In Vitro-In Vivo Correlation and Translation to the Clinical Outcome
for CJ-13,610, a Novel Inhibitor of 5-Lipoxygenase

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5-LOX, 5-lipoxygenase; IVIVC, in vitro-in vivo correlation; DDI, drug-drug interactions; P450, cytochrome P450; LTB4, leukotriene B4; FMO, flavin-monooxygenase; Clintr, intrinsic clearance; Clh, hepatic clearance; Vmax, maximal velocity; Km,app, apparent Michaelis-Menten constant; AUC, area under the concentration-time curve; MRT, mean residence time; Vdss, volume of distribution at steady-state; HLM, human liver microsomes; RLM, rat liver microsomes; DLM, dog liver microsomes; SSS, single-species allometric scaling
Abstract

The metabolism of the 5-lipoxygenase (5-LOX) inhibitor, CJ-13,610, was investigated in liver microsomes from human and pre-clinical species, in effort to compare metabolite profiles, and evaluate the in vitro-in vivo correlation (IVIVC) for metabolic clearance. Overall, the metabolite profile of CJ-13,610 was comparable across the species tested, with multiple oxidative metabolites observed, including sulfoxidation. The sulfoxidation kinetics characterized in rat, dog, and human liver microsomes (HLM) indicated a low apparent Michaelis-Menten constant (K_m,app) of 4-5 µM. Results from cDNA-expressed P450 studies indicated that the metabolism in HLM was primarily mediated by P450 3A4 and 3A5. A subsequent in vitro study utilizing ketoconazole as an inhibitor of CJ-13,610 sulfoxidation corroborated the CYP3A4/5-mediated pathway (IC_{50} = 7 nM). Assessment of multiple methods for predicting the human pharmacokinetic profile observed with CJ-13,610 following a 30 mg single oral dose indicated that clearance scaled from human liver microsomes yielded a better prediction when coupled with a V_{dss} term that was scaled from dog (AUC and half-life within 1.3-fold of actual), versus a V_{dss} term obtained from rat. Single-species allometric scaling of clearance and V_{dss} from dog pharmacokinetic studies was equally predictive, while scaling from rat resulted in under-predictions of both AUC and C_{max}. Results from these studies support the strategy of predicting human pharmacokinetics using human liver microsomal intrinsic clearance data. Importantly, results from the present investigation enabled the selection of alternative drug candidates from the chemical series via in vitro screening, while subsequently eliminating costly routine preclinical in vivo studies.
**Introduction**

CJ-13,610, 4-(3-(4-(2-methyl-1H-imidazol-1-yl)phenylthio)phenyl)-tetrahydro-2H-pyran-4-carboxamide (Figure 1) is a novel reversible inhibitor of 5-lipoxygenase (5-LOX) (Fischer et al., 2004), a critical enzyme involved in the initial step of the arachidonic acid (AA) cascade that results in the ultimate formation of numerous pro-inflammatory bioactive leukotrienes such as LTA₄, B₄, C₄, D₄ and E₄ (Samuelsson, 1983). Leukotrienes have been clearly shown to be potent chemoattractants for neutrophils, eosinophils and monocytes in response to inflammatory stimuli, and thus it is expected that inhibition of the 5-LOX enzyme may lead to successful therapeutic intervention in several inflammatory based diseases, including asthma, rheumatoid arthritis, and cardiovascular disease (Werz and Steinhilber, 2006).

Zileuton (Zyflo®) is currently the only 5-LOX inhibitor available on the market for the treatment of asthma (Liu et al, 1996; Wenzel and Kamada, 1996). While efficacious in the treatment of asthma, zileuton is fraught with issues such as a high daily dose (2400 mg/day), and deleterious effects on the liver, requiring repeated monitoring of liver enzyme levels. In addition, zileuton has been shown to be a mechanism-based inactivator of P450 1A2 (Lu et al., 2003), resulting in the potential for drug-drug interactions (DDI) with co-administered P450 1A2 substrates such as theophylline (Granneman et al., 1995). An alternative therapeutic strategy includes use of LTB₄ receptor antagonists, such as Singulair (Montelukast®), which has been shown to be effective and safe in the treatment of asthma, including the pediatric population (Reiss et al., 1996; Becker, 2000). In lieu of the favorable safety profile of LTB₄ receptor antagonists such as Singulair, upstream inhibition of the 5-LOX enzyme, theoretically
preventing formation of all of the aforementioned leukotrienes, presents a potential opportunity for enhanced clinical efficacy in multiple therapeutic areas. Thus, there remains a desire for a potent 5-LOX inhibitor that can be administered to patients at a lower therapeutic dose while maintaining the desired safety of LTB₄ receptor antagonists.

The use of in vitro biotransformation systems to predict the pharmacokinetic behavior of drug molecules remains an intense area of research within the drug discovery continuum, with much of the seminal work initiated by Houston and Iwatsubo (Houston 1994; Iwatsubo et al., 1997). In particular, the use of hepatic microsomes as an in vitro tool for quantitatively predicting metabolic clearance, as well as the contribution of specific drug metabolizing enzymes, is routinely applied in drug discovery to diagnose liabilities associated with drug disposition prior to nomination of drug candidates for development. To this end, the primary objectives of the present investigations were to (1) profile the in vitro metabolic pathways of CJ-13,610 in liver microsomes from multiple species, (2) compare the kinetics of liver microsomal metabolism in vitro and the in vivo distribution of CJ-13,610 across species in effort to assess in vitro-in vivo correlation, and (3) estimate the contribution of the specific human enzymes involved in the metabolism of CJ-13,610. This work was a retrospective analysis to investigate potential methodologies for predicting the disposition properties of CJ-13,610 as it relates to the potential for pharmacokinetic variability and drug-drug interaction risk of this potential therapeutic in humans. Results from these analyses will prove useful for any drug discovery program evaluating new chemical entities within this chemical class of compounds.
Materials and Methods

Chemicals. CJ-13,610 and the sulfoxide metabolite standard (CP-680179) were obtained from the Pfizer Global Research and Development chemical bank. Potassium phosphate buffer, NADPH, magnesium chloride, tolbutamide, bupropion, coumarin, dextromethorphan, diclofenac, midazolam, omeprazole, paclitaxel, astemizole, tacrine, ketoconazole, and benzydamine were purchased from Sigma-Aldrich (St. Louis, MO). Pooled human liver microsomes (50 donors), dog liver microsomes, and recombinant human cytochrome P450 2B6, 2C8, 2J2 and 3A5 Supersomes® were purchased from BD Biosciences (San Jose, CA). Rat liver microsomes were purchased from Xenotech LLC (Kansas City, MO), and recombinant human cytochrome P450 1A2, 2D6, 2C9, 2C19 and 3A4 Baculosomes® were purchased from Invitrogen (Carlsbad, CA). All other chemicals were obtained from commercial sources and were of the highest purity available.

In Vitro Incubations. The intrinsic clearance of CJ-13,610 (1 µM) was compared in human (0.8 mg/mL), rat (0.5 mg/mL), and dog (0.5 mg/mL) liver microsomes using substrate depletion methodologies. Incubations were performed in 100 mM potassium phosphate pH 7.4 buffer (1 mM MgCl₂) at 37 °C, and were initiated after a 5 min pre-incubation period by addition of NADPH (1 mM). At selected time intervals (0, 2, 5, 10, 20, and 30 min), 50 µl aliquots were taken and subsequently placed into a 96-well plate containing 150 µl of cold acetonitrile with internal standard (0.2 µM tolbutamide). Plates were then centrifuged at 3000 rpm (4°C) for 10 min, and the supernatant was transferred to a separate 96-well plate for LC/MS/MS analysis. The in vitro half-life ($t_{1/2}$) was calculated by linear regression of the natural log (ln) percent (%) remaining of substrate as a function of incubation time using GraphPad Prism (GraphPad Software Inc., San
Diego, CA) software according to the equation $t_{1/2} = \ln 2 / \text{slope}$. In effort to identify the enzymes involved in the metabolism of CJ-13,610 (1 μM), incubations with recombinant heterologously expressed P450 enzymes (150 pmol/mL, 15 pmol/mL for 3A4), and recombinant human FMO3 (50 μg/mL) were conducted in the presence of 100 mM potassium phosphate pH 7.4 buffer (1 mM MgCl₂) and NADPH (1 mM) in a total volume of 1 mL (0.2 mL for FMO3 incubation). Reactions were initiated by addition of NADPH (1 mM), and incubated on an Eppendorf Thermomixer™ at 37°C for 30 min. Incubations with FMO3 underwent a 5 min pre-incubation period in the presence of NADPH, and the reactions were initiated by addition of CJ-13,610, or benzydamine as positive control. Incubations were terminated by addition of ice-cold acetonitrile containing 0.2 μM tolbutamide, and centrifuged at 3000 rpm for 10 min. Samples were subsequently analyzed by LC/MS/MS for determination of substrate depletion following transfer of supernatant to separate 96-well plate. Positive control probe substrates for each individual P450 were included to confirm enzyme activity. Inhibition of CJ-13,610 sulfoxidation by the P450 3A inhibitor ketoconazole was tested with incubation conditions identical to enzyme kinetic studies described above in human liver microsomes, except that ketoconazole (0-5 μM) was also included in the incubation, with each concentration in triplicate. CJ-13,610 was incubated at a single concentration equal to the estimated $K_m$ (5 μM) and the total organic in each incubation was 1% (v/v), and the sulfoxide metabolite (CP-680179) was measured by LC/MS/MS.

**Plasma and Microsomal Protein Binding.** Plasma protein binding and non-specific microsomal binding in human, rat and dog was performed in triplicate using a 96-well equilibrium dialysis apparatus according to published methods (Banker et al., 2003). To
prepare the dialysis block, dialysis membranes were soaked in phosphate buffered saline pH 7.4 for 60 min. Following hydration, membranes were rinsed with 100 mM phosphate pH 7.4 buffer (1 mM MgCl₂), separated and allowed to soak for a minimum of 1 hour. Human, rat, and dog liver microsomes were diluted to a final concentration of 0.8, 0.5 and 0.5 mg/mL in 100 mM phosphate buffer pH 7.4, respectively. Heparinized human, rat, and dog plasma (Bioreclamation) was collected from at least three male and female fasted donors and frozen at -70°C until use. The pH of the plasma was determined and adjusted to pH 7.4 by drop-wise addition of dilute phosphoric acid. The final concentration of CJ-13,610 was 1 µM in both microsomal binding (ketoconazole and midazolam as controls), and plasma protein binding (S-warfarin as control) studies.

The conditioned membrane strips were then placed into the 96-well dialysis apparatus. The dialysate side of the 96-well equilibrium dialysis block was loaded with 150 µl of 100 mM phosphate buffer pH 7.4 to prevent dehydration of the membrane. The same volume of plasma or diluted microsomes spiked with compound was placed into the sample side. Once the dialysis block was loaded with plasma or microsomal samples and buffer, an adhesive sealing film was used to cover the block to prevent evaporation. The equilibrium block was incubated at 37°C for 4 hours in a 5% CO₂ incubator to ensure equilibrium conditions were achieved, verified in preliminary experiments. Following the incubation, a 50 µl aliquot of the post-dialysis plasma (or microsomes) and buffer samples were placed in a 96 well microtiter plate and then precipitated with three volumes of an acetonitrile containing internal standard. The extraction plate was centrifuged for 5 min at 3000 rpm and 90 µl was transferred to a fresh analytical plate for LC/MS/MS analysis.
In Vitro Metabolite Profiling of CJ-13,610. The in vitro metabolite profile of CJ-13,610 was investigated in rat, dog and human liver microsomes. A potassium phosphate buffered 1 mL reaction (100 mM, pH 7.4) with CJ-13,610 (20 µM), liver microsomes (1 mg/mL), and MgCl₂ (1 mM) was initiated by addition of NADPH (2 mM) and incubated at 37 °C for 60 min in 13 x 100 mm borosilicate glass test tubes. Protein was precipitated by addition of two volumes of cold acetonitrile, and the resulting mixture underwent centrifugation at 3000 rpm (4° C) for 15 min. The supernatants were drawn off, placed into clean glass test tubes, and subsequently dried under a gentle stream of nitrogen (N₂) gas. Dried samples were then reconstituted in 200 µl of mobile phase (85:15 (v/v) ammonium formate (10 mM, pH 4.1)/acetonitrile) for LC/MS/MS analysis.

Enzyme Kinetics. The kinetics of CJ-13,610 sulfoxidation was investigated in rat, dog, and human liver microsomes. Preliminary experiments established the linear conditions for CJ-13,610 sulfoxidation with respect to incubation time (0 to 20 min) and protein concentration (0 to 0.5 mg/mL microsomal protein). Briefly, multiple concentrations of CJ-13,610 (0-100 µM) in triplicate were incubated in 100 mM potassium phosphate pH 7.4 buffer (1 mM MgCl₂) with rat liver microsomes (0.05 mg/mL), human liver microsomes (0.05 mg/mL), and dog liver microsomes (0.1 mg/mL) for 15 min at 37 °C in a shaking water bath (total volume 200 µl) following addition of 2 mM NADPH. Incubation reactions were subsequently quenched by addition of 200 µl ice-cold acetonitrile containing tolbutamide (0.2 µM) as internal standard. Incubation plates were then centrifuged at 3000 rpm (4 °C), and supernatant was transferred to an analytical 96-well plate for LC/MS/MS analysis.
Pharmacokinetic Studies. Male Sprague-Dawley rats were purchased from Charles River Laboratories (Wilmington, DE) and acclimated to their surroundings for approximately one week with food and water provided ad libitum. On the day prior to study, animals were anesthetized with Isoflurane (to effect) and then implanted with BASi vascular catheters in the carotid artery and jugular vein. Animals were acclimated in Culex cages overnight prior to dosing. Patency of the carotid artery catheter was maintained using the “tend” function of Culex ABS. Three rats were dosed intravenously (i.v.) with a 10% ethanol/56% PEG-400/34% phosphate buffered saline pH 7.4 at 0.5 mg/kg via the jugular vein catheter at a dose volume of 0.5 mL/kg. Blood collections were performed by the Culex pre-dose, and at 2 min, 5 min, 15 min, 30 min, and 1, 2, 4, 6, 8, 12, 18 and 24 hrs post dose, and samples were collected into chilled heparinized tubes, centrifuged for 10 minutes at 3000 rpm, and the resulting plasma aliquoted to 96-well plates for LC/MS/MS analysis. Male beagle dogs (approximately 1-3 years of age and weighed between 9 - 12 kg at study initiation) were obtained from Marshall Farms (North Rose, New York). Dogs (n=3) were dosed i.v. using a butterfly tubing set and syringe into the cephalic vein. The i.v. dose was administered at 0.1 mg/kg (1 mg/mL) in 10% EtOH/50% PEG-400/40% phosphate buffered saline pH 7.4. The dogs were placed in metabolism cages following dosing and for 24 hours post dose. All dogs were fasted overnight, but water was allowed ad libitum. Food was returned 4 hours post dose. All dogs were weighed prior to dosing. A catheter was then inserted into the cephalic vein in one leg of each dog for blood collection. Blood was collected pre-dose and at 5 min, 15 min, 30 min, 1, 2, 4, 6, 8, 10, and 24 hours post dose. Blood was obtained from the catheter for collection times up to two hours. After two hours, the catheters were
removed and blood was collected from the jugular vein for the remaining time points using a needle and syringe. Approximately 0.6 mL of blood was collected at each time point into lithium heparin blood tubes, mixed thoroughly and chilled. Plasma was obtained by centrifugation at 5200 rpm for 15 minutes at 4°C. In addition, urine samples were collected at 0-10 hours, and 10-24 hours post dose. Plasma and urine samples were stored at -10° C until analysis by LC/MS/MS. All animal studies were approved by the St. Louis Pfizer Institutional Animal Care and Use Committee. The animal care and use program is fully accredited by the Association for Assessment and Accreditation of Laboratory Animal Care, International.

**Dosing of CJ-13,610 in Humans.** The Phase I first-in-human (FIH) study was a placebo-controlled, randomized parallel group study of increasing doses of CJ-13,610 administered sequentially, at least one week apart. The study protocol and informed consent documents were approved by the Institutional IRB. All subjects signed an approved written informed consent prior to any study-related activities for this clinical study. A higher dose was given only if the preceding dose did not result in any safety concerns. There were 6 subjects in each dose group, with 4 randomized to active study drug and 2 to a matching placebo. CJ-13,610 was administered as a solution to fasted male subjects at doses of 1, 3, 10, 30, 100 and 300 mg. A light meal was provided 4 hours after dosing. Blood samples were collected at intervals for up to 96 hours post dose for the assay of plasma CJ-13,610 and plasma LTB₄. Urine was collected from 0-24 hours and 25-96 hours, the volume measured and aliquots sent to Pfizer labs for bioanalysis of CJ-13,610. Blood pressure, pulse rate, respiratory rate and oral temperature were
measured at intervals for 24 hours and subsequently, every 24 hours for the duration of the study.

**Liquid Chromatography/Mass Spectrometry Analysis.** CJ-13,610 and sulfoxide (CP-780179) were both analyzed on a PE Sciex API-3000 triple-quadrupole instrument. The mass spectrometer was equipped with an electrospray ionization (ESI) interface connected in line with a Shimadzu LC20AD pump and a Leap Technologies CTC PAL (Leap Technologies, Carrboro, NC) auto-sampler. Analytes were separated using a Zorbax 3.5 µm Eclipse Plus C_{18} 2.1 x 50 mm column with a gradient elution profile. Mobile phase was flowing at 0.45 ml/min, and the gradient was initiated and held for the first 0.8 min at 95%A: 5%B (A: 0.1% formic acid in H_{2}O, B: 0.1% formic acid in acetonitrile), and was then ramped linearly to 5%A: 95%B over the next 2.3 min and held for 1.2 min. The profile was then immediately returned to initial conditions and allowed to re-equilibrate for 2 min. The source temperature was set to 400°C and mass spectral analyses were performed using multiple reaction monitoring (MRM), with transitions for CJ-13,610 (m/z 393.9/319.3) or sulfoxide metabolite (m/z 410.1/158.3) utilizing a turbo ion spray source in positive ionization mode (3.5 kV spray voltage). The limit of quantitation (signal-to-noise, 3:1) for CJ-13,610 was 0.0015 to 0.0024 µM for in vivo studies (rat, dog, and human), and 0.0049 µM for in vitro studies. The limit of quantitation of CP-680179 for enzyme kinetic studies was 0.0024 µM. Low, medium and high quality control (QC) samples were included in all bioanalyses, and each QC was calculated to be within 15% of nominal concentrations (data not shown). All data were analyzed using PE Sciex Analyst 1.4.2 software. For in vitro profiling of CJ-13,610 metabolites, an Agilent 1100 HPLC system (Agilent Technologies, Palo Alto, CA) was
coupled to a Discovery® C_{18} column (5 µ, 3.8 x 150 mm; Supelco, Bellefonte, PA). Solvent A was 10 mM ammonium formate (pH 4.1) and solvent B was acetonitrile. The initial mobile phase was 85:15 A:B (v/v) and by linear gradient transitioned to 20:80 A:B over 20 min. The flow rate was 0.40 mL/min. The HPLC eluent was introduced via electrospray ionization directly into a Finnigan LCQ™ Deca XPLPLUS ion trap mass spectrometer (Thermoelectron Corporation, San Jose, CA) operated in the positive ion mode. Ionization was assisted with sheath and auxiliary gas (nitrogen) set at 70 and 15 psi., respectively. The electrospray voltage was set at 5 kV with the heated ion transfer capillary set at 350°C and 15 V. Relative collision energies of 25-40% were used when performing MS/MS operations with the ion trap.

**Enzyme Kinetic and Pharmacokinetic Analysis.** In vitro intrinsic clearance (Cl_{int}) was calculated from rat, dog, and human liver microsomes using substrate depletion data and the standard values of 45 mg microsomal protein/gram liver for all species, and 40, 32, and 20 gram liver/kg body weight for rat, dog, and human, respectively. In turn, hepatic clearance (CL_{h}) was predicted using the well-stirred model factoring in plasma protein binding (f_{u}), non-specific microsomal binding (f_{u(microsomes)}), and liver blood flow (Q) (70, 40, and 20.7 mL/min/kg for rat, dog, and human, respectively), according to the following equation (equation 1):

\[
CL_{h} = \frac{Q \cdot f_{u} \cdot CL_{int}}{f_{u(microsomes)}} \cdot \frac{1}{\frac{f_{u(microsomes)}}{Q + f_{u} \cdot CL_{int}}}
\] (1)
Enzyme kinetic parameters for CJ-13,610 sulfoxidation were estimated using nonlinear regression within GraphPad Prism (GraphPad Software Inc., San Diego, CA) software, and the following standard Michaelis-Menten velocity equation (equation 2):

\[ v = \frac{V_{\text{max}} \cdot [S]}{K_m + [S]} \]  

(2)

Intrinsic clearance from enzyme kinetic studies (Cl\text{'int}) was defined according to equation 3:

\[ Cl'_{\text{int}} = \frac{V_{\text{max}}}{K_m} \]  

(3)

Inhibition of CJ-13,610 sulfoxidation in human liver microsomes was expressed as IC\text{50}, and estimated according to equation 4, where “Top” is the maximum percent control activity and “Bottom” is the minimum percent control activity.

\[ Y = Bottom + \frac{Top - Bottom}{1 + 10^{(X - \log IC_{50})}} \]  

(4)

Pharmacokinetic parameters following intravenous and oral dosing to rats and dogs, and oral dosing to humans were estimated using noncompartmental analysis methods (WinNonlin v5.2, Pharsight, Palo Alto, CA). Total body clearance was calculated using the following standard equation (equation 5), where AUC is area under the plasma concentration-time curve, calculated using the linear trapezoidal rule:

\[ \text{clearance} = \frac{Dose}{AUC_{(0-\text{inf})}} \]  

(5)

Volume of distribution at steady-state (Vd\text{ss}) was calculated using the following equation (equation 6), where MRT is Mean Residence Time, and Clearance is total body clearance:
Human pharmacokinetic simulations were conducted using WinNonlin and a one compartment first order absorption and elimination (no lag) pharmacokinetic model. Human clearance was either predicted using hepatic clearance (CL\textsubscript{h}) estimated from in vitro human liver microsomal experiments as described above, or from single-species scaling from rat and dog pharmacokinetic studies (Hosea et al., 2009) using equation 7:

\[ \text{CL}_{\text{iv}}^{\text{predicted}} = \left( \frac{\text{BW}_{\text{human}}}{\text{BW}_{\text{animal}}} \right)^{0.75} \cdot \left( \frac{\text{CL}_{\text{iv}}}{\text{BW}_{\text{animal}}} \right) \cdot \left( \frac{\text{Fu}_{\text{human}}}{\text{BW}_{\text{human}}} \right) \]  

(7)

Where CL\textsubscript{iv} is clearance following intravenous administration, BW is body weight, and Fu is fraction unbound in plasma. Volume of distribution (V\textsubscript{dss}) was scaled and predicted using pharmacokinetic data generated from i.v. studies in rat and dog using equation 8:

\[ V_{d}^{\text{predicted}} = V_{d_{\text{animal}}} \cdot \frac{\text{Fu}_{\text{human}}}{\text{Fu}_{\text{animal}}} \]  

(8)

Absorption rate (k\textsubscript{a}, hr\textsuperscript{-1}) for human pharmacokinetic simulations was estimated using modeled rat oral pharmacokinetic data, consistent with reported data suggesting use of rats for predicting human absorption of orally administered drugs (Chiou and Barve, 1998).
Results

In Vitro Metabolism of CJ-13,610. The rates of in vitro liver microsomal metabolism of CJ-13,610 in rat, dog, and human liver microsomes, as well as plasma and microsomal binding are presented in Table 1. The in vitro intrinsic clearance (Cl_{int}) determined from substrate depletion methodologies was highest for rat, and lowest for dog (rat>human>dog), while plasma protein binding appeared to be highest in rat (f_u=0.07). The predicted hepatic clearance (Cl_h) using the well-stirred model over-estimated plasma clearance when neither plasma nor non-specific microsomal protein binding was considered. Non-specific binding to liver microsomes in vitro appears to be low (f_u(microsomes) ~ 0.8) and thus, did not have a major impact on the calculated hepatic clearance values (Table 1). However, factoring in both plasma and microsomal binding resulted in hepatic clearance (CL_h) estimates that more closely resembled total plasma clearance observed following intravenous dosing to rats and dogs (Table 1).

Characterization of CJ-13,610 and Metabolites In Vitro in Liver Microsomes. The LC/MS/UV data demonstrating the metabolic profile of CJ-13,610 in human liver microsomes is provided in Figure 2A. The protonated [M+H]^+ molecular ion for CJ-13,610 was observed at m/z 394. Fragmentation of CJ-13,610 produced a key fragment ion at m/z 349 that corresponded to the loss of the amide moiety (Figure 2B). A subsequent ion produced from m/z 349 was observed at m/z 320 and corresponded to the further fragmentation of the tetrahydropyran moiety. The fragment ion at m/z 189 corresponded to the cleavage of the arylsulfide and proved useful in the identification of metabolites of CJ-13,610 (e.g., M5). Overall, the metabolite profile was consistent across
the species tested (rat, dog, and human, data not shown). The proposed metabolic pathways of CJ-13,610 in human is depicted in Figure 3.

**Metabolite M1.** Oxidation of the imidazole moiety resulted in the proposed acetamidine metabolite, M1 ([M+H]+ at \(m/z\) 370), based in part on the major fragmentation ion at \(m/z\) 353 that corresponded to a loss of 17 Da (NH\(_3\)). The loss of ammonia was not observed in the parent CJ-13,610 mass spectrum; furthermore, the presence of an ion at \(m/z\) 325 (-45 Da) indicated the amide moiety was intact.

**Metabolite M2.** The oxidized metabolite producing a [M+H]+ at \(m/z\) 410 was proposed as the sulfoxide, M2. A loss of both 18 Da (-H\(_2\)O) and 17 Da (-OH) is consistent with sulfoxidation. The presence of an ion at \(m/z\) 365 indicated that the amide moiety was intact. The fragmentation pattern of M2 was consistent with the synthetically prepared sulfoxide standard (CP-680179).

**Metabolite M3.** The metabolite producing an [M+H]+ at \(m/z\) 410 was proposed to bear a hydroxylation at the tetrahydropyran moiety (M3). The major dehydrated ion at \(m/z\) 392 (50 % relative abundance) was indicative of an aliphatic hydroxylation. The presence of an ion at \(m/z\) 365 indicated that the amide moiety was intact.

**Metabolite M4.** The metabolite bearing an addition of 32 Da over that of parent CJ-13,610 produced an [M+H]+ at \(m/z\) 426. The presence of fragment ions at 408 and 407 Da was indicative of a loss of H\(_2\)O and hydroxyl (-OH) and similar to that observed with M2. The ion at \(m/z\) 220 represented an oxidation of the fragment ion of CJ-13,610 at \(m/z\) 189. Together these data indicate M4 to be a sulfone metabolite (a secondary metabolite of M2).
Metabolite M5. The metabolite bearing an addition of 34 Da over that of parent CJ-13,610 produced an [M+H]⁺ at m/z 428. Proposed as the dihydrodiimidazole metabolite, M5, the collision-induced fragment at m/z 370 (-58 Da) indicated a biotransformation event at the 4,5 position of the imidazole moiety; incidently producing a protonated fragment ion isobaric to that of M1. A secondary fragment of the ion at m/z 370, was observed at m/z 353, and corresponded to the loss of ammonia (-17 Da) from the resulting acetamidine moiety.

Metabolite M6. The dihydropyran metabolite M6 produced an [M+H]⁺ at m/z 392. The collision-induced fragmentation of this metabolite produced an ion at m/z 364, indicating a facile loss of ethylene (-28 Da) from the dihydropyran moiety. The loss of formamide at m/z 348 and the cleavage of the arylsulfide linkage indicated the remainder of the compound had been unaltered.

Metabolite M7. The metabolite producing an [M+H]⁺ at m/z 408 bore biotransformation events of the arylsulfide (sulfoxidation) and the tetrahydropyran moieties. Consistent with that of M2, a major fragment ion observed was at m/z 391 and corresponded to the loss of 17 Da (-OH), a pattern consistent with sulfoxidation. The overall mass of the remainder scaffold being 2 Da less than that of M2 and parent CJ-13,610 implicated the corresponding oxidation of the pyran moiety to the dihydropyran oxidation state.

CJ-13,610 Sulfoxidation Kinetics. The sulfoxide metabolite of CJ-13,610 (CP-680179) was available as a synthetic standard, which afforded the ability to compare the enzyme kinetics for this predominant metabolic pathway across species. The enzyme kinetics of CJ-13,610 sulfoxidation in human liver microsomes was described by the classical
Michaelis-Menten equation (Figure 4), with the apparent kinetic constants shown in Table 1 for rat, dog and human. The apparent Michaelis-Menten constant (K_{m,app}) was similar across species (4.1 to 5.4 μM). However, maximal rates (V_{max}) varied by greater than three-fold, with the highest V_{max} observed in rat liver microsomes (RLMs). Intrinsic clearance (Cl{int}') was determined by dividing V_{max} by K_{m,app} (Equation 3), and the rank order was rat > human > dog (Table 1). Interestingly, Cl{int}' estimates from sulfoxidation kinetics closely resembled Cl_{int} values derived from substrate depletion studies (Table 1).

**Cytochrome P450 Identification.** Data illustrating substrate depletion (percent remaining) of CJ-13,610 following incubation for 30 min with a battery of recombinant human P450s (1A2, 2A6, 2B6, 2C8, 2C9, 2C19, 2D6, 2J2, 3A4, 3A5), recombinant human FMO3, and human liver microsomes are shown in Figure 5A. It is apparent that P450 3A4 and 3A5 play a predominant role in the metabolism of CJ-13,610, while P450s 2C8 and 2D6 demonstrated minimal activity towards CJ-13,610 (84% ± 0.07, and 85% ± 0.21 remaining, respectively). Negligible activity was observed for the remaining P450 enzymes tested. Considering the sulfoxidation of CJ-13,610 was observed as a predominant pathway of metabolism, the role of human flavin monooxygenase (hFMO3) was investigated. Surprisingly, hFMO3 did not show activity towards CJ-13,610, while the positive control substrate, benzydamine, was rapidly metabolized (data not shown).

Lastly, ketoconazole, an established potent inhibitor of P450 3A in vitro, was co-incubated with CJ-13,610 in human liver microsomes (Figure 5B), and shown to potently inhibit the formation of the sulfoxide metabolite from CJ-13,610 (IC_{50} = 7 nM).

**Pre-Clinical and Clinical Pharmacokinetics of CJ-13,610.** The pharmacokinetics of CJ-13,610 in rats and dogs were modeled employing a non-compartmental analysis.
approach. Pharmacokinetics in the rat were described by a total body clearance of 11.5 mL/min/kg, an apparent volume of distribution at steady-state (Vd_{ss}) of 3.6 L/kg, and a mean residence time (MRT) of 5.2 hours. Pharmacokinetics in the dog were described by a total body clearance of 4.4 mL/min/kg, a volume of distribution at steady-state (Vd_{ss}) of 2.9 L/kg, and a mean residence time (MRT) of 11 hours. Less than 1% of parent CJ-13,610 was recovered in urine following intravenous dosing in both rats and dogs, which indicated negligible urinary clearance in the disposition of CJ-13,610. Pharmacokinetic parameters following single oral doses of CJ-13,610 in humans (n=4) are shown in Table 3. Exposure as estimated by AUC and C_{max}, was linear and proportional up to a dose of 300 mg (Figure 6), and the overall mean plasma elimination half-life (T_{1/2}) was comparable at all dose levels (Table 3).

**Pharmacokinetic Simulations.** In an effort to model the human pharmacokinetic profile of CJ-13,610, multiple methods were applied, ranging from in vitro scaling to single-species allometric scaling from rat and dog. The primary objective of this investigation was to demonstrate that scaling in vitro intrinsic clearance using human liver microsomes would accurately estimate clearance in human subjects. In support of this approach, an in vitro-in vivo correlation using liver microsomal intrinsic clearance was assessed in rat and dog. As demonstrated in Table 1, predicted hepatic clearance (CL_{h}) in rat and dog very closely resembled total plasma clearance observed in pharmacokinetic studies (within 2-fold). When clearance was scaled from either human liver microsomes (Vd_{ss} scaled from dog) or single-species allometric scaling from dog (CL = 3.2 mL/min/kg), the pharmacokinetic profile in humans was closely predicted, with the AUC following a 30 mg single oral dose predicted within 10%, roughly comparable to the observed
standard deviation in exposure observed between four subjects (Table 4 and Figure 7). Similarly, the predicted maximal concentration following oral dose ($C_{\text{max}}$), and half-life was predicted within 1 to 1.5 standard deviations of observed pharmacokinetic parameters (Table 4). When $V_{dss}$ scaled from rat pharmacokinetics studies (6.7 L/kg) was combined with clearance scaled from human liver microsomes, the AUC was closely predicted, but the $C_{\text{max}}$ was under-predicted, and the half-life was over-predicted. Single-species allometric scaling of clearance and $V_{dss}$ from rat pharmacokinetic studies was less accurate, with the predicted AUC accounting for roughly 60% of that observed in human subjects, and the predicted $C_{\text{max}}$ being ~33% of the observed value (Table 4 and Figure 7).
Discussion

CJ-13,610 is a novel reversible inhibitor of 5-lipoxygenase (5-LOX), a critical enzyme in the arachidonic acid cascade that catalyzes the initial step in the ultimate formation of numerous pro-inflammatory leukotrienes. Preclinical pharmacology data suggests that chemical modulation of the 5-LOX pathway by CJ-13,610 has potential therapeutic benefits in disease areas such as asthma, liver fibrosis, and pain (Werz and Steinhilber, 2006; Horrillo et al., 2007; Zweifel et al., 2008; Cortes-Burgos et al., 2009). In the investigations described herein, in vitro studies were conducted with CJ-13,610 to delineate the in vitro hepatic metabolism of this compound in an effort to evaluate the in vitro-in vivo correlation for metabolic clearance, and enable the prediction of the pharmacokinetic profile of CJ-13,610 observed in humans following single-dose administration.

Methodologies for scaling of in vitro metabolism data to predict the in vivo behavior of drugs have been proposed for many years (Houston, 1994; Iwatsubo et al., 1997; Obach, 2001), and there are numerous examples of successful predictions of the in vivo pharmacokinetic properties of drugs using in vitro data (Obach, 2000; Obach et al., 2007; Gilissen et al., 2000; Kuperman et al., 2001). When investigating this type of in vitro-in vivo relationship using liver microsomes, there are some requisite assumptions; (1) clearance must be primarily mediated by phase I metabolism in the liver (e.g. P450, FMO), and (2) not susceptible to other non-P450 mediated metabolism or excretion. Indeed, results of metabolism studies of CJ-13,610 in humans demonstrated that this compound possesses the aforementioned properties. Specifically, our data indicated that the in vitro biotransformation of CJ-13,610 results primarily in metabolites arising from...
s-oxidation (M2, Figure 2a) and imidazole oxidation (M1, Figure 2a), while oxidation of the tetrahydropyran moiety was also observed (M3) as a principal biotransformation pathway in CJ-13,610 metabolism (Figure 3). Importantly, metabolic profiling studies in rat and dog microsomes also indicate that the aforementioned biotransformation pathways significantly contribute to the hepatic metabolism of CJ-13,610 (data not shown).

Intrinsic clearance data generated using the traditional substrate depletion approach and the well-stirred model (equation 1) demonstrated that predicted hepatic clearance (Cl\textsubscript{h}), when corrected for both plasma protein and non-specific microsomal binding, was comparable to total plasma clearance (within 1.6-fold) observed for both rat and dog (Table 1). Microsomal binding has been reported to potentially impact estimates of hepatic clearance when using microsomal fractions, especially for basic lipophilic amines (Obach, 1997, 1999). While binding to plasma proteins was moderately high across species tested (f\textsubscript{u} = 0.07 to 0.13), non-specific binding to microsomes was low (f\textsubscript{u(microsomes)} ~0.8), and did not have a dramatic impact on the calculated hepatic clearance (still within 2-fold of total plasma clearance). Nonetheless, when correcting for both plasma and non-specific microsomal binding, estimates of hepatic clearance were more representative of total in vivo clearance (Table 1).

Interestingly, intrinsic clearance (Cl\textsuperscript{′}\textsubscript{int}) for the sulfoxidation of CJ-13,610 calculated by Equation 3 following enzyme kinetic analysis, closely resembled total intrinsic clearance calculated from substrate depletion methodologies. While sulfoxidation was not the only metabolic pathway observed in liver microsomes following incubations with CJ-13,610 (20 µM) for metabolite profiling (Figure 3), the
observation that intrinsic clearance calculated by $V_{\text{max}}/K_m$ for the sulfoxidation pathway was comparable to that calculated by substrate depletion methodologies (e.g. total metabolism) suggests that sulfoxidation is the predominant metabolic pathway at lower and more relevant concentrations (e.g. ~1 µM). In fact, the sulfoxide has been reported to be the predominant circulating metabolite in plasma from humans following oral administration (Dalvie et al., 2009).

Experiments were also conducted in effort to identify which liver microsomal enzymes were involved in the metabolism of CJ-13,610. When incubated with a battery of recombinantly expressed cytochrome P450 enzymes, it appeared that the P450 3A family (P450 3A4 and 3A5) were predominantly involved in the overall metabolism of CJ-13,610. In addition, when co-incubated with the potent P450 3A inhibitor ketoconazole, sulfoxidation of CJ-13,610 was inhibited with an estimated IC$_{50}$ of 7 nM, closely resembling the inhibitory potency of ketoconazole towards known selective probe substrates of P450 3A4 such as midazolam and testosterone (Racha et al., 2003). With P450 3A being the predominant metabolic pathway, as suggested by our in vitro studies, CJ-13,610 could be at risk for victim drug-drug interactions if co-administered with potent inhibitors of P450 3A4 such as azole antifungals or antimicrobials such as erythromycin. Clinical drug-drug interaction studies would have to be conducted to determine the magnitude of this potential interaction. Interestingly, human flavin monooxygenase 3 (hFMO3), a microsomal enzyme that typically oxidizes nucleophilic heteroatoms, did not appear to be involved in the sulfoxidation of CJ-13,610 (Figure 5A), while benzydamine, a reported probe substrate for FMO3 (Stormer et al., 2000), was efficiently metabolized (data not shown). This was somewhat surprising when
considering the rapid oxidation of the sulfur by P450 3A4 and 3A5, and literature reports confirming that sulfur-containing drug molecules may be substrates for FMO3, including cimetidine, methimazole, and methyltolylsulfide (Cashman, 2000). Unfortunately, detailed structure-activity relationship (SAR) differences between cytochrome P450 and FMO enzymes have not been thoroughly investigated, so the chemical properties of CJ-13,610 that preclude a role for hFMO3 are not evident at this time. Recombinant human FMO1 and FMO5 were not tested for CJ-13,610 sulfoxidation activity, since hFMO3 is the primary isoform present in human liver.

The pharmacokinetic properties of CJ-13,610 were characterized in an early clinical program with healthy subjects following single-dose administration. Exposure was linear and proportional up to a dose of 300 mg (Figure 6). With this data available, we chose to retrospectively evaluate various scaling methods to see which may be the most optimal for predicting the pharmacokinetic profile of CJ-13,610 in humans, which would be tremendously valuable for any discovery programs working with this chemical class of compounds. Firstly, based on the favorable in vitro-in vivo correlation for clearance observed with rat and dog (Table 1), we chose to scale clearance from human liver microsomes, and use volume of distribution from rat and dog single-species allometric scaling to compare simulations to the observed pharmacokinetic profile following a 30 mg single oral dose. This dose was arbitrarily selected for simulation, considering that dose-linearity was observed with CJ-13,610 up to 300 mg. Due to a larger Vdss scaled from rat (6.7 L/kg), the half-life when using HLM to predict clearance was longer (21.7 hrs) than observed (13.4 hrs), even though the AUC was closely predicted (Table 4). This resulted in projected Cmax (51 ng/mL) that was roughly one-
third the observed $C_{\text{max}}$ (144 ng/mL). However, when clearance from HLM was scaled to humans and $V_{\text{dss}}$ was scaled from dog, the pharmacokinetic profile of CJ-13,610 was predicted within 10\% for AUC, resulting in a favorable half-life projection of 10.4 hrs (Table 4). In this case, it appears then, that dog would be a preferred species for pharmacokinetic characterization and more specifically, for predicting $V_{\text{dss}}$ in humans. A report that was recently published by colleagues at Pfizer suggested that allometric scaling from a single species (e.g. single-species scaling, SSS) may be just as successful as full allometric scaling using multiple species (Hosea et al., 2009). Therefore, these methods were also investigated for their predictive ability. When attempting to predict the pharmacokinetic profile of CJ-13,610 using rat SSS, the AUC and $C_{\text{max}}$ were underpredicted, due to the higher projected clearance and $V_{\text{dss}}$, although the projected half-life closely predicted the observed (Table 4). Interestingly, when the same single-species approach was taken using dog, the pharmacokinetic profile for CJ-13,610 was predicted with comparable success compared to using in vitro HLM data, due to similar scaled clearance (3.2 mL/min/kg from dog SSS).

In summary, CJ-13,610 appears to be metabolized in vitro exclusively by the cytochrome P450 family of drug metabolizing enzymes, and specifically the CYP3A subfamily. Oxidation of the sulfur heteroatom was the predominant metabolic pathway and was consistently observed across the species tested. Overall, a good in vitro-in vivo correlation was observed in rat and dog, and the human pharmacokinetic profile of CJ-13,610 observed in the clinic following single oral dose was closely predicted using traditional scaling methods from human liver microsomes. This data provides confidence moving forward that scaling in vitro intrinsic clearance data from HLM may be a
successful approach for predicting clearance in humans for this chemical series. It is anticipated that structurally related analogs within the same chemical class will likely possess similar metabolic clearance routes, and thus the use of predicted hepatic clearance derived from human liver microsomes may result in a high probability of successful pharmacokinetic predictions in humans, and serve as an efficient method for selection of additional drug candidates with suitable pharmacokinetic properties.
Acknowledgements

The authors would like to thank the in vivo group (Kathy Hotz, Steve Wene, and Lesley Albin) for conducting pharmacokinetic studies for CJ-13,610, Michael Baratta and I. Rochelle Riley for determining the plasma and microsomal protein binding for CJ-13,610, and Connie Wagner for conducting phenotyping experiments for CJ-13,610.
References


Kuperman AV, Kalgutkar AS, Marfat A, Chambers RJ and Liston TE (2001) Pharmacokinetics and Metabolism of a Cysteinyl Leukotriene-1 Receptor Antagonist


Footnotes

1Current Address: Boehringer-Ingelheim Pharmaceuticals Inc., Ridgefield, Connecticut

2Current Address: Seventh Wave Laboratories, Chesterfield, Missouri

3Results from rat studies indicated a negligible role for hepatobiliary transport in the disposition of CJ-13,610 (data not shown).
Legends for Figures

Figure 1. Structure of CJ-13,610.

Figure 2. A) Representative LC/MS (I) and HPLC/UV (II) profiles of CJ-13,610 metabolites obtained from human liver microsomes, and B) the mass spectrum of CJ-13,610 obtained by LC/MS/MS. Conditions are as described in the Materials and Methods section.

Figure 3. Proposed metabolic scheme of CJ-13,610 in human liver microsomes.

Figure 4. Substrate saturation plots of CJ-13,610 sulfoxidation in rat (■), dog (▲) and human (●) liver microsomes. Incubation conditions consistent with Michaelis-Menten steady-state enzyme kinetics are described in the Materials and Methods section.

Figure 5. In vitro characterization of the enzymes involved in the metabolism of CJ-13,610. A) Activity across a panel of recombinantly expressed P450 enzymes, recombinant human FMO3, and HLMs, and B) Inhibition of CJ-13,610 sulfoxidation in human liver microsomes by the CYP3A inhibitor ketoconazole (IC50 = 7 nM).

Figure 6. Linearity of pharmacokinetic parameters AUC and Cmax observed following single-dose oral administration of CJ-13,610 to human subjects (n=4).

Figure 7. Simulated human pharmacokinetic profile of CJ-13,610 using various scaling methodologies compared to the clinical human pharmacokinetic profile following a single 30 mg oral dose to normal healthy subjects (n=4). An absorption rate constant (ka) of 0.83 hr⁻¹ (estimated by modeling rat oral pharmacokinetic data) was used for human pharmacokinetic simulations, and clearance and Vdss parameters used in simulations are summarized in Table 4. All simulations were conducted using WinNonlin 5.2.
Table 1. Summary of substrate depletion kinetics of CJ-13,610, and enzyme kinetic parameters for the sulfoxidation metabolic pathway in rat, dog and human liver microsomes. Numbers in parenthesis indicate standard error (SE) of nonlinear regression analysis.

Reaction velocities were estimated as described in Materials and Methods.

<table>
<thead>
<tr>
<th>Substrate Depletion</th>
<th>Rat</th>
<th>Dog</th>
<th>Human</th>
</tr>
</thead>
<tbody>
<tr>
<td>In vitro half-life (min)</td>
<td>26</td>
<td>71</td>
<td>28</td>
</tr>
<tr>
<td>f_{u(plasma)}</td>
<td>0.07</td>
<td>0.12</td>
<td>0.13</td>
</tr>
<tr>
<td>f_{u(microsomes)}</td>
<td>0.81</td>
<td>0.79</td>
<td>0.83</td>
</tr>
<tr>
<td>Cl_{int}</td>
<td>54</td>
<td>19</td>
<td>31</td>
</tr>
<tr>
<td>(µl/min/mg microsomal protein)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Predicted CL_{h} (mL/min/kg)^a</td>
<td>40.6</td>
<td>16.5</td>
<td>11.7</td>
</tr>
<tr>
<td>Predicted CL_{h} (mL/min/kg)^b</td>
<td>6.2</td>
<td>3.1</td>
<td>3.1</td>
</tr>
<tr>
<td>Predicted CL_{h} (mL/min/kg)^c</td>
<td>7.4</td>
<td>3.8</td>
<td>3.6</td>
</tr>
<tr>
<td>Cl_{plasma} (mL/min/kg)</td>
<td>11.5</td>
<td>4.4</td>
<td>-</td>
</tr>
</tbody>
</table>

| Enzyme Kinetics (CJ-13,610 Sulfoxidation) | |
| K_{m,app} (µM) | 4.1 (0.55) | 5.4 (0.32) | 4.8 (0.59) |
| V_{max} (pmol/min/mg protein) | 247 (8.1) | 77.9 (1.1) | 184 (5.4) |
| Cl'_{int} d | 60 | 14 | 38 |
| (µl/min/mg microsomal protein) |        |         |         |

^a No plasma or microsomal binding correction (Equation 1)

^b Corrected for plasma protein binding only (Equation 1)

^c Corrected for both plasma and non-specific microsomal binding (Equation 1)

^d Calculated using Equation 3.

CL_{h} = hepatic clearance
Table 2. Summary of estimated pharmacokinetic parameters (SD) after intravenous administration of CJ-13,610 to rats and dogs (n=3) using non-compartmental pharmacokinetic analysis.

<table>
<thead>
<tr>
<th>PK Parameter</th>
<th>Rat</th>
<th>Dog</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dose (mg/kg)</td>
<td>0.5</td>
<td>0.1</td>
</tr>
<tr>
<td>AUC (µM*hr)</td>
<td>1.83 (0.11)</td>
<td>0.94 (0.04)</td>
</tr>
<tr>
<td>Clearance (mL/min/kg)</td>
<td>11.5 (0.69)</td>
<td>4.4 (0.17)</td>
</tr>
<tr>
<td>Vdss (L/kg)</td>
<td>3.6 (0.17)</td>
<td>2.9 (0.29)</td>
</tr>
<tr>
<td>MRT (hours)</td>
<td>5.2 (0.4)</td>
<td>11 (1.5)</td>
</tr>
</tbody>
</table>
Table 3. Summary of mean (standard deviation, SD) pharmacokinetics of CJ-13,610 in healthy fasted male subjects following single oral doses administered as solution.

<table>
<thead>
<tr>
<th>Dose (mg)</th>
<th>AUC(0–∞)$^a$ (ng·hr/mL)</th>
<th>C$_{\text{max}}$$^a$ (ng/mL)</th>
<th>T$_{\text{max}}$ (hr)</th>
<th>T$_{1/2}$ (hr)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>NC$^b$</td>
<td>2.1 (0.6)</td>
<td>6.8</td>
<td>NC$^b$</td>
</tr>
<tr>
<td>3</td>
<td>249 (73)</td>
<td>11 (3)</td>
<td>5.0</td>
<td>14.8</td>
</tr>
<tr>
<td>10</td>
<td>783 (279)</td>
<td>34 (10)</td>
<td>4.5</td>
<td>16.2</td>
</tr>
<tr>
<td>30</td>
<td>1970 (220)</td>
<td>144 (34)</td>
<td>1.8</td>
<td>13.4</td>
</tr>
<tr>
<td>100</td>
<td>6240 (2520)</td>
<td>684 (263)</td>
<td>1.5</td>
<td>13.1</td>
</tr>
<tr>
<td>300</td>
<td>19700 (4000)</td>
<td>2110 (1000)</td>
<td>3.5</td>
<td>14.6</td>
</tr>
</tbody>
</table>

$^a$ Mean and SD are geometric for AUC and C$_{\text{max}}$

$^b$ NC indicates not calculated due to insufficient data
Table 4. Comparison of simulated human pharmacokinetic parameters using various scaling methodologies for clearance and volume of distribution (Vdss) and pharmacokinetics (mean, SD) of CJ-13,610 after a 30 mg single oral dose to healthy male subjects (n=4).

<table>
<thead>
<tr>
<th>Scaling Methodology</th>
<th>Clearance (mL/min/kg)</th>
<th>Vdss (L/kg)</th>
<th>AUC (0-inf) (ng*hr/ml)</th>
<th>Cmax (ng/mL)</th>
<th>T1/2 (hrs)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Human liver microsomes (Vdss scaled from rat)</td>
<td>a 3.6</td>
<td>6.7</td>
<td>1802</td>
<td>51</td>
<td>21.7</td>
</tr>
<tr>
<td>Human liver microsomes (Vdss scaled from dog)</td>
<td>a 3.6</td>
<td>3.2</td>
<td>1802</td>
<td>97</td>
<td>10.4</td>
</tr>
<tr>
<td>Scaled from rat PK</td>
<td>b 5.3</td>
<td>6.7</td>
<td>1222</td>
<td>48</td>
<td>14.7</td>
</tr>
<tr>
<td>Scaled from dog PK</td>
<td>b 3.2</td>
<td>3.2</td>
<td>2014</td>
<td>98</td>
<td>11.6</td>
</tr>
<tr>
<td>Single Oral Dose (30 mg)</td>
<td>-</td>
<td>-</td>
<td>1970 (220)</td>
<td>144 (34)</td>
<td>13.4 (2.1)</td>
</tr>
</tbody>
</table>

aPredicted clearance in human using Equation 1.

bPredicted clearance in human using Equation 7.

cPredicted Vdss in human from either rat or dog using Equation 8.

All pharmacokinetic simulations were conducted using WinNonlin, and an absorption rate constant (ka) of 0.83 hr\(^{-1}\) (estimated by modeling rat oral pharmacokinetic data) was used. High oral bioavailability was also assumed.
Figure 6

The graph shows the relationship between AUC(0-∞) ng/hr/mL and Dose (mg) for 0, 100, 200, and 300 mg. The AUC(0-∞) increases linearly with increasing dose. Similarly, the graph for C_max (ng/mL) also shows a linear increase with increasing dose.
Figure 7

CJ-13,610 Plasma Concentration (ng/mL) vs. Time (hr)

- HLM (Vdss from rat PK)
- HLM (Vdss from dog PK)
- Scaled from rat PK
- Scaled from dog PK
- Single oral dose 30 mg (n=4)