARCH ARTICLE

Safety, Tolerability, and Pharmacokinetics of Etamicastat, a Novel Dopamine-β-Hydroxylase Inhibitor, in a Rising Multiple-Dose Study in Young Healthy Subjects

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Abstract

Background: Activation of the sympathetic nervous system is an important feature in hypertension and congestive heart failure. A strategy for directly modulating sympathetic nerve function is to reduce the biosynthesis of norepinephrine (noradrenaline) via inhibition of dopamine-β-hydroxylase (DβH).

Objective: To assess the safety, tolerability, and pharmacokinetics of etamicastat (BIA 5-453), a new DβH inhibitor, following repeated dosing.

Methods: A double-blind, randomized, placebo-controlled study was conducted in healthy young male volunteers. Participants received once-daily doses of placebo or etamicastat 25, 50, 100, 200, 400, or 600 mg, for 10 days.

Results: Etamicastat underwent N-acetylation to its metabolite BIA 5-961. Etamicastat and BIA 5-961 maximum concentrations were achieved at 1–3 and 2–4 hours, respectively, after dosing. Elimination half-lives ranged from 18.1 to 25.7 hours for etamicastat and 6.7 to 22.5 hours for BIA 5-961. Both etamicastat and BIA 5-961 followed linear pharmacokinetics. The extent of systemic exposure to etamicastat and BIA 5-961 increased in an approximately dose-proportional manner, and steady-state plasma concentrations were attained up to 9 days of dosing. Etamicastat accumulated in plasma following repeated administration. The mean observed accumulation ratio was 1.3–1.9 for etamicastat and 1.3–1.6 for BIA 5-961. Approximately 40% of the etamicastat dose was recovered in urine in the form of parent compound and BIA 5-961. There was a high variability in pharmacokinetic parameters, attributable to different N-acetyltransferase-2 (NAT2) phenotype. Urinary excretion of norepinephrine decreased following repeated administration of etamicastat. Etamicastat was generally well tolerated. There was no serious

adverse event or clinically significant abnormality in clinical laboratory tests, vital signs, or ECG parameters.

Conclusion: Etamicastat was well tolerated. Etamicastat undergoes N-acetylation, which is markedly influenced by NAT2 phenotype. NAT2 genotyping could be a step toward personalized medicine for etamicastat.

Trial Registration: EudraCT No. 2007-004142-33

Background

Activation of the sympathetic nervous system is a common finding in congestive heart failure and hypertension.[1-6] Adrenoceptor antagonists can be used to inhibit such sympathetic activation, but a proportion of patients do not tolerate the immediate hemodynamic impact, particularly in heart failure.[7] An alternative strategy is to inhibit dopamine-β-hydroxylase (DβH; EC 1.14.17.1), a mono-oxygenase that catalyses the conversion of dopamine into norepinephrine (noradrenaline) in the catecholamine biosynthetic pathway.[8] Gradual sympathetic modulation by DβH inhibitors as opposed to abrupt inhibition observed with β-adrenoceptor blockers (β-blockers) could decrease the hemodynamic negative impact.[9] Furthermore, inhibition of DβH also increases dopamine release,[10,11] which can improve renal function by causing renal vasodilatation and inducing diuresis and natriuresis.[9,12,13]

Several DβH inhibitors have been thus far reported;[14-16] however, both first- and second-generation DβH inhibitors were found to have low potency, poor DβH selectivity, and relevant toxic effects.[17] Nepicastat (RS-25560-197),[8] a third-generation DβH inhibitor, was found to have much greater potency and to be devoid of some of the problems associated with first- and second-generation inhibitors. However, nepicastat was found to cross the blood-brain barrier and to cause potentially significant CNS-related adverse events (AEs).[18] Therefore, there still remains an unmet clinical need for a potent, safe, and peripherally selective DβH inhibitor.

Etamicastat [BIA 5-453; (R)-5-(2-aminoethyl)-1-(6,8-difluorochroman-3-yl)-1,3-dihydroimidazole-2-thione hydrochloride] (figure 1) is intended to act as a reversible inhibitor of peripheral DβH with limited access to the brain.[17] Following oral administration of etamicastat, a blood pressure-lowering effect was observed in studies performed with spontaneously hypertensive rats (SHR). Both the systolic and diastolic blood pressure (but not the heart rate) were decreased in SHR rats in a dose-dependent manner, an effect not observed in normotensive control rats.[19] Chronically administered etamicastat in drinking water also significantly reduced both blood pressure and norepinephrine urinary excretion in SHR rats. In parallel, the urinary excretion of dopamine increased significantly but only in SHR rats.[20]

Etamicastat safety, tolerability, and pharmacokinetics were investigated in a previous entry-into-man single-dose, double-blind, randomized, placebo-controlled study in healthy subjects in
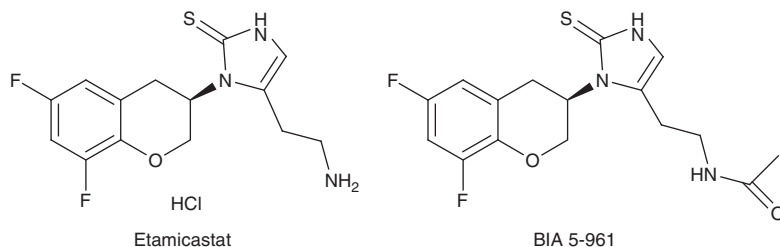

Fig. 1. Structural formulae of etamicastat (BIA 5-453) and its acetylated metabolite BIA 5-961.

the dose range 2–1200 mg.[21] Maximum plasma concentrations (C_{max}) occurred at 1–3 hours after dosing. Elimination was bi-compartmental, characterized by a first short early elimination half-life ($t_{1/2}$) followed by a longer $t_{1/2}$ of 16–20 hours for etamicastat doses ≥100 mg. In a food interaction study in healthy subjects, the absorption of etamicastat was delayed by the presence of food, but no significant effect was observed in the extent of systemic exposure to etamicastat, as assessed by the area under the plasma concentration-time curve (AUC).[22]

N-Acetyltransferase (NAT) is one of the major hepatic phase II enzymes involved in drug metabolism. Humans express two functional NAT isoforms: NAT1 and NAT2.[23] Data from a previous single-dose study showed that N-acetylation by NAT2 appeared to be the main metabolic pathway of etamicastat.[21] Plasma exposure to the N-acetylated metabolite (BIA 5-961), which was shown to be inactive in *in vitro* and *in vivo* preclinical experiments (e.g. following intraperitoneal administration in mice and rats [BIAL unpublished observation]), increased in a dose proportional manner.

A high interindividual variability of pharmacokinetic parameters of etamicastat and BIA 5-961 was observed following single doses of etamicastat in healthy subjects.[21] Pharmacogenomic data showed that such variability is mainly dependent upon the NAT2 phenotype (rapid or slow acetylating ability).[21] Etamicastat was well tolerated at all the investigated dose levels. In the present work, we describe the results of a study aiming to evaluate the safety, tolerability, and pharmacokinetics of multiple-dose regimens of etamicastat in young healthy male volunteers.

Population and Methods

Study Design and Ethics Compliance

This was a phase I, double-blind, randomized, placebo-controlled, multiple-dose study investigating etamicastat oral doses of 25, 50, 100, 200, 400, and 600 mg administered once-daily for 10 days to sequential groups of young healthy male subjects. The study was conducted according to the principles of the Declaration of Helsinki and the Good Clinical Practice (ICH) guidelines. An Independent Ethics Committee (Comité de Protection des Personnes [CCP] Ouest VI, Brest, France) reviewed and approved the study protocol and the subject information. Written informed consent was obtained for each subject prior to enrolment in the study. Subjects were required to sign a specific pharmacogenetic informed consent form to allow NAT1 and NAT2 genotyping and phenotyping.

Participants

Healthy male volunteers aged 18–45 years were eligible for this study. Health status was confirmed by medical history, physical examination and vital signs, electrocardiogram (ECG), and laboratory workup, including hematology, plasma biochemistry, urinalysis, and hepatitis B, hepatitis C, and HIV serology performed at screening.

Volunteers were excluded from participation if they (i) had any clinically relevant disorder; (ii) smoked more than 10 cigarettes per day; (iii) had a history of drug abuse or alcoholism within 1 year prior to admission; (iv) had an ECG corrected QT (QTc) interval reading at screening or enrolment >450 ms; (v) had positive serologic results for HIV antibodies, hepatitis B surface antigen, and/or hepatitis C virus antibodies; (vi) had positive results of urine drug screen (amphetamines, barbiturates, benzodiazepines, cannabinoids, cocaine, methadone, opiates, 3,4-methylenedioxymethamphetamine [ecstasy]) at screening and admission; (vii) had used any investigational drug within 90 days or prescription drug within 30 days prior to admission; (viii) had consumed any caffeine-containing products (e.g. coffee, tea, chocolate, or soda) in excess of six cups per day (or equivalent), grapefruit, grapefruit-containing products, or alcoholic beverages within 72 hours prior to admission; or (ix) had used any over-the-counter drugs including herbal supplements (except for the occasional use of acetaminophen [paracetamol], acetylsalicylic acid [aspirin] and vitamins within the recommended daily allowance) within 7 days prior to admission. No concomitant

medication was allowed during the study unless required to treat an AE.

Interventions

Participants underwent health status screening within 28 days before admission to the study. Following confirmation of eligibility, within each group of eight volunteers, two subjects were randomized to receive placebo and the remaining six subjects to receive etamicastat. Etamicastat doses of 25, 50, 100, 200, 400, and 600 mg were investigated in ascending order and the decision to proceed to the next higher dose was made on the basis of tolerability assessments of the previous dose level. Due to the occurrence of a maculopapular rash of probable infectious origin in three of eight subjects in group 4 (placebo/etamicastat 200 mg), this group was repeated to rule out drug hypersensitivity before escalating to the next dose level.

Subjects were admitted to the research facility from day −2 (2 days before the first dose of investigational product) until approximately 72 hours after the last dosing. Subjects were then discharged from the research facility and instructed to return for a follow-up visit 4–7 days later. At admission, medical history, physical examination, and laboratory safety tests were updated. Supine and standing blood pressure and heart rate measurements and computerized 12-lead ECG recordings were obtained at frequent intervals during admission. Clinical laboratory tests were repeated on days 4 and 10, and 72 hours after the last dose (discharge). At the follow-up visit, the medical history was updated and clinical laboratory safety tests performed.

Etamicastat was administered once daily, in the morning (between 7:30am and 9:00am), for 10 days (days 1–10). Capsules of etamicastat or placebo identical in appearance were used. The doses of investigational product were administered with 250 mL of water following an overnight fasting of at least 8 hours. Subjects remained fasted for at least 4 hours post-dose on day 1 and day 10, and for at least 2 hours post-dose on the remaining treatment days.

A diet low in monoamines was adopted. The following food was not permitted from 3 days prior first dosing through 72 hours after last dosing:

- drinks – chocolate, cocoa, beer, alcohol, orange juice;
- fruits – banana, pineapple, orange, grapefruit, grapes, strawberries;
- dry fruits/nuts – almonds, peanuts, pistachios, prunes;
- vegetables – tomatoes, eggplants, broad beans;
- meat and fish – smoked fish, liver, brains; and
- cheese and vanilla-flavored desserts.

A normal sodium diet (NaCl 7 g/day) was provided.

Safety Assessments

Safety assessments consisted of AE monitoring, physical examinations, vital signs measurements, digital 12-lead ECG recordings, and clinical laboratory tests. During admission, blood pressure and heart rate measurements using a Dinamap® (GE Healthcare, Chalfont St. Giles, UK) blood pressure monitor using the oscillometric method and 12-lead digital ECG recordings were taken in the supine position, after resting for at least 10 minutes, at the following times: (i) day −1 (the day prior to first dosing) – time 0 (24 hours before first dosing) and 1, 2, 3, 4, 6, 8, 10, 12, and 16 hours after; (ii) day 1 (day of first dosing) – pre-dose and 1, 2, 3, 4, 6, 8, 10, 12, and 16 hours post-dose; (iii) from day 2 to day 9 – pre-dose and 2 hours post-dose; and (iv) day 10 (day of last dosing) – pre-dose and 1, 2, 3, 4, 6, 8, 10, 12, 16, 24, 48, and 72 hours post-dose. Digital ECGs were performed in triplicate (with an interval of 5 minutes, with a difference of at least 1 minute between each of the three recordings). The investigator was responsible for providing the interpretation of all ECGs. The results included heart rate, PR interval, QRS interval, QT interval, and QTc interval, with comments on normality or abnormality. Two corrections of the QT interval were investigated: Fridericia's correction (QTcF) and Bazett's correction (QTcB). The primary method of correction was QTcF. A manual reading of the digital ECGs was conducted to assess prolongation of QT/QTc intervals.

All clinical AEs were monitored throughout the entire study period. Their severity (intensity) was categorized according a three-point scale (mild, moderate, and severe), and the causality (potential relationship to drug) was assessed by the investigator before breaking the blinding.

Pharmacokinetic Assessments and Bioanalytical Methods

Blood samples (3 mL) for determination of plasma concentrations of etamicastat and its metabolites were taken, by direct venepuncture or an intravenous catheter, into lithium-heparin tubes at the following times on days 1 and 10: pre-dose, then at 0.5, 1, 2, 3, 4, 5, 6, 8, 10, 12, and 16 hours post-dose. Further blood samples were taken at 24, 36, 48, and 72 hours post-last dose. Pre-dose blood samples were also taken on days 2–9. After collection, blood samples were centrifuged at approximately $1500\,g$ for 10 minutes at 4°C. The resulting plasma was then separated into four aliquots of 250 μL and stored at −80°C until required for analysis. Urine samples for pharmacokinetic assessments were collected at pre-dose and over the intervals 0–4, 4–8, 8–12, and 12–24 hours post-dose on day 1, and at pre-dose and over the intervals 0–4, 4–8, 8–12, 12–24, 24–36, 36–48, 48–60, and 60–72 hours after the last dose (day 10). Four aliquots of 500 μL were prepared and stored at −80°C until required for analysis.

For the preparation of calibration samples, etamicastat and BIA 5-961 were dissolved in methanol to a final concentration of 250 μg/mL (method in plasma) or 1250 μg/mL (method in urine). For the preparation of quality control (QC) samples, a second set of stock solutions was prepared. For calibration and QC samples, working solutions in methanol were added to plasma using a ratio of 2/98 (v/v). For the preparation of the internal standard (ISTD) solution, reference standard (BIA 5-1058; molecular formula $C_{21}H_{21}F_2N_3OS$) was dissolved in methanol to a concentration of 1000 μg/mL. Further dilutions to a final concentration of 555 ng/mL were done using methanol.

Plasma samples were vortexed and centrifuged for 20 minutes at approximately $3362\,g$ after un-

assisted thawing at room temperature. The method involved the addition of 300 μL of acetonitrile/ethanol (50/50, v/v) containing 555 ng/mL of ISTD and 300 μL of acetonitrile to 100 μL of plasma. After protein precipitation at room temperature, samples were centrifuged for 20 minutes at approximately $2773\,g$ and 8°C. The supernatant was transferred into a deep well plate. An aliquot of 10 μL of the supernatant was injected onto the liquid chromatography coupled to mass spectrometric detection (LC-MS/MS) system. The analysis was done by separation with an injection volume of 10 μL (10 μL in a 10 μL sample pool) in reversed phase chromatography (column temperature 50°C) followed by mass detection spectrometry.

Urine samples were vortexed and centrifuged for 20 minutes at approximately $3362\,g$ after unassisted thawing at room temperature. To an aliquot of 20 μL urine, 80 μL lithium-heparin plasma was added and precipitated by 300 μL of ISTD solution. After protein precipitation and short mixing at room temperature, samples were centrifuged for 20 minutes at approximately $2773\,g$ and 8°C. The supernatant (250 μL) was transferred into an ultrafiltration filter plate and centrifuged for about 2 hours at approximately $2773\,g$ and 20°C. An aliquot of 5 μL of the supernatant was injected onto the LC-MS/MS system. The analysis was done by separation with an injection volume of 5 μL (5 μL in a 10 μL sample pool) in reversed phase chromatography (column temperature 50°C) followed by mass detection spectrometry.

Plasma and urine concentrations of etamicastat and its metabolite BIA 5-961 were determined using validated methods consisting of LC-MS/MS. The LC-MS/MS system consisted of a Rheos 2200 pump (Flux Instruments, Basel, Switzerland), a PAL autosampler (CTC Analytics AG, Zurich, Switzerland) and a TSQ Quantum triple-stage quadrupole mass spectrometer (Thermo Fisher Scientific, San Jose, CA, USA). Separation was achieved using a Luna C8 (2), 2.0×5.0 mm, 5 μm analytical column (Phenomenex, Torrance, CA, USA), a C8, 2.0×5.0 mm, 5 μm precolumn filter (Phenomenex, Torrance, CA, USA), a Gecko 2000 column heater (CIL, Courbevoise, France) at 50°C and a mobile phase

(0.5 mL/min) consisting of water containing 1.0% formic acid (phase A) and acetonitrile containing 1.0% formic acid (phase B). The MS detector was operated in positive ion mode.

Calibration curves – over the nominal concentration ranges of 5–5000 ng/mL for plasma assay or 25–25000 ng/mL for urine assay – and a set of QC samples (triplicates over three concentration levels) were analyzed with each batch of study samples. The QC samples were used to monitor the performance of the assay. In plasma, the data for the QC samples showed that the overall imprecision of the method, measured by the coefficient of variation, was ≤6.0% for etamicastat and ≤6.9% for BIA 5-961. The overall accuracy (as the proportion of the determined value in relation to the true or nominal value) ranged from 93.5% to 98.0% for etamicastat, and 93.5% to 98.0% for BIA 5-961. In urine, the overall imprecision was ≤10.0% for etamicastat and ≤7.8% for BIA 5-961, and the overall accuracy ranged from 99.5% to 99.7% for etamicastat and 97.2% to 100.8% for BIA 5-961. The lower limit of quantification of the assay (LLOQ) was 5 ng/mL in plasma and 25 ng/mL in urine. Etamicastat, BIA 5-961 and reference standard (BIA 5-1058) were supplied by BIAL (Laboratory of Chemistry, S. Mamede do Coronado, Portugal).

Pharmacodynamic Assessments

Urine for the assessment of urinary levels of norepinephrine, dopamine, epinephrine and homovanillic acid was collected on day 1 at pre-dose and 0–4, 4–8, 8–12, and 12–24 hours post-dose, and on day 10 at pre-dose and 0–4, 4–8, 8–12, 12–24, 24–36, 36–48, 48–60, and 60–72 hours post-dose. After collection, 3 mL aliquots of acidified urine were prepared and stored at −80°C. The determination of urine levels of catecholamines was carried out by high performance LC with electrochemical detection using a validated method. The LC electrochemical detection system consisted of a pump (Gilson 302) and a stainless steel 5-μm ODS2 column (Biophase; Bioanalytical Systems, West Lafayette, IN, USA), measuring 250 mm in length and 4.6 mm in diameter; samples were injected by means of an automatic sample injector (Triathlon-Spark Holland, Emmen, the Netherlands). The mobile phase was a degassed solution consisting of citric acid (0.1 mmol/L), sodium octylsulfate (0.5 mmol/L), sodium acetate (0.1 mol/L), Na2EDTA (0.17 mmol/L), dibutylamine (1 mmol/L), and methanol (10% v/v), adjusted to pH 3.5 with perchloric acid (2 mol/L), and pumped at a rate of 1.0 mL/min. The detection was carried out electrochemically with a glassy carbon electrode, an Ag/AgCl reference electrode, and an amperometric detector (Intro-Antec Leyden for norepinephrine, dopamine, epinephrine, and Gilson 141 for homovanillic acid); the detector cell was operated at 0.75 V. The lower limits of detection of norepinephrine, dopamine, epinephrine, and homovanillic acid were 22, 19, 37, and 500 ng/L, respectively.

Determination of Subjects NAT1 and NAT2 Genotypes

The *NAT1* and *NAT2* genes were genotyped for all subjects participating in the current study. Genomic DNA was extracted from total venous blood. For NAT1, eight single nucleotide polymorphisms (SNPs) were analyzed (190 C > T, 445 G > A, 459 G > A, 559 C > T, 560 G > A, 640 T > G, 1088 T > A, and 1095 C > A) and the corresponding genotypes determined. Associated genotypes were defined according to the literature.[17,24] Subjects were classified NAT1 rapid (fast) acetylators if they carried the NAT1*10 (except NAT1*10/*14) or NAT1*11 alleles, normal acetylators if they carried the NAT1*4 or NAT1*3 alleles or the NAT1*10/*14 genotype, and poor (slow) acetylators if they carried the NAT1*14 (except NAT1*10/*14) or NAT1*17 alleles. For NAT2, four coding SNPs were analyzed (191 G > A, 341 T > C, 590 G > A, and 857 G > A) and the corresponding genotypes determined. Associated genotypes were defined according to the literature.[25,26] The NAT2*4 allele encodes for a fully active enzyme and is considered the wild type (rapid acetylator) allele. Subjects were classified NAT2 rapid acetylators if they carried the NAT2*4/*4 allele or the NAT2*4/*5, NAT2*4/*6 or NAT2*4/*7 genotype, and poor acetylators if they carried the NAT2*5/*5, NAT2*6/*6 or NAT2*7/*7 alleles,

Table I. Main demographic details by treatment dose group

Parameter	Placebo (n = 15)	Etamicastat dose group					
		25 mg (n = 6)	50 mg (n = 6)	100 mg (n = 6)	200 mg (n = 12)	400 mg (n = 6)	600 mg (n = 6)
Mean age ± SD [y] (range)	30.6 ± 6.4 (18–40)	26.5 ± 6.7 (21–39)	36.0 ± 4.5 (31–43)	31.5 ± 4.9 (27–39)	31.3 ± 7.9 (20–45)	31.0 ± 8.7 (22–44)	28.8 ± 6.5 (23–40)
Mean height ± SD [cm] (range)	175.8 ± 8.0 (158–186)	175.7 ± 3.3 (173–182)	175.2 ± 5.2 (170–183)	176.8 ± 9.3 (168–191)	177.8 ± 6.9 (170–193)	177.3 ± 6.7 (168–187)	177.2 ± 5.6 (167–183)
Mean BMI ± SD [kg/m^2] (range)	22.5 ± 2.9 (19.0–29.4)	23.0 ± 2.8 (19.4–26.4)	23.1 ± 1.3 (20.8–24.7)	22.5 ± 2.1 (20.3–25.7)	24.7 ± 2.8 (20.0–28.4)	23.9 ± 1.5 (22.0–26.1)	22.3 ± 2.4 (19.4–25.5)
Race [n (%)]							
Caucasian	13 (86.7)	6 (100)	5 (83.3)	4 (66.7)	6 (50)	5 (83.3)	6 (100)
other	2 (13.3)	0	1 (16.7)	2 (33.3)	6 (50)	1 (16.7)	0

BMI = body mass index; **SD** = standard deviation.

or the NAT2*5/*6, NAT2*5/*7 or NAT2*6/*7 genotype.

Analyses

For this exploratory study, no formal sample size calculation was performed. Eight subjects per treatment (six subjects assigned to active treat- ment and two subjects assigned to placebo) are a standard number of subjects per treatment group in early clinical trials in healthy subjects and were considered compatible with a reasonable clinical interpretation and descriptive statistics.

AEs were tabulated and summarized accord- ing to the Medical Dictionary for Regulatory Activities (MedDRA). Blood pressure, heart rate,

Table II. Number (%) of subjects reporting any treatment-emergent adverse event

MedDRA preferred term	Placebo (n = 15)	Etamicastat dose group					
		25 mg (n = 6)	50 mg (n = 6)	100 mg (n = 6)	200 mg (n = 12)	400 mg (n = 6)	600 mg (n = 6)
Conjunctivitis	1 (6.7)						
Diarrhea							5 (83.3)
Flatulence	1 (6.7)						1 (16.7)
Nausea					1 (8.3)		1 (16.7)
Vomiting					1 (8.3)		
Pyrexia					1 (8.3)		
Ear infection					1 (8.3)		
Nasopharyngitis		1 (16.7)		1 (16.7)			
Rhinitis	1 (6.7)	1 (16.7)		2 (33.3)			
Pain in extremity					1 (8.3)		
Dizziness				1 (16.7)			
Headache	1 (6.7)				3 (25.0)	1 (16.7)	
Syncope, vasovagal					1 (8.3)		
Pruritus					1 (8.3)		
Rash papular					3 (25.0)		
Urticaria							1 (16.7)
Total[a]	**3 (20.0)**	**2 (33.3)**	**0**	**4 (66.7)**	**5 (41.7)**	**1 (16.7)**	**6 (100)**

a   Some subjects reported more than one adverse event.

MedDRA = Medical Dictionary for Regulatory Activities.

vital signs, and clinical laboratory data were summarized using descriptive statistics. For the laboratory safety data, out-of-range values were flagged in the data listings and a list of abnormal values was presented. Any clinically significant abnormality was considered as an AE.

The following parameters were calculated for supine systolic (SBP) and diastolic (DBP) blood pressure, ECG-measured heart rate and QTcF: maximum observed effect (E_{max}), time to E_{max} (t_{Emax}) and area under the effect-time curve over 24 hours (AUC_{EC}). The parameters were summarized using descriptive statistics. E_{max} and ΔAUC_{EC} (difference in AUC_{EC} in relation to AUC_{EC} on day −1) were compared at day 1 and day 10 between dose groups by using a one-way analysis of variance with dose group as the fixed effect. If global treatment effect was significant, a multiple-comparisons Dunnett's test was performed to compare each dose group versus placebo. E_{max} at day 10 was tested versus E_{max} at day 1 using a paired Student's t-test in each dose group.

The following pharmacokinetic parameters for etamicastat and its metabolites were derived by non-compartmental analysis from the individual plasma concentration-time profiles: time of occurrence of C_{max} (t_{max}); AUC from time zero to the last sampling time at which concentrations were at or above the limit of quantification (AUC_t) and AUC over 24 hours (AUC_{24}), both calculated by the trapezoidal rule, and AUC from time zero to infinity (AUC_∞), calculated from $AUC_t + (C_{last}/\lambda_z)$, where C_{last} is the last quantifiable concentration; the apparent terminal rate constant (λ_z) and corresponding terminal $t_{1/2}$ calculated from $\ln 2/\lambda_z$; observed accumulation ratio (R_O), calculated from AUC_{24} (day 10)/AUC_{24} (day 1); and theoretical accumulation ratio (R_T), calculated from $1/(1 − e^{-\lambda_z \bullet \tau})$, where τ is the dosing interval. The volume of distribution (V/F) and the renal clearance of drug from plasma (CL_R), calculated from Ae/AUC_∞, where Ae is the cumulative amount of drug excreted in the urine, were also derived. Nominal sampling times were used for the pharmacokinetic analysis. Plasma concentrations below the limit of quantification of the assay were taken as zero for all calculations. All calculations were made using raw data.

Summary statistics of all data were reported, as appropriate, using the geometric mean, arithmetic mean, standard deviation (SD), coefficient of variation (CV%), standard error of the mean (SEM), median, and range (minimum and maximum). A nonlinear power model was used to assess dose proportionality of C_{max} and AUC_{24}. The relationship is written as a power function: parameter $= a \bullet dose^b$, where a is a constant, b is the proportionality constant, and parameter is C_{max} or AUC_{24}. Linearization of this relationship gives: $\log$ parameter $= \log a + \log dose \bullet b$. The relationship is dose proportional when $b = 1$. The exponent of the power function, with 95% confidence intervals (95% CI), was fitted to the in-
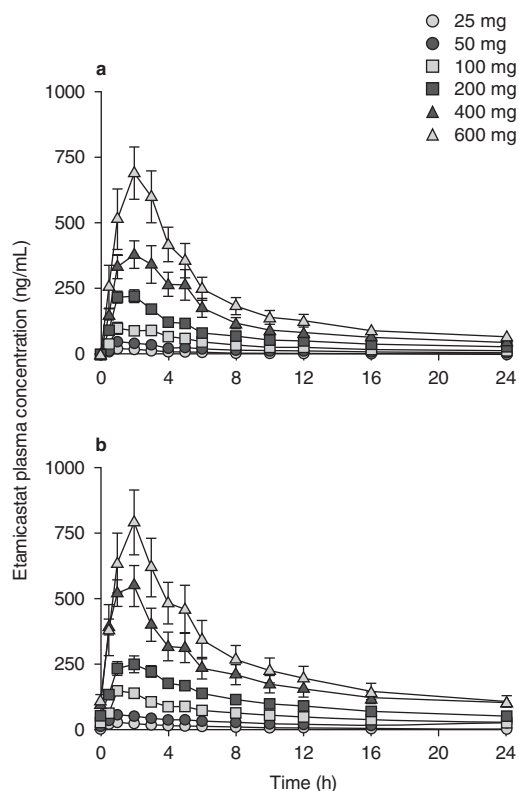

Fig. 2. Plasma concentration (mean ± SEM) vs time profiles of etamicastat following single (day 1 [**a**]) and repeated (day 10 [**b**]) oral once-daily administration of etamicastat in the dose range 25–600 mg (etamicastat 200 mg: n = 12; other dose groups: n = 6 per dose group).

Table III. Pharmacokinetic parameters of etamicastat following the first dose (day 1) and the final dose (day 10) of repeated administration of etamicastat to healthy subjects[a]

Parameter	Dose	Etamicastat dose group					
		25 mg (n=6)	50 mg (n=6)	100 mg (n=6)	200 mg (n=12)	400 mg (n=6)	600 mg (n=6)
C_{max} (ng/mL)	First	23±7	56±17	128±40	251±72	468±99	722±265
	Last	32±12	65±28	153±40	275±87	596±154	866±280
t_{max} (h)	First	1.5 (0.5–2.0)	1.0 (1.0–3.0)	1.5 (1.0–3.0)	1.5 (1.0–4.0)	3.0 (1.0–5.0)	2.0 (1.0–3.0)
	Last	1.0 (0.5–2.0)	1.0 (1.0–2.0)	1.0 (1.0–2.0)	2.0 (1.0–3.0)	2.0 (1.0–2.0)	2.0 (1.0–2.0)
AUC_t (ng • h/mL)	First	147±108	399±157	862±283	1746±521	3095±1056	4804±1829
	Last	397±394	1018±719	2302±867	4125±1437	7466±2865	9126±4195
AUC_{24} (ng • h/mL)	First	165±99	405±150	863±284	1748±522	3097±1057	4806±1830
	Last	307±183	664±328	1460±4733	2662±886	4957±1860	6484±2848
AUC_∞ (ng • h/mL)	Last	562±491	1334±776	2740±1168	4601±1597	8094±3145	9826±4605
$t_{1/2}$ (h)	First	8.7±7.6	13.7±3.2	13.0±3.0	14.7±1.9	13.7±2.1	13.0±3.3
	Last	18.1±11.6	25.6±6.2	25.7±3.6	22.2±2.9	20.3±2.6	20.3±1.6
R_T	Last	1.7±0.7	2.1±0.4	2.1±0.2	1.9±0.2	1.8±0.1	1.8±0.1
R_O	Last	1.9±0.3	1.6±0.3	1.7±0.1	1.6±0.2	1.6±0.1	1.3±0.2
CL_R (L/h)	Last	15.2±5.5	11.9±1.9	14.6±2.1	16.0±3.1	13.2±2.0	15.2±1.7
V/F (L)	Last	343±148	440±125	540±119	517±135	393±107	445±40

a   Data are given as mean±SD (median and range for t_{max}).

AUC_{24} = area under the plasma concentration-time curve from time 0 to 24 hours; AUC_t = area under the plasma concentration-time curve from time zero to time t; AUC_∞ = area under the plasma concentration-time curve from time zero to infinity; C_{max} = maximum concentration; CL_R = renal clearance; R_O = observed accumulation ratio; R_T = theoretical accumulation ratio; $t_{1/2}$ = elimination half-life; t_{max} = time to maximum concentration; V/F = volume of distribution.

dividual C_{max} and AUC_{24}. For determination of time to steady-state of plasma concentrations, a pairwise comparison for pre-dose concentrations between day 2 and day 10 was performed by dose group using a paired Student's t-test. Values at each period and cumulated values of urinary catecholamines were summarized using descriptive statistics by dose group.

The pharmacokinetic parameters were calculated with WinNonlin (version 5.0.1, Pharsight Corporation, Mountain View, CA, USA). Statistical calculations were performed with SAS® Software (version 8.2, SAS Institute Inc., Cary, NC, USA). The α risk p-values reported are two-sided and the statistical significance nominal limit was set to 0.05.

Results

Study Population

Of 94 participants screened, 37 were deemed ineligible because they met exclusion criteria or failed to show up at the start of the study. A total of 57 male volunteers, aged 18–45 (median = 30) years were enrolled in the study. The demographic characteristics of the study population by treatment group are summarized in table I. One subject administered placebo withdrew his consent and was replaced. No relevant differences in demographic characteristics were found between treatment groups.

Safety Results

During the course of the study, a total of 32 treatment-emergent AEs were reported by 21 subjects (table II). The most frequent treatment-emergent AE was diarrhea. There were no serious AEs. All AEs were mild to moderate in intensity. An outbreak of maculopapular rash occurred in three subjects administered etamicastat 200 mg. Subjects had elevated antistreptolysin O titers, and antibiotics were prescribed. In the three subjects, histamine, tryptase C3, C4, and C1q were normal, there was no hypereosinophilia and

there was a moderate increase of C-reactive pro-
tein. Although an infection appeared to be a
plausible cause, drug hypersensitivity could not
be ruled out. Thus, a decision was made to repeat
the placebo/etamicastat 200 mg group before es-
calating to the subsequent dose level (placebo/
etamicastat 400 mg).

Clinical laboratory evaluations indicated no
clinically significant abnormality. There was no
statistically significant change in QTcF. There
was no evidence for prolongation of the QT in-
terval. All QTcF values remained below the limit
of 0.450 seconds. There were no apparent trends
in ECG parameters over time, and there were no
apparent differences between dose groups.

Pharmacokinetic Results

Due to the occurrence of rash, the tenth dose
was skipped in two subjects administered etami-
castat 200 mg, and the pharmacokinetic profiling
planned for day 10 was performed after day 9
dosing. All other subjects of the pharmacokinetic
population completed the treatment period as
planned.

Mean etamicastat plasma concentration-time
profiles following the first and last doses of
etamicastat are displayed in figure 2, and the
corresponding pharmacokinetic parameters are
presented in table III. As expected, etamicastat
was shown to be metabolized to BIA 5-961.
Plasma concentration-time profiles of BIA 5-961
are displayed in figure 3, and the corresponding
mean pharmacokinetic parameters are presented
in table IV. Figure 4 displays the mean trough
(pre-dose) plasma concentrations of etamicastat
and BIA 5-961 during the treatment period.

Etamicastat C_{max} was reached 1–3 hours post-
dose and declined thereafter, with an approx-
imate mean apparent $t_{1/2}$ of 9–15 hours following
the first dose of etamicastat and 18–26 hours
following repeated administration. C_{max} of BIA
5-961 was reached 2–4 hours post-dose and de-
clined thereafter, with a mean $t_{1/2}$ of 4–8 hours
following the first dose of etamicastat and 7–23
hours following repeated administration. Sys-
temic exposure to etamicastat and BIA 5-961 in-
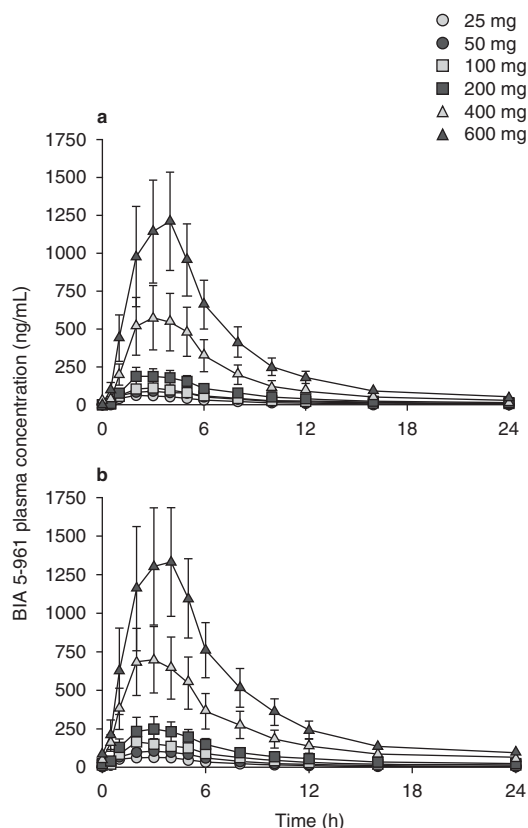creased with increasing doses on both day 1 and

Fig. 3. Plasma concentration (mean ± SEM) vs time profiles of BIA
5-961 following single (day 1 [**a**]) and repeated (day 10 [**b**]) oral once-
daily administration of etamicastat in the dose range 25–600 mg
(etamicastat 200 mg: n = 12; other dose groups: n = 6 per dose
group).

day 10 (table V and figure 5). The apparent dose-
proportional increase in systemic exposure was
confirmed by the statistical analysis using an ex-
ponential regression model. Also, the comparison
of etamicastat and BIA 5-961 pharmacokinetic
parameters after log-transformation and dose-
normalization showed that C_{max}, AUC_t and
AUC_{24} following the first dose of etamicastat,
and C_{max}, AUC_t, AUC_{24}, and AUC_{∞} following
the last dose were not significantly different be-
tween dose groups.

The R_O indicating the extent to which etami-
castat accumulated relative to the first dose
ranged from 1.3 to 1.9. This ratio was lower than
R_T, which ranged from 1.7 to 2.1. This is in

accordance with the apparent $t_{1/2}$ of 9–15 hours following etamicastat first dose and 18–26 hours following etamicastat last dose. The R_O for BIA 5-961 was also relatively low, ranging from 1.3 to 1.6.

The one-way analysis of variance using trough (pre-dose) etamicastat plasma concentrations from day 2 to day 10 showed that steady state was reached at day 9 (200 mg), day 8 (400 mg) and before day 6 for the remaining dose groups. For BIA 5-961, steady state was reached at day 9 (200 and 600 mg), day 8 (25 mg), and before day 6 for the remaining groups.

The cumulative amounts of etamicastat and BIA 5-961 recovered in urine are displayed in figure 6. Urine drug recovery occurred predominantly over the first 12 hours after etamicastat administration. The excretion rate of etamicastat ranged from 0.3 ± 0.1 mg/h (25 mg) to 8.5 ± 3.2 mg/h (600 mg) following the first dose and from 0.4 ± 0.2 mg/h (25 mg) to 10.6 ± 2.8 mg/h (600 mg) following the last dose. For BIA 5-961, the excretion rate ranged from 0.7 ± 0.6 mg/h (25 mg) to

10.4 ± 6.9 mg/h (600 mg) and from 0.753 ± 0.560 mg/h (25 mg) to 15.2 ± 9.8 mg/h (600 mg), respectively. Renal clearance was relatively similar at the different dose levels, ranging from 11.9 to 16.0 L/h for etamicastat and from 11.3 to 14.8 L/h for BIA 5-961.

The interindividual variability in the pharmacokinetic parameters was high for both etamicastat and BIA 5-961, with mean CV close to 100% in some parameters. This variability is due to the different subjects' NAT2 phenotype (fast/slow acetylating ability), which affects the rate of biotransformation of etamicastat into BIA 5-961. Table VI presents the distribution of the different subjects' NAT1 and NAT2 phenotypes within each etamicastat dose group. No relevant effect of NAT1 phenotype on etamicastat pharmacokinetics was apparent. Etamicastat pharmacokinetics was shown to be markedly affected by the NAT2 status (figure 7). The extent of systemic exposure to etamicastat in NAT2 slow acetylators was 1.5–6.7 times higher than in NAT2 fast acetylators. BIA 5-961 exposure was

Table IV. Pharmacokinetic parameters of BIA 5-961 following the first dose (day 1) and the final dose (day 10) of repeated administration of etamicastat to healthy subjects[a]

Parameter	Dose	Etamicastat dose group					
		25 mg (n=6)	50 mg (n=6)	100 mg (n=6)	200 mg (n=12)	400 mg (n=6)	600 mg (n=6)
C_{max} (ng/mL)	First	65±55	105±67	119±47	209±212	625±516	1286±865
	Last	66±45	114±73	166±103	264±279	731±545	1420±989
t_{max} (h)	First	2.5 (1.0–3.0)	3.0 (1.0–5.0)	2.5 (2.0–4.0)	2.0 (2.0–5.0)	3.5 (2.0–5.0)	3.5 (2.0–5.0)
	Last	3.0 (2.0–4.0)	2.5 (2.0–4.0)	2.0 (2.0–4.0)	4.0 (2.0–5.0)	2.5 (2.0–3.0)	3.5 (2.0–4.0)
AUC_t (ng·h/mL)	First	403±352	685±389	838±328	1518±1239	4111±3311	8291±5172
	Last	548±315	924±461	1634±768	2699±2011	7017±5313	12203±7229
AUC_{24} (ng·h/mL)	First	427±340	694±377	838±329	1519±1240	4113±3312	8293±5173
	Last	513±327	820±398	1286±582	2070±1707	5603±4239	10297±6560
AUC_∞ (ng·h/mL)	Last	615±355	1069±524	1853±905	2926±2032	7356±5477	12516±7277
$t_{1/2}$ (h)	First	3.9±1.1	5.2±1.6	6.4±0.8	7.8±1.1	7.5±1.0	7.0±1.6
	Last	6.7±3.0	15.8±7.1	19.5±8.9	22.5±4.4	18.3±2.3	17.0±2.5
R_T	Last	1.1±0.1	1.6±0.4	1.8±0.5	1.9±0.3	1.77±0.1	1.6±0.1
R_O	Last	1.6±0.3	1.3±0.5	1.5±0.2	1.3±0.2	1.4±0.2	1.3±0.1
CL_R (L/h)	Last	14.8±4.4	11.6±1.1	12.2±3.4	12.7±3.3	11.3±2.0	13.6±1.8

a Data are given as mean ± SD (median and range for t_{max}).

AUC_{24} = area under the plasma concentration-time curve from time 0 to 24 hours; AUC_t = area under the plasma concentration-time curve from time zero to time t; AUC_∞ = area under the plasma concentration-time curve from time zero to infinity; C_{max} = maximum concentration; CL_R = renal clearance; R_O = observed accumulation ratio; R_T = theoretical accumulation ratio; $t_{1/2}$ = elimination half-life; t_{max} = time to maximum concentration.
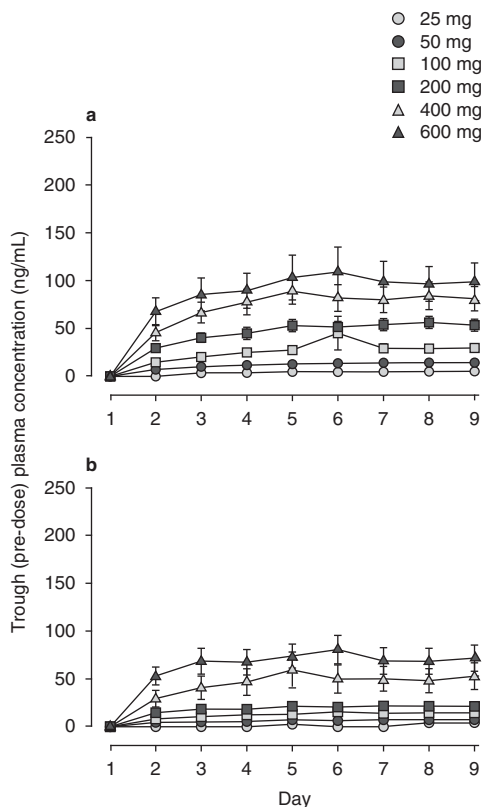
Fig. 4. Trough (pre-dose) plasma concentrations (mean ± SEM) of (**a**) etamicastat and (**b**) BIA 5-961 during oral once-daily administration of etamicastat in the dose range 25–600 mg (etamicastat 200 mg: n = 12; other dose groups: n = 6 per dose group).

1.5–3.5 times higher in rapid NAT2 acetylators. These results explain the high variability observed in the main etamicastat and BIA 5-961 pharmacokinetic parameters.

Pharmacodynamic Results

The cumulative amounts of norepinephrine recovered in urine over a 24-hour interval post-dose on day 1 (first dose) and day 10 (last dose) are displayed in figure 8. No relevant change from day 1 to day 10 was observed in subjects administered placebo ($29.6 \pm 12.4\,\mu g$ on day 10 vs $33.1 \pm 10.5\,\mu g$ on day 1). Following repeated etamicastat administration, urinary excretion of norepinephrine decreased, which is consistent with the claimed DβH inhibitory effect of etamicastat. In relation to the day 1 value, urinary excretion of norepinephrine at day 10 decreased by 24%, 18%, 36%, 34%, 32%, and 34% following repeated administration of etamicastat 25, 50, 100, 200, 400, and 600 mg, respectively. No relevant changes were found in the urinary excretion of homovanillic acid, epinephrine and dopamine in the different dose groups.

Due to the expected pharmacologic effect of etamicastat, supine SBP, DBP, and ECG-measured heart rate (HR) measurements were considered as pharmacodynamic variables. Following the first dose of placebo or etamicastat 25, 50, 100, 200, 400, and 600 mg, no significant changes in SBP, DBP, or HR were observed. Following repeated dosing, SBP and HR did not significantly change at any dose levels, but a significant decrease in DBP occurred with etamicastat 100 mg and 200 mg doses. In relation to day 1, DBP E_{max} and AUC_{EC} decreased by 5.6 mmHg ($p < 0.01$) and 223 mmHg • h ($p < 0.05$) following etamicastat 100 mg and by 5.9 mmHg ($p < 0.05$) and 193 mmHg • h ($p < 0.05$) following etamicastat 200 mg, respectively.
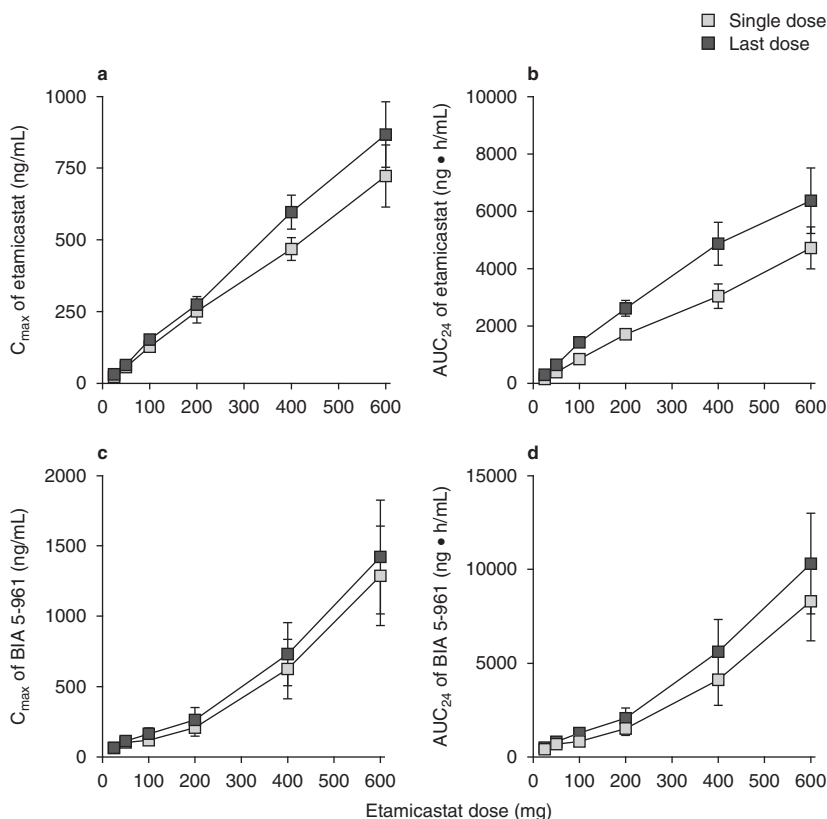
Table V. Proportionality of maximum concentration (C_{max}) and area under the plasma concentration-time curve from time 0 to 24 hours (AUC_{24}) of etamicastat and BIA 5-961 following the first dose (day 1) and the final dose (day 10) of repeated administration of etamicastat to healthy subjects

Etamicastat dose (mg)	Fold increase in dose	Etamicastat				BIA 5-961			
		fold increase in C_{max}		fold increase in AUC_{24}		fold increase in C_{max}		fold increase in AUC_{24}	
		first dose	last dose	first dose	last dose	first dose	last dose	first dose	last dose
25 (n = 6)	1	1	1	1	1	1	1	1	1
50 (n = 6)	2.0	2.4	2.0	2.5	2.2	1.6	1.7	1.6	1.6
100 (n = 6)	2.0	2.3	2.4	2.1	2.2	1.1	2.5	1.2	1.6
200 (n = 12)	2.0	2.0	1.8	2.0	1.8	1.7	1.6	1.8	1.6
400 (n = 6)	2.0	2.9	2.2	1.8	1.9	3.0	2.9	2.7	2.7
600 (n = 6)	1.5	1.5	1.5	1.6	1.3	2.1	1.9	2.0	1.8

Fig. 5. Maximum plasma concentrations (C_{max}) and area under the concentration-time curve from time 0 to 24 hours (AUC_{24}) values (mean ± SEM) of (**a** and **b**) etamicastat and (**c** and **d**) BIA 5-961 following single (day 1) and repeated (day 10) oral once-daily administration of etamicastat in the dose range 25–600 mg (etamicastat 200 mg: n = 12; other dose groups: n = 6 per dose group).

Discussion

This trial constituted the second study with etamicastat, a new DβH inhibitor, in humans. The objectives of the study were to evaluate the safety, tolerability, and pharmacokinetics of etamicastat in healthy male adults following repeated administration. The study followed a standard design commonly used in studies with identical purposes. Etamicastat administered orally at doses up to 600 mg once daily for 10 days appeared to be well tolerated. All AEs were mild to moderate in intensity. Most subjects (83%) in the 600 mg etamicastat group reported diarrhea as an AE. Diarrhea has also been reported at high etamicastat doses in preclinical studies. DβH gene variants that cause low plasma DβH have also been associated

with an increased propensity to report diarrhea.[27] The data show that a marked decrease of DβH activity can predispose to the occurrence of diarrhea. A papular rash of plausible infectious origin occurred in three subjects under treatment with etamicastat 200 mg. There were no serious AEs or clinically significant abnormalities in clinical laboratory tests, vital signs, or ECG parameters.

Consistent with the results from a previous study in which etamicastat single doses of 2–1200 mg were administered to young healthy subjects,[21] etamicastat was shown to be metabolized to its N-acetylated metabolite BIA 5-961 following single and repeated administration of 25–600 mg once-daily doses. C_{max} of etamicastat and BIA 5-961 were achieved at 1–3 hours and 2–4 hours after dosing, respectively. Thereafter, etamicastat
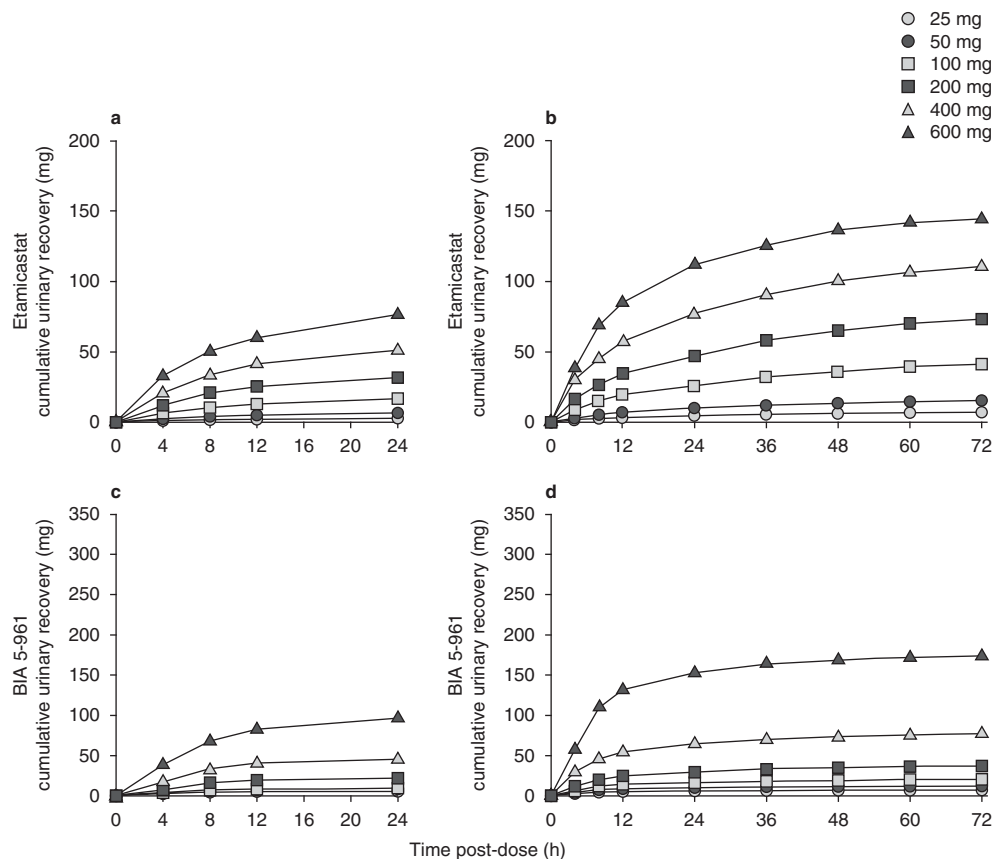
Fig. 6. Mean cumulative urinary excretion of etamicastat following single (day 1 [**a**]) and repeated (day 10 [**b**]), and BIA 5-961 following single (day 1 [**c**]) and repeated (day 10 [**d**]) oral once-daily administration of etamicastat in the dose range 25–600 mg (etamicastat 200 mg: n = 12; other dose groups: n = 6 per dose group).

plasma concentrations declined with a mean apparent $t_{1/2}$ of 9–15 hours following the first dose and 18–26 hours following the last dose. Estimated $t_{1/2}$ values for BIA 5-961 were slightly shorter than those of the parent drug. Both etamicastat and BIA 5-961 followed approximate linear pharmacokinetics over the dose range studied. The extent of systemic exposure to etamicastat increased in an approximately dose-proportional manner following single and repeated administration. Etamicastat accumulated in plasma following repeated administration. The mean R_O was 1.3–1.9 for etamicastat and 1.3–1.6 for BIA 5-961. The identical accumulation ratio of BIA 5-961 is consistent with the slightly shorter $t_{1/2}$ of BIA 5-961 compared with that of etamicastat.

Steady-state etamicastat and BIA 5-961 plasma concentrations were reached up to 9 days of etamicastat repeated administration.

Table VI. N-acetyltransferase (NAT)-1 and NAT2 phenotype by etamicastat dose group

Etamicastat dose (mg)	No. of subjects				
	NAT1			NAT2	
	slow	normal	fast	slow	fast
25 (n = 6)	0	4	2	3	3
50 (n = 6)	0	2	4	3	3
100 (n = 6)	0	4	2	3	3
200 (n = 12)	0	6	6	7	5
400 (n = 6)	1	4	1	1	5
600 (n = 6)	1	1	4	4	2

Approximately 45% of the etamicastat dose was recovered in urine collected up to 24 hours post-dose in the form of parent drug and N-acetylated metabolite. These data are consistent with those from a study aiming to characterize the absorption, distribution, metabolism, and excretion of etamicastat and metabolites following an oral dose of [14C]-labeled etamicastat (BIAL unpublished observation). Eleven days after administration, approximately 95% of the administered radioactivity had been excreted, two-thirds in urine and one-third in feces. Biotransformation of etamicastat occurred mainly via N-acetylation, forming BIA 5-961, the major metabolite. However, glucuronidation, oxidation, oxidative deamination, and de-sulfation were also involved.

Renal clearance of etamicastat from plasma following repeated oral administration of etamicastat was 11.9–16.0 L/h, which is higher than the glomerular filtration rate (7.6 L/h).

N-Acetylation is the major metabolic pathway for etamicastat. Humans express two functional NAT isoforms, NAT1 and NAT2. NAT1 has broad tissue distribution whereas NAT2 is more restricted, with higher levels of expression in the liver and red blood cells.[23] NAT exhibits different substrate specificities. Human NAT1 and NAT2 loci are highly polymorphic, with more than 25 alleles identified in each locus.[28,29] Within this polymorphic population, different phenotypes have been identified for both forms, NAT1 and NAT2. As an important metabolizing en-
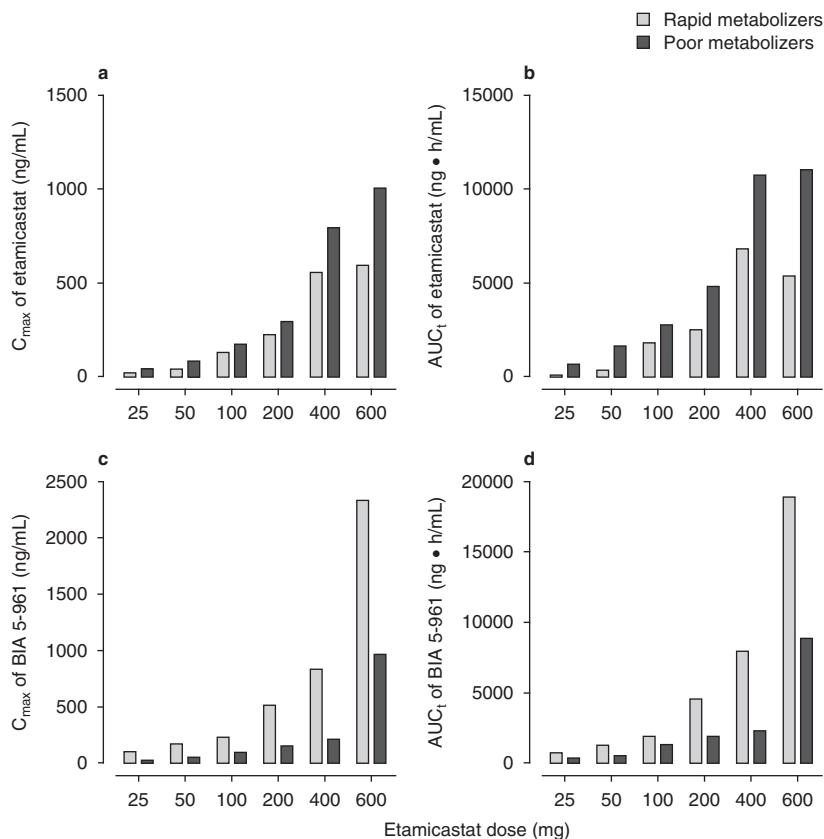

Fig. 7. Mean maximum plasma concentrations (C_{max}) and area under the concentration-time curve from time zero to the last sampling time at which concentrations were at or above the limit of quantification (AUC_t) of (**a** and **b**) etamicastat and (**c** and **d**) BIA 5-961 in rapid or poor N-acetyltransferase-2 acetylators following single (day 1) and repeated (day 10) oral once-daily administration of etamicastat in the dose range 25–600 mg (etamicastat 200 mg: n = 12; other dose groups: n = 6 per dose group).

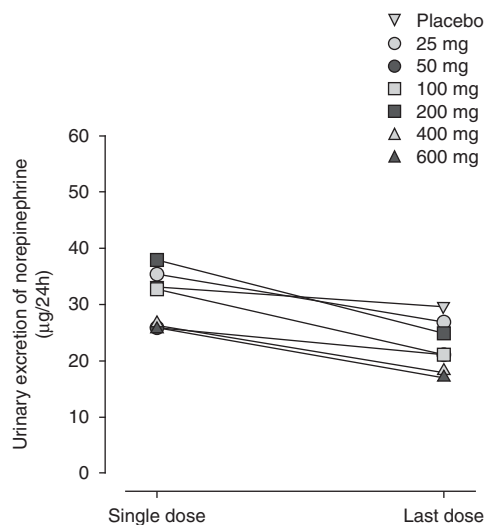**Fig. 8.** Mean cumulative urinary excretion of norepinephrine (nor-adrenaline) during a 24-hour period following the first (day 1) and last (day 10) doses of a once-daily regimen of placebo and etamicastat in the dose range 25–600 mg (placebo: n = 14; etamicastat 200 mg: n = 12; other dose groups: n = 6 per dose group).

zyme in humans, the polymorphism of human NAT expression, especially NAT2, raises concerns about drug-drug interactions during clinical use. In *in vitro* studies (BIAL unpublished observation), etamicastat N-acetylation in cytosolic fraction showed a good correlation with sulfamethazine NAT activity, a selective substrate to NAT2, but not with *p*-aminosalicylic acid, a selective substrate to NAT1, suggesting that etamicastat N-acetylation is predominantly driven by NAT2. These data are consistent with the results from the current study and those of a previous single-dose study in healthy subjects,[21] which showed no apparent major influence of NAT1 phenotype on etamicastat pharmacokinetics. However, the contribution of NAT1 to etamicastat metabolism should not be completely excluded, since NAT2 is expressed predominantly in liver whereas NAT1 is expressed ubiquitously.[23]

As demonstrated in figure 7, the extent of systemic exposure to etamicastat differed markedly between NAT2 poor and rapid acetylators. The extent of systemic exposure to etamicastat in NAT2 poor acetylators was 1.5–6.7 times higher than that observed in rapid acetylators and, inversely, NAT2 rapid acetylators showed a 1.5- to

3.5-fold increase in systemic exposure to the N-acetylated metabolite, confirming the involvement of NAT2 in the biotransformation of etamicastat into BIA 5-961. The magnitude of the effect of NAT2 phenotype on etamicastat pharmacokinetics is a strong argument for genotyping participants in further clinical trials with etamicastat. Effective clinical translation would establish etamicastat pharmacogenomics as a step toward personalized medicine for etamicastat.

Similar to a previous single-dose study,[21] the distribution of fast and slow NAT2 acetylators was not equal in all dose groups in the current study, and differences in the proportion of NAT2 fast/slow acetylators complicated the interpretation of the results (i.e. the dose-proportionality assessment) and were a cause of variability. To facilitate the interpretation of the results, stratification according to NAT2 phenotype could be considered in future studies with etamicastat.

DβH catalyzes the conversion of dopamine into norepinephrine. Consistently with the claimed DβH inhibitory effect by etamicastat, urinary excretion of norepinephrine decreased following repeated administration of etamicastat. For doses of etamicastat 100 mg and above, the decrease in norepinephrine urinary excretion was 32–36%. It should be taken into account, however, that the reported effect may be underestimated, since day 1 values do not correspond to a true baseline because they might have been influenced by the effect of the first dose of etamicastat. No relevant change from day 1 to day 10 was found in the other urinary catecholamines in the different dose groups.

A statistically significant decrease was found in supine DBP following repeated administration of etamicastat 100 mg and 200 mg. Although consistent with the claimed pharmacologic effect, this effect, however, should be interpreted with caution because no clear dose-response relationship was observed and the number of subjects in each dose group is small.

Conclusions

In conclusion, etamicastat was well tolerated and was shown to be metabolized to the

N-acetylated metabolite BIA 5-961, following single and multiple doses of etamicastat 25–600 mg. Etamicastat and its metabolite followed linear pharmacokinetics, and steady-state plasma concentrations were attained up to 9 days of dosing. Etamicastat pharmacokinetics are markedly affected by the NAT2 phenotype, and NAT2 genotyping could be a step toward personalized medicine for etamicastat. Consistently with the expected pharmacologic effect, urinary excretion of norepinephrine decreased following repeated administration of etamicastat.

Acknowledgments

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