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ORIGINAL ARTICLE

Pharmacokinetic characterization of a natural product–inspired novel MEK1 inhibitor E6201 in preclinical species

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Abstract
Purpose E6201 is a natural product–inspired novel inhibitor of mitogen-activated protein kinase/extracellular signal–regulated kinase kinase-1 (MEK1) and other kinases and is currently under development as an anticancer (parenteral administration) and antipsoriasis agent (topical application). In vitro and in vivo preclinical studies were performed to characterize the pharmacokinetics of E6201. Allometric scaling was applied to predict human pharmacokinetics of E6201.
Methods In vitro metabolism studies for CYP induction and CYP inhibition were conducted using human hepatocytes and microsomes, respectively. Metabolic stability using microsomes and protein-binding studies using pooled plasma were performed for mice, rats, dogs, and human. Pharmacokinetics of E6201 and its isomeric metabolite, ER-813010, in mice, rats, and dogs was determined following single IV administration of E6201 at three dose levels. Bioanalysis was performed using LC/MS/MS. Pharmacokinetic parameters were determined using non-compartmental analysis, and allometric scaling with a two-compartment model was used to predict E6201 pharmacokinetics in humans.
Results E6201 showed high plasma protein binding (>95%), and metabolic stability half-life ranged from 36 to 89 min across species. In vitro CYP inhibition (CYP1A2, 2C9, 2C19, 2D6, 2E1, and 3A) and CYP induction (CYP1A, 3A, 2C9, and 2C19) suggested no inhibitory or induction effect on the tested human CYPs up to 10 μM of E6201. Pharmacokinetics of E6201 in mice, rats, and dogs was characterized by mean clearance ranging from 3.45 to 10.92 L/h/kg, distribution volume ranging from 0.63 to 13.09 L/kg, and elimination half-life ranging from 0.4 to 1.6 h. ER-813010 was detected in all species with metabolite to parent exposure ratio (AUC_R) ranging from 3.1 to 33.4% and exhibited fast elimination (<3 h). The allometry predicted high clearance and large volume of distribution of E6201 in humans and was in general in good agreement with the observed first human subject pharmacokinetics.
Conclusions E6201 exhibited high clearance, high to moderate distribution, and fast elimination in preclinical species. In vitro results suggested that E6201 has low risk of drug–drug interactions due to CYP inhibition and induction in humans. In the first-in-man study, E6201 exhibited high clearance, which was well predicted by allometric scaling.

Keywords E6201 · Pharmacokinetics · MEK1 inhibitor · Protein binding · CYP induction · CYP inhibition

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Introduction

E6201, a totally synthetic analog of LL-Z1640-2 (in the zearelenone natural product family, [3]), is a novel inhibitor of mitogen-activated protein kinase/extracellular signal–regulated kinase kinase-1 (MEK1) and other kinases [11, 14]. It was found to be effective as an anti-inflammatory and antihyperproliferative agent both in vitro and in vivo [5]. It suppressed cancer cell growth with IC_{50} values in low nanomolar (nM) range [11, 14]. The chemical structures of E6201 and its isomeric metabolite, ER-813010, are shown in Fig. 1. Currently, E6201 is under development as an anticancer (parenteral administration) and antipsoriasis agent (topical application).

In cancer, the RAS/RAF/MEK/ERK signaling pathway has been viewed as a pathway to target for developing novel anticancer therapies, based on its central role in regulating growth and survival of cells in a broad spectrum of human cancers [15, 16]. Importantly, mitogen-activated protein kinase/extracellular signal–regulated kinase kinase-1 (MEK1) is downstream of RAS and RAF proteins, which are often mutated [2] and abnormally active in tumors. Therefore, MEK1 inhibition is an attractive target for anticancer therapy. A series of in vivo xenograft animal studies have demonstrated antitumor activity of E6201 with potent activity against V600E BRAF-mutated human cancer xenografts [11, 18] prompting us to further develop E6201.

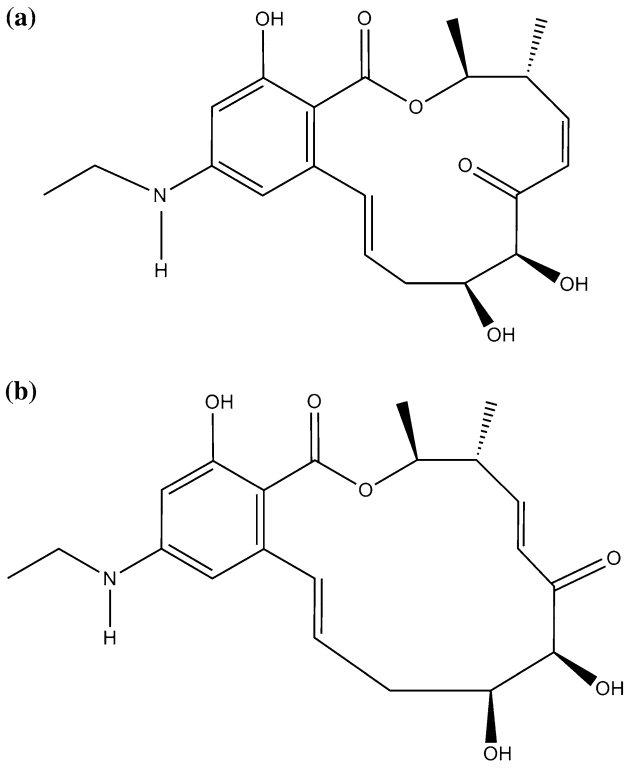

Fig. 1 Chemical structure of (**a**) E6201 and (**b**) isomeric metabolite, ER-813010

Preclinical pharmacokinetics and metabolism characterization of a potential candidate compound is an integral part of drug discovery and development [8]. This manuscript describes the preclinical pharmacokinetic characterization of E6201 in mice, rats, and dogs. In vitro metabolism and plasma protein-binding studies of E6201 are also described. Utilizing the preclinical data, allometric scaling was applied to predict human pharmacokinetics of E6201.

Materials and methods

Materials

E6201 and its isomeric metabolite, ER-813010, were synthesized by the Pharmaceutical Science and Technology Core Function Unit (CFU) of Eisai Inc. (Andover, MA, USA). Blank mouse, rat, and dog plasma were purchased from Bioreclamation Inc. (Westbury, NY, USA), and blank human plasma was purchased from Biological Specialty Corporation (Colmar, PA, USA). Pooled mouse, rat, dog, and human liver microsomes were purchased from BD Biosciences (Woburn, MA, USA). Monohydroxylated warfarin metabolites (6- and 7-hydroxywarfarin), (±)-bufuralol, (±)-1′-hydroxybufuralol, chlorzoxazone, 6-hydroxychlorzoxazone, (±)-4′-hydroxymephenytoin, 1′- and 4-hydroxymidazolam were purchased from BD Biosciences. Albendazole, allyl sulfide, α-naphthoflavone, ketoconazole, magnesium chloride, mebendazole, midazolam, niflumic acid, NADPH, R-propranolol, quinidine, sulfaphenazole, tranylcypromine, triazolam, TRIZMA, and racemic-warfarin were obtained from Sigma Chemical Corp. (St. Louis, MO, USA). S-Mephenytoin was purchased from BIOMOL Research Laboratories Inc. (Plymouth Meeting, PA, USA). Optically pure R- and S-warfarin were prepared from racemic mixtures by a differential crystallization method (enantiomer purity ≥98%). All chemicals, reagents, and solvents used were of either analytical or HPLC grade. Millipore water (Millipore Corporation, Billerica, MA, USA) was used in all experiments.

In vitro plasma protein binding

The in vitro plasma protein binding of E6201 in mouse, rat, dog, and human plasma ($n = 3$ per concentration per species) was determined using equilibrium dialysis. The binding was determined at 100, 500, 1,000, 3,000, 10,000, and 30,000 ng/mL of E6201. The spiked plasma was placed on one side of the membrane with phosphate-buffered saline on the other side. The system was incubated

at approximately 37°C on an orbital shaker for 4 h. Aliquots from plasma and buffer chambers were collected, appropriately labeled, extracted and were analyzed using LC/MS/MS.

In vitro metabolic stability, CYP inhibition and CYP induction studies

The in vitro stability of E6201 was investigated in incubations with liver microsomal fractions from mouse, rat, dog, and human. The half-life ($t_{1/2}$) of E6201 depletion ($n = 3$) was determined under the following final conditions: E6201 (5 μM); microsomes (2 mg/mL); in pH 7.4 Tris buffer (50 mM) containing 15 mM magnesium chloride. Incubations were performed in test tubes at approximately 37°C. Incubations were initiated by the addition of NADPH and were quenched at various time points (0, 5, 15, 30, 45, 60, or 120 min) by the addition of an equal volume (0.25 mL) of methanol. The supernatants were analyzed using LC/MS/MS.

The inhibition potential of E6201 on CYP1A2, 2C9, 2C19, 2D6, 2E1, and 3A activities was assessed using pooled human liver microsomes. The effect of E6201 on the metabolic rates of CYP-specific probe reactions was measured in triplicate under the following final conditions: E6201 (1, 5, or 10 μM); microsomes (2 mg/mL); R-warfarin (2 mM); S-warfarin (200 μM); S-mephenytoin (200 μM); bufuralol (4 μM); chlorzoxazone (200 μM); midazolam (40 μM); in pH 7.4 Tris buffer (50 mM) containing 15 mM magnesium chloride. CYP-selective inhibitors (α-naphthoflavone, tranylcypromine, sulfaphenazole, quinidine, allyl sulfide, and ketoconazole) served as positive controls. Incubations were performed in test tubes at approximately 37°C. Incubations were initiated by the addition of NADPH and were quenched at respective times (10 min for CYP3A-mediated midazolam 1′-and 4-hydroxylations, 15 min for CYP2D6-mediated bufuralol 1′-hydroxylation, 30 min for CYP2E1-mediated chlorzoxazone 6-hydroxylation, 60 min for CYP1A2-mediated R-warfarin 6-hydroxylation, CYP2C9-mediated S-warfarin 7-hydroxylation and CYP2C19-mediated S-mephenytoin 4′-hydroxylation) by the addition of an equal volume (0.25 mL) of methanol. The supernatants were analyzed using LC/MS/MS.

Primary human hepatocytes were used to evaluate the potential of E6201 to induce CYPs at 1, 5, or 10 μM concentrations of E6201. Potential inductions of CYP1A2, 3A, 2C9, and 2C19 were assessed using CYP form-specific metabolism activities determined by LC/MS/MS and by Western immunoblotting using anti-human CYP antibodies [6, 9, 17]. The positive controls for induction were 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD; 20 nM) for CYP1A and rifampicin (10-50 μM) for CYP2C and CYP3A [12, 13, 19]. Methanol was used as the vehicle (negative) control. The treatment period was 72 h with media, treatment replacement occurring every 24 h.

Pharmacokinetic experiments

Pharmacokinetic studies were performed in accordance with the Institutional Animal Care and Use Committee (IACUC) of Eisai (for mouse and rat studies) or IACUC, or equivalent committee, of the Contract Research Organisation (CRO) for the dog study.

Mouse

Male BALB/c mice (Charles River Laboratories, Kingston, NY, USA) weighing 22–24 g at the start of the study were administered 1, 5, and 10 mg/kg of E6201 IV (20% (w/v) hydroxypropyl-β-cyclodextrin solution) via tail vein injection ($n = 3$/time point/dose group) at 10 mL/kg dosing volume. Blood samples were collected by cardiac puncture at 5, 15, 30 min, 1, 2, 4, 6, and 8 h postdose with EDTA as the anticoagulant and placed on ice. Harvested plasma samples were appropriately labeled and stored at approximately −20°C pending LC/MS/MS analysis.

Rat

Male Sprague–Dawley rats (Hilltop, Scottdale, PA, USA), weighing 226–250 g at the start of the study, were administered 1, 5, and 10 mg/kg of E6201 IV via tail vein injection ($n = 3$/dose group). The rats were jugular vein cannulated to facilitate multiple sampling from a single animal. The formulation and dosing volume for rat were identical to that of mouse study. Blood samples were collected at 5, 15, 30 min, 1, 2, 4, 6, and 8 h postdose with EDTA as the anticoagulant and placed on ice. Harvested plasma samples were appropriately labeled and stored at approximately −20°C pending LC/MS/MS analysis.

Dog

Four male beagle dogs (Marshall Farms; North Rose, NY, USA) weighing 8–10 kg at the start of the study, each identified by a numbered ear tattoo, were administered E6201 at 0.5, 1.5, and 5 mg/kg IV in a dose-escalating crossover design with a 1-week washout between doses. E6201 was dissolved in a 20% (w/v) hydroxypropyl-β-cyclodextrin solution and administered at the dosing volume of 5 mL/kg. The IV doses were administered through an indwelling catheter, inserted into a cephalic or

saphenous vein, as a short infusion over a period of 5 min. Blood samples were collected from the jugular vein at predose, 5, 15, 30 min, 1, 2, 4, 6, 8, 12, and 24 h postdose with sodium heparin as the anticoagulant and placed on ice. Harvested plasma samples were appropriately labeled and stored at approximately −70°C pending LC/MS/MS analysis.

Bioanalysis

Samples obtained from the pharmacokinetic studies were assayed for E6201 and its isomeric metabolite, ER-813010, concentration using LC/MS/MS. A structurally similar analog was used as an internal standard (IS, Eisai proprietary compound) for sample analysis. For pharmacokinetic samples, plasma was mixed with IS and diluted with 500 μL of water. The samples were then extracted by liquid–liquid extraction using 5 mL of ethyl acetate. The organic phase was separated and dried under nitrogen. The samples were reconstituted in 200 μL of methanol/water (50/50) solution and injected onto the LC/MS/MS. The protein-binding samples (100 μL) were processed by protein precipitation with 200 μL of methanol containing IS solution and the clear supernatant was injected onto the LC/MS/MS. The HPLC systems consisted of either a Waters 2695 Separations Module system (Milford, MA), an Agilent HPLC (Agilent Technologies, Santa Clara, CA), or a Shimadzu HPLC system (Shimadzu Scientific Instruments, Columbia, MD). Samples were injected on a C18 Waters YMC^{TM} ODS-AM (23 × 2.0 mm) column maintained at approximately 25°C at a flow rate of 0.3 mL/min with a gradient elution. Samples were injected using the autosampler maintained at approximately 4°C. The mobile phase consisted of 0.1% (v/v) formic acid in water (A) and 0.1% (v/v) formic acid in methanol (B). The initial mobile phase composition was 65% A/35% B and was changed to 100% B over 5 min and held constant for an additional 2 min postinjection. The mobile phase composition then returned to initial conditions with 2.9 min for column re-equilibration with a total analysis time of 10 min. The HPLC was interfaced to a Micromass Quattro Ultima (Micromass Limited, Beverly, MA) or an AB Sciex API2000/API4000 (Foster City, CA) triple quadrupole mass spectrometer using electrospray ionization under positive ion mode. Data acquisition utilized multiple reaction monitoring (MRM). E6201 and ER-813010 were monitored at precursor ion m/z 390.2 and product ion m/z 232.0, and the IS was monitored at precursor ion m/z 450.2 and product ion m/z 274.0. The retention times for E6201, ER-813010, and IS were approximately 5.6, 5.2, and 5.6 min, respectively. Quantitation was based on a linear regression with a $1/x^2$ weighting factor of the peak area ratios of E6201 to IS or ER-813010 to IS versus concentration.

Pharmacokinetic data analysis and allometric scaling

The plasma concentration versus time data of E6201 and ER-813010 following E6201 IV administration was analyzed using non-compartmental approach (WinNonlin v. 4.0.1 [Pharsight; Mountain View, CA, USA]). The area under the curve (AUC) from time zero to the last quantifiable time point postdose (AUC_{0-t}) was calculated using the trapezoidal method. The $AUC_{0-\infty}$ was calculated as $AUC_{0-t} + AUC_{extrap}$, where AUC_{extrap} represents the extrapolated AUC from the last quantifiable time point (C_{last}) to infinity and was calculated as C_{last}/λ_z. Lambda z (λ_z), the slope of the concentration versus time curve during the terminal phase, was determined by linear regression and used for determining the elimination half-life ($t_{1/2}$) as $\ln 2/\lambda_z$. The observed area under the first-moment plasma concentration versus time curve ($AUMC_{0-t}$) was calculated by the trapezoidal rule, while the estimated area under the first-moment plasma concentration versus time curve from time zero to infinity ($AUMC_{0-\infty}$) was calculated as:

$$AUMC_{0-\infty} = AUMC_{0-t} + \frac{C_{last} \cdot t_{last}}{\lambda_z} + \frac{C_{last}}{\lambda_z^2}$$

The total body clearance (CL) and the volume of distribution at steady state (V_{ss}) were calculated as dose/$AUC_{0-\infty}$ and $CL \cdot MRT_{0-\infty}$, respectively. The $MRT_{0-\infty}$ was determined as a ratio of $AUMC_{0-\infty}$ to $AUC_{0-\infty}$.

For allometric scaling, mean concentration versus time data from mice, rats, dogs, and rabbits (unpublished in house data) following E6201 IV administration was simultaneously modeled using a two-compartment body model with allometric scaling coefficients. Data were modeled using Scientist v. 2.0.1 (Micromath Inc, Salt Lake City, Utah, USA) as described by Gabrielsson and Weiner [4]. For predicting CL, allometric scaling with correction factor as suggested by the rule of exponent by Mahmood was applied [10]. The following allometric equation was applied.

$$Y = a \cdot W^b$$

where Y is pharmacokinetic parameter of interest (clearance or volume), W is the average weight of species in kg, and a and b are the coefficients and exponent of the allometric equation, respectively. The model-predicted parameters were used to predict human clearance and distribution volume and simulate the human pharmacokinetic profile at the proposed starting dose for Phase I clinical trial.

Results

In vitro studies

The mean plasma protein binding of E6201 ranged from 98.3 to 99.0% in mouse, 98.7 to 99.4% in rat, 98.0 to 98.7% in dog, and 98.2 to 98.4% in human plasma across 100–30000 ng/mL (Table 1).

The microsomal half-life as determined in NADPH-dependent E6201 depletion using a first-order decay equation in microsomal preparations was 84, 89, 70, and 36 min for mouse, rat, dog, and human, respectively.

No inhibition of major human CYPs (CYP1A2, 2C9, 2C19, 2D6, 2E1, and 3A) was detected in pooled human liver microsomes at 1–10 μM of E6201 (Table 2). The CYP-selective positive controls (data not shown) showed appropriate inhibitory effects for the respective probe reactions.

E6201 did not induce CYP3A, 2C9, or 2C19 at 1, 5, and 10 μM E6201 concentrations in human hepatocytes (Table 3). One donor (donor D at 10 μM E6201) showed slight CYP1A induction (~12.8% above vehicle), which was 3.7% of the induction observed with the positive control (20 nM TCDD). The results from Western blots (data not shown) also showed no detectable increase in CYP1A, 3A, 2C9, and 2C19 protein levels for any of the donors used in the induction assay.

Pharmacokinetics in mice, rats, and dogs

Following IV administration in mice (Fig. 2), E6201 exhibited high plasma clearance (mean CL ranged from 5.68 to 7.46 L/h/kg, Table 4). The V_{ss} was high and ranged from 3.12 to 4.83 L/kg. The mean elimination half-life ($t_{1/2}$) ranged from 0.4 to 1.0 h, indicating fast elimination of E6201 in mice. A nearly dose proportional increase in exposure with respect to dose of E6201 was observed in mice (Table 4). At 1 mg/kg dose, low levels of ER-813010 could be observed. Detectable levels of ER-813010 appeared in mouse plasma from 0.25 to 1 h and could be measured up to 4 or 6 h postdose of E6201. The mean elimination half-life ($t_{1/2}$) of ER-813010 ranged from 0.9 to 1.4 h in mice (Table 5). The metabolite to parent exposure ratio (AUC_R) was <4% in mice across the three doses.

In rats, following IV administration (Fig. 3), the systemic plasma CL of E6201 was high (mean CL ranged from 4.67 to 10.92 L/h/kg, Table 4), and the V_{ss} was high (mean V_{ss} ranged from 3.66 to 13.09 L/kg, Table 4). The mean $t_{1/2}$ ranged from 0.8 to 1.3 h. The exposure to E6201 in rats increased proportionally from 1 to 5 mg/kg, but more than proportionally from 5 to 10 mg/kg. In rats, measurable levels of ER-813010 appeared in plasma at 0.25 h and could be measured up to 8 h (4 h at 1 mg/kg dose) postdose of E6201. The mean elimination half-life ($t_{1/2}$) of ER-813010 ranged from 1.2 to 2.2 h in rats (Table 5). The AUC_R was >20% in rats.

Following IV administration of E6201 in dogs (Fig. 4), the systemic plasma CL of E6201 was high (mean CL ranged from 2.11 to 3.45 L/h/kg, Table 4). The V_{ss} was moderate (mean V_{ss} ranged from 0.63 to 1.12 L/kg). The mean $t_{1/2}$ ranged from 0.6 to 1.6 h, suggesting fast elimination. The exposure to E6201 in dogs increased proportionally from 0.5 to 1.5 mg/kg, but less than proportionally from 1.5 to 5.0 mg/kg, (Table 5). Measurable levels of ER-813010 appeared in dog plasma at 0.25 h and could be monitored up to 12 h (4 h at 0.5 mg/kg dose) postdose of E6201. The mean elimination half-life ($t_{1/2}$) of ER-813010 ranged from 0.9 to 2.9 h in dogs across doses (Table 5). The AUC_R was >29% in dogs.

Allometric scaling and prediction of human pharmacokinetics

Human pharmacokinetics of E6201 was predicted using allometric scaling approach [4, 10]. A two-compartmental model with parameterization in terms of clearance (CL, Q) and volume of distribution (V_1, V_2) best described E6201 pharmacokinetics in all preclinical species. The allometry predicted an average plasma CL of 3.44 L/h/kg, an intercompartmental CL (Q) of 0.11 L/h/kg, a volume of distribution in the central compartment (V_1) of 7.29 L/kg, and a volume of distribution in the peripheral compartment (V_2)

Table 1 Plasma protein binding of E6201

Concentration of E6201 (ng/mL)	% protein binding (mean ± SD; $n = 3$)			
	Mouse	Rat	Dog	Human
100	98.3 ± 0.1	98.7 ± 0.2	98.0 ± 0.1	98.3 ± 0.3
500	98.7 ± 0.3	98.9 ± 0.2	98.0 ± 0.3	98.3 ± 0.2
1,000	98.8 ± 0.4	99.4 ± 0.1	98.0 ± 1.2	98.4 ± 0.2
3,000	98.9 ± 0.3	99.2 ± 0.1	98.7 ± 3.3	98.4 ± 0.2
10,000	98.7 ± 0.3	99.2 ± 0.1	98.4 ± 0.4	98.2 ± 0.2
30,000	99.0 ± 0.2	99.1 ± 0.1	98.6 ± 0.2	NT

SD standard deviation, *NT* not tested

Table 2 Evaluation of human CYP inhibition potential by E6201

CYP450	Metabolite formation rate (pmol/min/well)[a]			
	Vehicle	E6201 concentration (μM)		
		1	5	10
CYP1A2[b]	5.7 ± 1.3	5.9 ± 1.3	4.6 ± 0.3	4.5 ± 0.8
CYP2C19[c]	38.4 ± 0.8	40.8 ± 2.7	37.9 ± 1.1	38.2 ± 2.2
CYP2C9[d]	8.3 ± 1.9	9.3 ± 0.1	8.1 ± 2.0	6.5 ± 0.4
CYP2D6[e]	14.3 ± 0.5	14.3 ± 1.0	13.6 ± 0.2	13.2 ± 0.5
CYP2E1[f]	456.9 ± 18.4	446.5 ± 13.7	452.5 ± 13.7	448.2 ± 19.9
CYP3A[g]	78.9 ± 4.0	82.1 ± 1.7	85.6 ± 4.0	84.6 ± 3.1
CYP3A[h]	456.3 ± 31.7	470.9 ± 15.0	509.0 ± 39.5	519.1 ± 29.7

Positive controls for respective CYP450 enzymes

[a] Mean ± SD ($n = 3$)

[b] R-warfarin 6-hydroxylation

[c] S-mephenytoin 4′-hydroxylation

[d] S-warfarin 7-hydroxylation

[e] bufuralol 1′-hydroxylation

[f] chlorzoxazone 6-hydroxylation

[g] midazolam 1′-hydroxylation

[h] midazolam 4-hydroxylation

Table 3 Evaluation of human CYP induction potential by E6201

Donor	Metabolite formation rate (pmol/min/well)[a]					
	Midazolam 1′-hydroxylation (CYP3A4/5)			Phenacetin *O*-deethylation (CYP1A)		
	A	B	C	D	E	F
Treatment						
Vehicle, MeOH	1.33 ± 0.07	1.45 ± 0.13	9.46 ± 0.73	79.7 ± 3.32	2.91 ± 0.29	14.6 ± 1.31
TCDD (20 nM)	NA	NA	NA	429 ± 5.00	11.1 ± 0.98	337 ± 62.6
RIF (50 μM)	21.7 ± 0.75	2.50 ± 0.10	47.1 ± 2.16	93.6 ± 2.98	6.69 ± 0.68	4.00 ± 2.22
E6201 (1 μM)	1.59 ± 0.04	<1.00[b]	7.86 ± 0.08	84.0 ± 1.86	<2.00[b]	6.42 ± 0.16
E6201 (5 μM)	2.31 ± 0.20	<1.00[b]	5.40 ± 0.13	87.3 ± 1.88	<2.00[b]	12.2 ± 0.90
E6201 (10 μM)	2.40 ± 0.09	<1.00[b]	7.03 ± 0.29	92.5 ± 2.62	2.14 ± 0.15	10.3 ± 0.79
Donor	Tolbutamide 4-methylhydroxylation (CYP2C9)			*S*-Mephenytoin 4′-hydroxylation (CYP2C19)		
	G	H	I	J	K	L
Treatment						
Vehicle, MeOH	6.15 ± 0.43	7.71 ± 1.31	22.3 ± 0.91	5.92 ± 0.06	6.96 ± 0.29	<0.50[b]
RIF (50 μM)	28.2 ± 0.84	26.1 ± 5.86	46.3 ± 1.60	24.5 ± 2.15	20.1 ± 0.68	1.77 ± 0.16
E6201 (1 μM)	6.75 ± 1.43	7.78 ± 3.10	23.7 ± 1.04	6.49 ± 0.35	10.6 ± 1.68	<0.50[b]
E6201 (5 μM)	7.46 ± 1.13	8.43 ± 1.12	26.3 ± 2.22	7.20 ± 0.27	11.1 ± 1.26	0.78 ± 0.06
E6201 (10 μM)	9.24 ± 0.80	11.1 ± 0.68	27.3 ± 1.46	8.29 ± 0.14	11.7 ± 0.79	<0.50[b]

MeOH methanol, *TCDD* 2,3,7,8-tetrachlorodibenzo-*p*-dioxin, *RIF* rifampicin, *NA* not applicable

[a] Mean ± standard deviation ($n = 3$)

[b] Below quantification limit. Lower limit is given as the maximum value

of 3.29 L/kg in humans. Allometric scaling–based simulation and the observed human pharmacokinetic profile from the first-in-man trial are shown in Fig. 5. The allometry-predicted CL was 1.9-fold higher than the observed CL in first human subject, while the V_1 was overpredicted by approximately 70-fold.

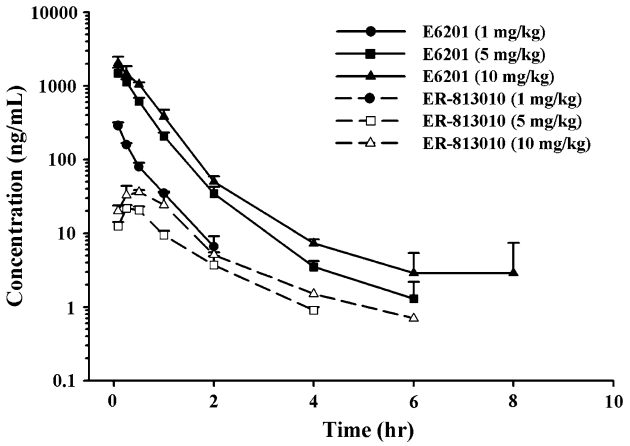

Fig. 2 Plasma concentration versus time (mean ± SD, $n = 3$) profiles of E6201 (*solid line*) and ER-813010 (*broken line*) following 1, 5, and 10 mg/kg IV administration of E6201 in mice

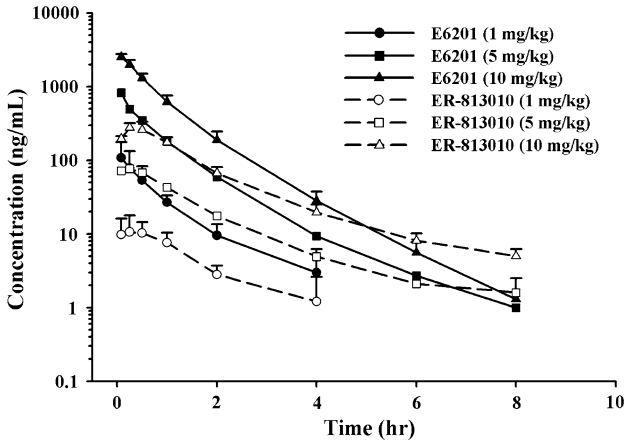

Fig. 3 Plasma concentration versus time (mean ± SD, $n = 3$) profiles of E6201 (*solid line*) and ER-813010 (*broken line*) following 1, 5, and 10 mg/kg IV administration of E6201 in rats

Table 4 Pharmacokinetic parameters of E6201 in mice, rats, and dogs following IV administration of E6201 ($n = 3$; $n = 4$ for dog)

Species	Dose (mg/kg)	$AUC_{0-\infty}$ (ng h/mL)	$AUC_{0-\infty}$/D (ng h/mL/D)	CL (L/h/kg)	V_{ss} (L/kg)	$t_{1/2}$ (h)
Mice	1	140.3	140.3	7.13	3.54	0.4
	5	879.7	175.9	5.68	3.12	0.6
	10	1,341.2	134.1	7.46	4.83	1.0
Rats[a]	1	97.3 ± 31.3	97.3 ± 31.3	10.92 ± 2.97	13.09 ± 11.05	0.9 ± 0.5
	5[b]	598.7 ± NC	119.7 ± NC	8.41 ± NC	7.73 ± NC	1.3 ± NC
	10	2,161.6 ± 249.2	216.2 ± 24.9	4.67 ± 0.52	3.66 ± 0.18	0.8 ± 0.2
Dogs[a]	0.5	246.6 ± 52.4	493.2 ± 104.9	2.11 ± 0.50	0.63 ± 0.37	0.6 ± 0.1
	1.5	629.0 ± 221.2	419.3 ± 147.4	2.62 ± 0.91	0.87 ± 0.41	1.1 ± 0.2
	5	1,489.7 ± 301.1	297.9 ± 60.2	3.45 ± 0.65	1.12 ± 0.36	1.6 ± 1.3

NC not calculated

[a] Mean ± standard deviation

[b] $n = 2$

Table 5 Pharmacokinetic parameters of ER-813010 in mice, rats, and dogs following IV administration of E6201 ($n = 3$; $n = 4$ for dog)

Species	Dose[a]	C_{max} (ng/mL)	t^{b}_{max} (h)	$AUC_{0-\infty}$ (ng h/mL)	$t_{1/2}$ (h)	AUC^{c}_{R} (%)
Mice	1	1.6	1.0	NC	NC	NC
	5	21.7	0.25	27.0	0.9	3.1
	10	36.1	0.50	50.2	1.4	3.7
Rats[d]	1	11.7 ± 5.4	0.25	19.8 ± 0.63	1.2 ± 0.8	20.3
	5[e]	76.6 ± NC	0.25	123.8 ± NC	2.2 ± NC	20.7
	10	282.5 ± 52.1	0.25	462.4 ± 94.8	1.8 ± 0.3	21.4
Dogs[d]	0.5	75.0 ± 24.5	0.25	71.8 ± 17.9	0.9 ± 0.1	29.1
	1.5	239.1 ± 82.1	0.25	210.2 ± 59.4	2.9 ± 1.0	33.4
	5	519.0 ± 81.1	0.25	467.2 ± 73.9	2.9 ± 0.2	31.4

NC not calculated

[a] Dose of E6201 in mg/kg

[b] Median value (except for mouse)

[c] AUC ratio calculated as $(AUC_{0-\infty,\ ER\text{-}813010}/AUC_{0-\infty,\ E6201}) \times 100$

[d] Mean ± standard deviation

[e] $n = 2$

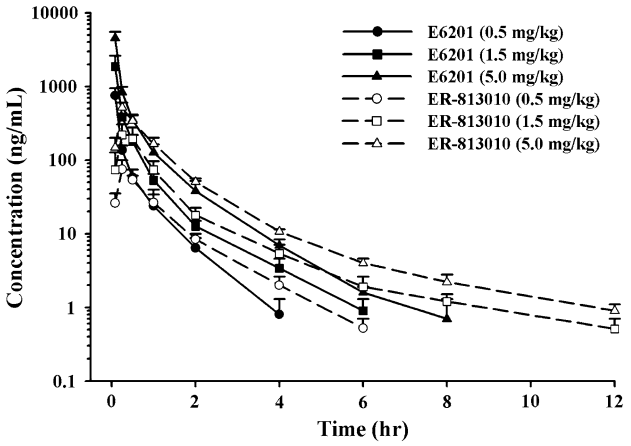

Fig. 4 Plasma concentration versus time (mean ± SD, $n = 4$) profiles of E6201 (*solid line*) and ER-813010 (*broken line*) following 0.5, 1.5, and 5 mg/kg IV administration of E6201 in dogs

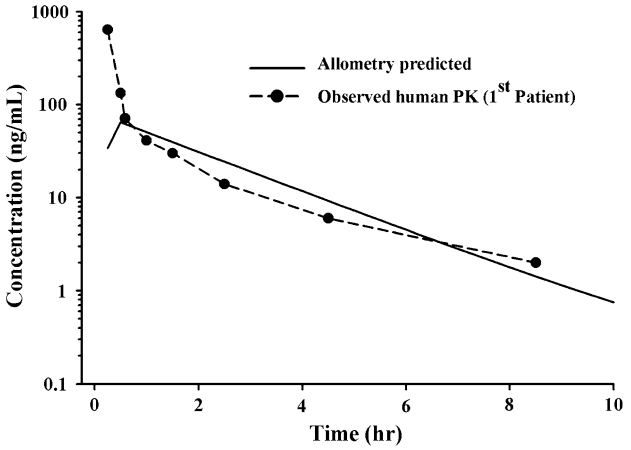

Fig. 5 Observed (*filled circle*) and simulated (*solid line*) plasma concentration versus time profile of E6201 following a 30-min IV infusion of E6201 (20 mg/m^2) in humans

Discussion

In the present work, the in vitro metabolism and pharmacokinetic characteristics of E6201, an anti-inflammatory and antihyperproliferative agent, were evaluated in preclinical species, and allometric scaling was applied to predict human pharmacokinetics.

The protein binding of E6201 (100–30,000 ng/mL) in mouse, rat, dog, and human plasma was high (>97%). In humans, mean plasma protein binding of E6201 ranged from 98.2 to 98.4% across the tested concentrations. Protein binding of E6201 was independent of the concentration and in general similar across the tested species. The in vitro metabolic stability studies of E6201 using microsomes exhibited species difference with human showing faster metabolic CL (microsomal half-life of 36 min) and rat with the highest metabolic stability (microsomal half-life of 89 min). However, there was a rapid conversion of E6201 to ER-813010 across all species tested in S9 fraction (unpublished in house data), suggesting that enzymes other than CYPs may be involved in E6201 metabolism. This may help explain the observed high plasma CL of E6201 in preclinical species.

Evaluation of CYP inhibition potential of E6201 using human microsomes resulted in no significant effects of E6201 on CYP isoform-selective activities. Thus, based on these data, E6201 is expected to have low potential for drug–drug interaction (DDI) in patients. Furthermore, no significant CYP induction was observed as determined using human hepatocytes. The minor CYP1A induction detected in one donor (donor D) did not approach the FDA's suggested threshold of 40% of the induction of the positive control and was only 12.8% greater than the vehicle [7]. In addition, the induction was only seen at the highest concentration of 10 μM. Therefore, based on the available data, the likelihood of DDI in patients due to E6201 CYP-mediated induction is anticipated to be low [1, 7].

The in vivo pharmacokinetics of E6201 in mice, rats, and dogs suggested high plasma CL of E6201 following IV administration. The distribution volume of E6201 was large in mice and rats, suggesting that E6201 distributes into extravascular tissues while in dogs the distribution was moderate. E6201 was rapidly eliminated with $t_{1/2}$ of <2 h in preclinical species.

The in vitro evaluation in a variety of human cancer cell line panel indicated that BRAF-mutated cell lines were sensitive to E6201 [11]. In mouse xenograft models, E6201 i.v administration (Q4D × 3 dosing regimen) resulted in a dose-dependent tumor growth delay or regression in three BRAF-mutated human cancer cell xenograft models and also demonstrated survival benefit in metastatic brain tumor model of BRAF-mutated MDA-MB-231 breast cancer cells [18]. Additionally, E6201 demonstrated a time-dependent inhibition of MEK 1 as measured by phospho-pRb in LOX tumors and decreased plasma IL-8 levels in V600E BRAF-mutated human melanoma-bearing mice at a single 40 mg/kg IV injection [18].

In the preclinical species, ER-813010, the isomeric metabolite of E6201, was observed across species as early as 0.25 h postdose, suggesting rapid in vivo conversion of E6201 to ER-813010. ER-813010 exhibited fast elimination ($t_{1/2}$ ranging from 0.9 to 2.9 h) similar to that of E6201 in all species. The AUC_R ratio of ER-813010 was >10% in all species except mice at the studied dose levels of E6201.

For humans, the pharmacokinetic parameters of E6201 were predicted using allometric scaling, and the prediction suggested high CL and large volume of distribution as observed in preclinical species. A comparison of first human subject pharmacokinetics from Phase I indicated

that allometry yielded a good prediction of human CL although the distribution volume was overpredicted several folds (Fig. 5).

Overall, the pharmacokinetics of E6201 in preclinical species was described by high CL with large to moderate distribution. E6201 showed high protein binding independent of concentration across species. No significant CYP inhibition and CYP induction were observed in vitro, suggesting low risk of DDIs with E6201 in humans. In the first-in-human study, E6201 exhibited high CL, which was well predicted by allometric scaling.

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Conflict of interest All authors (except Vipul Kumar and Zhi-Yi Zhang) are current employee of Eisai Inc. Vipul Kumar and Zhi-Yi Zhang were employee of Eisai Inc. at the time when some of this work was done.

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