Interplay of Dissolution, Solubility and Non-Sink Permeation
Determines the Oral Absorption of the Hedgehog Pathway Inhibitor, GDC-0449, in Dogs: An Investigation using Preclinical Studies and Physiologically- Based Pharmacokinetic Modeling

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List of abbreviations: AUC\(_{0-\infty}\): Area under the plasma concentration-time profile extrapolated to infinity; AUC\(_{0-24}\): Area under the plasma concentration-time profile from time 0 to 24 hours post-dose; AUC\(_{0-168}\): Area under the plasma concentration-time profile from time 0 to 168 hours post-dose; C\(_{\text{max}}\): highest observed plasma concentration; CL: plasma clearance; CL\(_{\text{biliary}}\): biliary clearance; IV: intravenous; PO: oral; t\(_{1/2}\): half-life; t\(_{\text{max}}\): time at which C\(_{\text{max}}\) is observed; V\(_{ss}\): Volume of distribution at steady-state.
ABSTRACT

Factors determining the pharmacokinetics of GDC-0449 (2-chloro-N-(4-chloro-3-(pyridine-2-yl)phenyl)-4-(methylsulfonyl)benzamide) were investigated using preclinical studies and physiologically-based pharmacokinetic (PBPK) modeling. Multiple dose studies where dogs were given twice daily oral (PO) doses of either 7.5 mg/kg or 25 mg/kg GDC-0449 showed less than dose proportional increases in exposure on Day 1. At steady-state, exposures were comparable between the two dose groups. Oral administration of activated charcoal to dogs receiving PO or intravenous (IV) GDC-0449 (25 mg) showed a more rapid decrease in plasma concentrations suggesting that the concentration gradient driving intestinal membrane permeation was reversible. The biliary clearance of GDC-0449 in dogs was low (0.04 ml/min/kg) and did not account for the majority of the estimated systemic clearance (~19% of systemic clearance). Similarly, in vitro studies using sandwich-cultured human hepatocytes showed negligible biliary excretion. The effect of particle size on oral absorption was demonstrated in a single dose study where 150 mg of GDC-0449 of two particle sizes was administered. An oral PBPK model was used to investigate mechanisms determining the oral pharmacokinetics of GDC-0449. The overall oral absorption of GDC-0449 appears dependent on the interplay between the dissolution and intestinal membrane permeation processes. A unique feature of GDC-0449 distinguishing it from other Biopharmaceutics Classification System II compounds was that incorporation of the effects of solubility rate-limited absorption and non-sink permeation on the intestinal membrane permeation process was necessary to describe its pharmacokinetic behavior.
INTRODUCTION

The hedgehog (Hh) signaling pathway regulates proliferation and differentiation during embryogenesis. Hh ligands bind to Patched (PTCH1), a transmembrane protein on target cells. In the absence of Hh, the role of PTCH1 is to inhibit the activity of Smoothened (SMO), a seven-transmembrane protein that serves as the signaling component of the pathway. Binding of Hh proteins to PTCH1 relieves this inhibition and initiates activation of SMO. The increase in SMO activity causes increases in activated forms of Gli, transcriptional factors which serve to regulate the expression of Hh target genes. Activation of the Hh pathway has been implicated in a number of cancers (Scales and de Sauvage, 2009). Mutations in the Hh receptor components, PTCH1 or SMO, result in constitutive pathway activation and have been identified in basal cell carcinoma (Hahn et al., 1996; Johnson et al., 1996) and medulloblastoma (Pietsch et al., 1997; Raffel et al., 1997; Vorechovsky et al., 1997). It has also been observed that aberrant Hh ligand production can contribute to the growth of other tumor types such as colorectal and pancreatic cancer (Yauch et al., 2008), prostate cancer (Fan et al., 2004), and B cell lymphoma (Dierks et al., 2007) through paracrine activation of the Hh pathway. Paracrine signaling typically involves ligand expressed on the cancer cells signaling adjacent stromal components in the case of solid tumors, or signaling from stromally produced Hh ligand to cancer cells in the case of hematopoietic cancers. The growing scientific data associating Hh signaling pathway activation with certain types of cancers has made this pathway an attractive target for the development of selective small molecule inhibitors.
GDC-0449, 2-chloro-N-(4-chloro-3-(pyridin-2-yl)phenyl)-4-(methylsulfonyl)benzamide (Figure 1) is a small molecule inhibitor of the Hh signaling pathway currently being developed at Genentech, Inc. It inhibits Hh signaling with IC₅₀s of 13 and 2.8 nM in Hh responsive cell lines derived from mouse (10T1/2) and human embryonic palatal mesenchyme cells, respectively. Hh signaling is blocked by GDC-0449 through binding to and inhibiting SMO (Yauch et al., 2009). Previously, we described the preclinical absorption, distribution, metabolism and excretion properties of GDC-0449 (Wong et al., 2009). The compound exhibited low plasma clearance in mouse, rat, and dog and moderate clearance in the monkey. These in vivo observations were consistent with in vitro metabolic stability studies performed using hepatocytes. Recently, the oral pharmacokinetics of GDC-0449 in humans has been described (Von Hoff et al., 2009; Ding et al., 2010). The clinical pharmacokinetics was characterized by remarkably high plasma exposures suggestive of a low systemic clearance. Of the preclinical species tested, the dog exhibits characteristics most similar to humans having the lowest plasma clearance and longest t₁/₂ in vivo, and showing virtually no turnover after a 3 hour incubation with dog and human hepatocytes (Wong et al., 2009). Detailed studies aimed at understanding the disposition of new chemical entities in humans are often difficult to perform due to obvious ethical restrictions. Here we describe the results of the preclinical studies and physiologically-based pharmacokinetic (PBPK) modeling using the dog to provide insight into understanding the pharmacokinetic characteristics of GDC-0449.
METHODS

In Vivo Pharmacokinetic Studies in Dogs

Bile-Duct Cannulated Dog Study: This study was performed to assess the role of biliary clearance on the in vivo disposition of GDC-0449 in dogs. At study initiation, dogs used weighed from 7.9 to 9.9 kg. Intact (n=4) and bile-duct cannulated (n=4) male beagle dogs (Wuxi Pharmatech Co. Ltd., Shanghai, China) were given a single 25 mg IV bolus dose of GDC 0449 (Genentech Inc., South San Francisco, CA) in 80% v/v polyethylene glycol 400 in water. Animals were not fasted prior to dosing. Blood samples (approximately 0.5 mL per sample) were collected from a peripheral vessel into tubes containing potassium ethylenediaminetetraacetic acid (K2EDTA) at the following timepoints: predose and 0.033, 0.083, 0.25, 0.5, 1, 2, 4, 8, 12, 24, 48, 72, 96, 120, 144, and 168 hours post dose. Samples were centrifuged within 1 hour of collection. Plasma was collected and kept frozen on dry ice before storage at approximately -70 °C. Bile was collected from bile-duct cannulated dogs at the following time intervals: predose, between 0 and 8 hours post dose, between 8 and 24 hours post dose, between 24 and 48 hours post dose, and between 48 and 72 hours post dose. Bile was collected into containers cooled by wet ice and volumes for different intervals were measured and recorded. All bile samples were stored at approximately -80°C. GDC-0449 in plasma and bile was measured using a liquid chromatography tandem mass spectrometric method (LC/MS/MS) (Wong et al., 2009).
Activated Charcoal Studies: The purpose of this study was to examine the effect of oral administration of activated charcoal on the oral and intravenous pharmacokinetics of GDC-0449 in dogs. At study initiation, dogs used weighed from 8.3 to 11.8 kg. Two groups of male beagle dogs (n=4 per group) (Covance, Kalamazoo, MI) received a single oral (PO) dose of GDC-0449 as a 25-mg capsule. A third group of dogs (n=4) received a single 25-mg IV dose of GDC-0449 in 80% PEG 400 via a cephalic vein. One group of orally dosed animals and all intravenously dosed animals received a 2 g/kg dose of activated charcoal (Sigma-Aldrich, St. Louis, MO) as a slurry, via oral gavage, at approximately 24, 27, 31, 36, 48, 60, 72, 84, and 96 hours following GDC-0449 administration. All animals were fasted overnight before dosing until approximately 4 hours following the GDC-0449 dose. At approximately 30 minutes before GDC-0449 administration, all animals received a single intramuscular injection of 0.024 mL/kg pentagastrin (6 μg/kg) in the left thigh. Blood samples (approximately 3 mL per sample) were collected from the jugular vein of each animal at the following timepoints: predose and 0.083, 0.25, 0.5, 1, 2, 4, 8, 12, 24, 72, 96, 120, and 168 hours post-dose. All blood samples were collected into tubes containing K2 EDTA and then chilled on ice until centrifugation. Samples were centrifuged within 1 hour of collection. Plasma was collected and kept frozen on dry ice before storage at approximately -70 °C. Concentrations of GDC-0449 in plasma were quantitated by LC/MS/MS (Wong et al., 2009).

Multiple Dose Pharmacokinetics of GDC-0449: Multiple dose oral pharmacokinetics of GDC-0449 was obtained from toxicology studies with GDC-0449. Male beagle dogs
were given twice-daily oral doses of 7.5 mg/kg (15 mg/kg/day) or 25 mg/kg (50 mg/kg/day) of GDC 0449, respectively, for 91 days. The two daily doses were administered 6 hours apart on each dosing day. At the initiation of the study, dogs were at least 8 months old and weighed 7.0 to 10.7 kg. Blood samples were collected for a 24 hour period starting after the administration of the first dose on days 1, 44, and 90 at predose (30 minutes before the first daily dose) and at 1, 3, 5.75, 6.5, 10, and 24 hours post dose. Blood samples (approximately 1 mL each) were collected from a jugular vein into tubes containing K$_2$EDTA and then chilled until centrifugation. Samples were centrifuged within 1 hour of collection. Plasma was collected and stored frozen at approximately -60°C to -80°C. Concentrations of GDC-0449 in plasma were quantitated using a validated LC/MS/MS method similar to one described previously (Wong et al., 2009). Results from Day 1 and Day 44 are presented in this manuscript as steady-state was achieved by Day 44.

**Oral Formulation Comparison Study:** The purpose of this study was to evaluate the impact of alterations in compound particle size on oral exposure of GDC-0449. At the initiation of this study, dogs weighed from 12.7 to 15.6 kg. Male beagle dogs (Covance, Kalamazoo, MI) were given an oral 150 mg dose of GDC-0449 as a single 150 mg capsule (n=6; smaller particle size; d(50) 21 µm) or as 25 and 125 mg capsules (n=6; larger particle size; d(50) 120 µm). Approximately 30 minutes prior to GDC-0449 administration, animals in both groups received a single 6 µg/kg intramuscular dose of pentagastrin (Sigma-Aldrich, St. Louis, MO) at 0.024 mL/kg. Animals were fasted overnight prior to dosing through approximately 4 hours postdose. Blood samples
(approximately 3 mL per sample) were collected from the jugular vein of each animal at the following timepoints: predose and 0.083, 0.25, 0.5, 1, 2, 4, 8, 12, 24, 72, 96, 120 and 168 hours postdose. All blood samples were collected into tubes containing K2EDTA and then chilled until centrifugation. Samples were centrifuged within 1 hour of collection. Plasma was collected and kept frozen on dry ice before storage at approximately -70 °C. Concentrations of GDC-0449 in plasma were quantitated by LC/MS/MS (Wong et al., 2009).

Pharmacokinetic Data Analysis

All pharmacokinetic parameters were calculated by noncompartmental methods as described by Gilbaldi and Perrier (1982). Pharmacokinetic parameters (aside from \( t_{\text{max}} \)) are reported as the mean ± SD. \( t_{\text{max}} \) is presented as the median along with the observed range in parentheses.

Physiologically-Based Pharmacokinetic (PBPK) Modeling

A nine intestinal segment oral absorption PBPK model was constructed in ModelMaker Version 4.0 (Oxford, UK) based upon a modified version of the advanced compartmental absorption and transit model (ACAT) (Agoram et al., 2001). The configuration of the described model is shown in Figure 2. The first segment represents the stomach followed by seven small intestine (SI) segments and the colon segment. Two compartments were assigned for each of the nine segments representing the gastrointestinal (GI) tract and
were designated to contain either solid (S) or dissolved (Dis) drug. Rate constants describing gastric emptying (K_S), small intestine compartmental transit (K_T), and colon emptying (K_C) were set 4 hr^{-1}, 3.85 hr^{-1}, and 0.0833 hr^{-1}, respectively, in order to reflect the GI transit times presented for the Beagle Model in GastroPlus (Simulations Plus, Inc., Lancaster, CA). Specific pH for the GI compartments were set at pH values presented for the Beagle Model in GastroPlus as follows: SI1 – pH 6.2; SI2 – pH 6.2; SI3 – pH 6.2; SI4 – pH 6.4; SI5 – pH 6.6; SI6 - pH 6.68; SI7 – pH 6.75; Colon – pH 6.45. The exception was the stomach compartment which was set to be similar to human gastric pH (pH-1.2) since our studies involved either pentagastrin pre-treated dogs or fed dogs which have stomach pH similar to man (Sagawa et al., 2009). Solubility of the stomach compartment was set at 0.49 mg/mL, the solubility of GDC-0449 in simulated gastric fluid at the stomach pH. The volume in the stomach compartment was set at 450 mL. Aside from the stomach, the volume of the lumen for each GI compartment was calculated using following equation: \( V_{\text{lumen}} = \pi LR^2 \), where L is the length of the intestinal section, R is the radius. A radius of 0.5 cm was used for all SI compartments and 2 cm was used for the colon. The following lengths (in cm) were used for each GI compartment as per the Beagle Model in GastroPlus: SI1 – 44.76; SI2 – 32.98; SI3 – 24.3; SI4 – 17.9; SI5 – 13.19; SI6 – 9.72; SI7 – 7.16; Colon – 6.25

**Dissolution Process**: Dissolution rate of solid drug in the oral absorption PBPK model was governed by the following equation based upon the Noyes-Whitney equation:
where $X_{\text{solid}}$ is the amount of undissolved GDC-0449 in the S compartments, $D$ is the GDC-0449 diffusion coefficient (default $= 10^{-4}$ cm$^2$/min), $\rho$ is the drug particle density (GDC-0449 = 1.34 g/cm$^3$), $r$ is the particle radius (adjusted based upon GDC-0449 form dosed: 20 µm for the multiple dose pharmacokinetics study, and 21 or 120 µm for the oral formulation comparison study), $h$ is the diffusion layer thickness (if $r < 30$ µm, $h = r$; if $r > 30$ µm, $h = 30$ µm; Sugano et al., 2007), $C_{\text{solubility,IF}}$ is the measured solubility in simulated intestinal fluid (FASSIF; GDC-0449 = 3 µg/mL at pH from 6.0 - 6.8 ), and $C_{\text{GItract}}$ is the concentration of dissolved GDC-0449 in the GI tract segment. Based upon the equation above, dissolution of GDC-0449 was assumed to be driven by the difference between the FASSIF solubility and the concentration of dissolved GDC-0449 in the GI segment.

**Intestinal Membrane Permeation Process**: The rate of membrane permeation of dissolved GDC-0449 from the GI tract into the system circulation in the PBPK model was governed by the following equation:

$$\frac{dX_{\text{solution,GItract}}}{dt} = -P_{\text{app}} \times A \times (f_{\text{mono}} \times C_{\text{GItract}} - C_{\text{unbound,sys}})$$  \hspace{1cm} \text{Equation 2}$$

where $X_{\text{solution,GItract}}$ is the dissolved drug in the GI tract segment, $P_{\text{app}}$ is the permeability coefficient of the drug ($60.1 \times 10^{-6}$ cm/sec based upon experimental data from Caco-2
cells; Additional Note: Efflux ratio in Caco-2 cells is 0.79 (Genentech Inc.), A is the surface total area available for absorption; $f_{mono}$ is the fraction of GDC-0449 that is not in micelles (equal to solubility in buffer divided by solubility in FASSIF), and $C_{unbound,sys}$ is the unbound concentration of GDC-0449 in the systemic circulation. An unbound fraction of 1.50% (determined ex vivo by equilibrium dialysis in 12 dogs) was used to determine unbound concentrations in simulations. For the oral formulation comparison study, the actual measured mean unbound fraction from animals from that study was used (2.77%).

If $f_{mono} \times C_{Gltract} > C_{solBuffer}$ (Solubility in buffer), then the rate of permeation would be governed by the following equation:

$$\frac{dX_{SolutionGItract}}{dt} = -P_{app} \times A \times \left( C_{solBuffer} - C_{unbound,sys} \right)$$  \hspace{1cm} \text{Equation 3}

Only the free monomer ($f_{mono} \times C_{Gltract}$; GDC-0449 not associated with micelles in FASSIF) was assumed to be available for diffusion across the intestinal membrane. The upper limit of free monomer concentration was set as the GDC-0449 aqueous solubility associated with the pH of the intestinal segment of interest. Aqueous solubility of GDC-0449 aqueous solubility was determined twice giving values of 0.1 and 0.4 µg/mL from pH 6.5 to 7.0 (Genentech Inc.). Both estimates of aqueous solubility produced PK profiles with similar phramacokinetic characteristics (data not shown). GDC-0449 aqueous solubility was set at 0.4 µg/mL for all simulations presented since this estimate was the most conservative for demonstrating non-sink permeation conditions. The total surface area available for absorption (A) was calculated as $(2\pi RL) \times \text{total amplification}$. 


factor. Mammals have been shown to have a total surface area amplification factor due to villi and microvilli of approximately 450 (Ferraris et al., 1989). An additional amplification factor of 3 was incorporated as dog is believed to have a higher permeability than humans (Parrott et al., 2009). Absorption was assumed to only occur in the small intestine.

For simulations performed under sink conditions only (SINK), equations describing membrane permeation (Equations 2 and 3) were modified as follows:

\[
\frac{dX_{\text{solutionGItract}}}{dt} = -P_{\text{app}} \times A \times (f_{\text{mono}} \times C_{\text{GItract}}) \quad \text{Equation 4}
\]

If \( f_{\text{mono}} \times C_{\text{GItract}} > C_{\text{solBuffer}} \)

\[
\frac{dX_{\text{solutionGItract}}}{dt} = -P_{\text{app}} \times A \times (C_{\text{solBuffer}}) \quad \text{Equation 5}
\]

For simulations where the effect of solubility rate-limited absorption (SRLA) on the permeation process was removed from the PBPK model, permeation was governed solely by Equation 2 (or Equation 4 where SINK conditions also apply) even under conditions where \( f_{\text{mono}} \times C_{\text{GItract}} > C_{\text{solBuffer}} \).

The maximum permeation extraction ratio (MPER), a measure of the maximum extraction of compound from the intestinal lumen into the systemic circulation under non-sink conditions is defined as follows:
Based upon the equation described above, under sink conditions, MPER would range from 0.9 to 1. Non-sink conditions for permeation would occur at MPER of < 0.9.

**Systemic Circulation:** The elimination rate constant (ke) from the systemic circulation in the PBPK model was set at 0.00997 hr⁻¹ based upon a t₁/₂ of 69.5 hours estimated from the bile-duct cannulated dog study (described above) during the first 72 hours post dose. Since bile was collected during this period, this t₁/₂ was assumed to be entirely due to systemic elimination of the compound with no influence from enterohepatic recycled compound. This t₁/₂ is virtually identical to the t₁/₂ estimated for bile-duct cannulated dogs using plasma-concentration data from the full duration of the study (See Table 1).

**In Vitro Study Using Sandwich-Cultured Human Hepatocytes**

B-CLEAR®-HU (Qualyst, Inc., Raleigh, North Carolina) sandwich-cultured fresh primary human hepatocytes 6-well plates were used to investigate the hepatobiliary disposition of GDC-0449. Briefly, the experiment involved pre-incubating sandwich-cultured hepatocytes in the Hank’s buffered salt solution with (+) or without (-) Ca²⁺ for approximately 10 minutes. Ca²⁺ free buffer has been shown to disrupt the tight junction between the canalicular lumen and the extracellular space causing substrate that is excreted into bile canalicular networks to diffuse back into the incubation media (Liu et
al., 1999). At the end of the pre-incubation period, GDC-0449 (5 or 50 μM) in Hank’s buffered salt solution (+) Ca²⁺ was added and incubated for approximately 20 minutes. At the end of the incubation, an aliquot of incubation buffer was taken for measurement of GDC-0449 by LC/MS/MS. The remaining buffer was aspirated, cells were washed three times with ice cold (+) Ca²⁺ buffer, and 1 mL of acetonitrile containing 0.188 μM of deuterated internal standard was added to each well and mixed for at least 20 minutes. The resulting lysate was collected and protein (Lowry et al., 1951) and GDC-0449 concentrations were assessed. GDC-0449 uptake was normalized by protein concentrations. Incubations were performed in three donors and in triplicate. The biliary excretion index (BEI) and in vitro biliary clearance (CL_biliary) were calculated as described by the following equations (Liu et al., 1999):

\[
BEI = \frac{\text{Uptake}_{(+)}\text{Ca}^{2+} - \text{Uptake}_{(-)}\text{Ca}^{2+}}{\text{Uptake}_{(+)}\text{Ca}^{2+}} \times 100 \quad \text{Equation 7}
\]

\[
CL_{\text{biliary}} = \frac{\text{Uptake}_{(+)}\text{Ca}^{2+} - \text{Uptake}_{(-)}\text{Ca}^{2+}}{\text{AUC}_{(\text{in vitro})}} \quad \text{Equation 8}
\]

where Uptake_{(+)}\text{Ca}^{2+} and Uptake_{(-)}\text{Ca}^{2+} are the cumulative uptake of GDC-0449 over the 20 minute incubation period for hepatocyte cultures pre-incubated in buffer with and without Ca²⁺, respectively. AUC_{(\text{in vitro})} is the area under the concentration-time curve of the sandwich culture incubations calculated by multiplying the average concentration of GDC-0449 at the start and end of the incubation by the duration of the incubation (i.e. 20 minutes). Taurocholate and digoxin were run as positive controls (data not shown).
RESULTS

Bile-Duct Cannulated Dog Study

Figure 3 is concentration-time profile of GDC-0449 following an intravenous dose of 25 mg administered to intact and bile-duct cannulated dogs. The estimated pharmacokinetic parameters are presented in Table 1. The mean plasma clearance (CL) of GDC 0449 in both intact and bile-duct cannulated dogs was very low, approximately 0.4 and 0.6% of hepatic blood flow, respectively. The mean volume of distribution (Vss) at steady state in intact and bile-duct cannulated dogs was approximately 1.6 and 1.9 times, respectively, of total body water volume (Davies and Morris, 1993). Half-life (t1/2) was long being approximately 80 hours in intact animals and approximately 68 hours in bile-duct cannulated dogs. The mean biliary clearance of GDC-0449 in bile-duct cannulated dogs was very low, at approximately 0.04 mL/min/kg, and was approximately 19% of plasma clearance in bile-duct cannulated dogs.

Activated Charcoal Studies:

Figure 4 presents a plot of the mean plasma concentration-time profile for dogs that received a 25 mg IV or PO dose of GDC 0449, with or without administration of activated charcoal. GDC-0449 concentrations at the start and end of charcoal administration (24 and 96 hours, respectively) and the area under the concentration-time profile up to 168 hours post-dose (AUC0-168) are presented in Table 2. The mean GDC 0449 concentration at 96 hours post dose for dogs given 25 mg of GDC-0449 orally (0.137 µM) was approximately 21.0% of the concentration observed at the 24 hour post
dose timepoint (0.650 µM). In contrast, PO administration of activated charcoal, from 24 to 96 hours post dose, to dogs given either a PO or IV dose of 25 mg GDC-0449 resulted in mean GDC-0449 concentrations at 96 hours (0.004 and 0.018 µM, respectively) that were approximately 0.5% of the concentrations observed at the 24 hour post dose timepoint (0.847 and 4.49 µM, respectively). Oral administration of activated charcoal to dogs given a 25 mg oral dose of GDC-0449 resulted in a modest reduction of AUC_{0-168} causing a decrease by approximately 50% (see Table 2). This was because a significant portion of the AUC_{0-168} originates from the time prior to the administration of charcoal (0 to 24 hours post-dose).

Intravenous data from intact dogs from the bile-duct cannulated dog study (described above) is also presented in Table 2 and Figure 4 for comparative purposes. When compared to the dogs given 25 mg IV GDC-0449 with oral activated charcoal, both the mean GDC-0449 concentration at 96 hours post-dose and the AUC_{0-168} were higher in the intact dogs from the bile-duct cannulated dog study.

**Multiple Dose Pharmacokinetics of GDC-0449:**

Estimates of oral pharmacokinetic parameters for GDC-0449 on Day 1 and Day 44 in male dogs given either 7.5 mg/kg BID or 25 mg/kg BID are presented in Table 3. Corresponding mean plasma concentration-time profiles are presented in Figure 5A and B. Increases in oral exposure were less than dose proportional on Day 1. An approximate three fold increase in dose (7.5 mg/kg BID to 25 mg/kg BID) resulted in only an approximate two fold increase in AUC_{0-24} and C_{max} (Table 3). GDC-0449 plasma concentrations on Day 1 showed no signs of decline by 24 hours for both dose groups.
Finally, an expected secondary increase in GDC-0449 plasma concentrations was observed on Day 1 following the administration of the second daily dose at 6 hours. In contrast to Day 1, AUC$_{0-24}$ and C$_{max}$ estimates were very similar for both dose groups on Day 44 (Table 3). GDC-0449 plasma concentration-time profiles on Day 44 were unusually flat showing little increase in GDC-0449 plasma concentration following the administration of the second daily dose. Continuous dosing of 7.5 mg/kg BID for 44 days resulted in accumulation of GDC-0449 with C$_{max}$ and AUC$_{0-24}$ estimates being approximately 5 and 6 fold higher, respectively, on Day 44 when compared to Day 1. The extent of accumulation was less for the 25 mg/kg BID dose group with C$_{max}$ and AUC$_{0-24}$ being approximately 2 and 3-fold higher, respectively, on Day 44 when compared to Day 1.

Predicted concentrations from simulations using the oral PBPK model show an approximately 2-fold increase in exposure with dose on Day 1 (Figure 5A). At steady-state (Figure 5B), this difference in exposure was almost nonexistent and is consistent with observed data. Figure 5C and D show the simulated maximum permeation extraction ratio (MPER) for Day 1 and Day 44. The simulations suggest non-sink conditions exist for the oral permeation GDC-0449 in dogs. In particular, non-sink permeation conditions were more prominent at steady-state (Day 44) where the MPER is < 0.5 throughout the entire 24 hours post dose for both dose levels.

Additional simulations were performed to assess the mechanistic influence of solubility–rate limited absorption and non-sink permeation on the less than dose proportional increases in exposure observed for GDC-0449 in dogs. Modifications were made to the equations describing the intestinal membrane permeation process of the full
PBPK model (as described in the Methods) by removing solubility rate-limited absorption conditions (NO SRLA), removing non-sink permeation conditions (SINK), and removing both non-sink permeation and solubility rate-limited absorption conditions (SINK and NO SRLA). Little effect was observed for the 7.5 mg/kg BID dose on Day 1 (Figure 6A) and at Steady-State (Day 44) (Figure 7A). In contrast, for the 25 mg/kg BID dose, incorporation of solubility-rate limited absorption into the PBPK model appeared necessary to better simulate the plasma profile on Day 1 (Figure 6B). Removal of solubility rate limited absorption from the model (“NO SRLA” and “SINK and NO SRLA” scenarios) resulted in a doubling of the average Day 1 concentrations of GDC-0449 causing a larger deviation from the observed concentrations. Removal of either or both solubility-rate limited absorption and non-sink membrane permeation conditions caused ~ 2 to 3.5 fold increases in the simulated average GDC-0449 steady-state (Day 44) concentrations (Figure 7B). The full model incorporating both conditions appeared provided the best simulation of the observed Day 44 data.

**Oral Formulation Comparison Study:**

The mean GDC-0449 plasma concentration-time profile in dogs from the oral formulation comparison study is shown in Figure 8 along with the simulated plasma concentration-time profiles generated using the oral PBPK model. The effect of particle size on the oral pharmacokinetics of GDC-0449 in male dogs is presented in Table 4. Estimates of AUC_{0-168} and C_{max} were both approximately 3-fold higher when particle size was reduced for GDC-0449. The median t_{max} was comparable between the two groups being 4.0 and 3.0 hours. However, the t_{max} appeared more variable for the in dogs given
GDC-0449 as larger particles. As seen in Figure 8, the PBPK model was able to nicely capture the observed particle size effect.

A simulation study using the oral PBPK model was performed to better understand the impact of changing dose and dose regimen on the observed particle size effect. For this purpose, simulations were performed at total doses of 600 mg (600 mg once daily (QD) and 150 mg QID (four times daily); Figure 9). The doses were chosen such that steady concentrations achieved in the simulation approached relevant concentrations that have been observed clinically. The effect of particle size on oral absorption was predicted to decrease with increasing dose when comparing the particle size effect following a single 150 mg dose (Figure 8) to the simulation of a single dose of 600 mg (Figure 9A). The effect of administering the 600 mg total dose over four 150 mg doses (i.e. 150 mg QID) was predicted to increase the effect of particle size (Figure 9A and 9C). The effect of particle size on oral absorption was predicted to be minimal at steady-state regardless of the dose regimen (Figure 9B and 9D).

Sandwich-Cultured Human Hepatocytes Studies

No biliary excretion was observed for GDC-0449 (at 5 and 50 µM) from sandwich-cultured human hepatocytes. Accordingly, estimates of BEI and CL_biliary for GDC-0449 were negligible suggesting that biliary elimination of GDC-0449 is either very low or absent.
DISCUSSION

The oral absorption of drugs is dependent on the sequential processes of dissolution and intestinal membrane permeation (Figure 10). The overall absorption characteristics are a result of the interaction between these two processes. The dissolution rate, described commonly by the Noyes-Whitney equation (Equation 1), is influenced by compound characteristics such as solubility in intestinal fluid, and more controllable properties such as particle size. The driving force for dissolution is the gradient between intestinal fluid solubility and compound concentration in the intestinal compartment of interest.

Following dissolution in the intestinal lumen, drug in solution exists as free molecules or is solubilized by bile micelles (Sugano et al., 2007). Solubilization in micelles can act to enhance intestinal membrane permeation by assisting the movement across the unstirred water layer adjacent to the intestinal membrane (Amidon et al., 1981). As micelle bound molecules are not available for membrane permeation, those carried across the unstirred water layer by micelles must be released prior to crossing the intestinal membrane. Thus, the driving force for membrane permeation is the concentration gradient between the free molecules in the intestinal lumen and the unbound molecules in the systemic circulation as described by Equations 2 and 3. For most drugs, the free molecule concentration in the lumen is much higher than the unbound concentrations in the systemic circulation such that membrane permeation occurs under “sink” conditions (see Equations 4 and 5). In these cases, the rate limiting step for permeation is movement across the unstirred water layer (Sugano et al., 2007).
Inhibition of the Hedgehog signaling pathway and its potential implications on the treatment of certain cancers has resulted in the synthesis of small molecule inhibitors of this pathway (Borzilla and Lippa 2005; Scales and de Sauvage, 2009). Previously, we reported the preclinical absorption, distribution, metabolism and excretion properties of GDC-0449, a small molecule inhibitor of the Hedgehog signaling pathway currently in Phase II clinical trials (Wong et al., 2009). GDC-0449 is extremely stable in dog and human hepatocytes displaying minimal turnover in 3 hour hepatocyte incubations. The systemic clearance of GDC-0449 is exceptionally low in dog being ~ 1% of hepatic blood flow (Davies and Morris, 1993) and this was reflected in the long terminal half-life. The systemic clearance in intact and bile-duct cannulated dogs in the current study is similarly low being ~ 0.4% and 0.6% of hepatic blood flow, respectively. Half-life in intact and bile-duct cannulated dogs was ~80 and 68 hours, respectively and was similar (within 2-fold biological variability) to the previously reported t1/2 of ~42 hours (Wong et al., 2009). The low systemic clearance and the associated long t1/2 in dogs contribute to high steady-state concentrations of GDC-0449 observed in the current study. In terms of the Biopharmaceutical Classification System (BCS), GDC-0449 is considered a BCS class II compound (Dahan et al., 2009) based upon its very low solubility and high Caco-2 permeability. The oral absorption of BCS class II compounds are usually limited by particle size effects or intestinal fluid solubility on the dissolution process. Membrane permeation is usually not considered rate limiting for BCS class II compounds (Takano et al., 2008).

Physiologically-based pharmacokinetic modeling is a useful tool to understand the interplay between different biological processes and their overall effect on the
disposition of a particular molecule over time (Theil et al., 2003). The complexity of the oral absorption process has made the use of physiological modeling widespread. In particular, models based upon the compartmental absorption transit (CAT) model described by Yu and Amidon (1999) have been used extensively. (Agoram et al., 2001; Parrott et al., 2009). In the current manuscript, we utilize a CAT model that divides to GI tract into 9 segments where solid and dissolved material are represented as two separate compartments (18 compartments total; Figure 2), and dissolution and permeation processes are consistent with the scenario described in Figure 10. The described oral PBPK model was used to investigate the factors influencing the oral absorption of GDC-0449.

The pharmaceutical and biological characteristics of GDC-0449 make it uniquely distinct from most BCS class II compounds. As discussed, for BCS class II compounds, membrane permeation is usually not considered rate limiting. The permeation process is often governed by a form of Equation 4 which implies that permeation occurs under sink conditions (Sugano et al., 2007). The permeation equation used in the current model (Equation 2) is based upon Fick’s Law of diffusion where the driving force is the concentration gradient between the free molecules in the intestinal lumen (i.e. molecules not associated with micelles) and the unbound molecules in the systemic circulation. Equation 3 “caps” the free molecule concentration at the aqueous solubility of GDC-0449 and serves to describe a solubility rate-limited scenario for the membrane permeation process. A combination of low compound solubility along with high steady-state unbound concentrations act in unison to reduce the concentration gradient that serves as the driving force for membrane permeation. Based upon the in vivo properties of GDC-
0449, the use of these equations that allow for nonlinear membrane permeation is appropriate.

Our simulations of the multiple dose studies show that inclusion of solubility rate-limited absorption in the permeation process is necessary to describe the steady-state oral pharmacokinetics of GDC-0449 at the doses administered. More unique is the non-sink permeation characteristics that are also required to describe steady-state concentrations. At steady-state, the unbound concentrations are such that significant non-sink permeation can exist which is illustrated in our simulations presented in Figure 5D. As mentioned above, the permeation process is driven by the concentration gradient between the free molecules in the intestinal lumen and the unbound molecules in the systemic circulation. The MPER is calculated based upon the maximum possible concentration gradient at a given plasma concentration since the upper limit of free concentration in the intestinal lumen (i.e. aqueous solubility) is used in its calculation. Regardless, the MPER ratio is well under 0.9 during the entire 24 hour interval at steady-state suggesting significant non-sink permeation working to decrease the movement of GDC-0449 across the intestinal membrane into the systemic circulation at both doses tested. Our studies with charcoal clearly demonstrate that the permeation across the intestinal membrane is dependent on a concentration gradient that can be reversed with the application of charcoal making a non-sink permeation situation entirely plausible.

The oral formulation comparison study demonstrates a particle size effect on the oral absorption of GDC-0449. This is consistent with previous observations for BCS class II compounds such as danazol, griseofulvin, and aprepitant (Takano et al., 2008). Results of our simulation study using the described oral PBPK model, suggest that
increasing dose and or applying multiple doses of GDC-0449 to steady-state reduces the effect of particle size on oral absorption. The predicted reduction in the particle size effect can be attributed to a shift from a situation where absorption is rate limited by dissolution to a situation where absorption is rate limited by solubility and/or the intestinal membrane permeation process where changes in particle size have minimal impact (Sugano et al., 2007).

Biliary clearance presents additional complexity in that compounds eliminated via the bile are reabsorbed and can have a significant impact on the shape of the plasma concentration-time profile. We investigated the contribution of biliary clearance to the disposition of GDC-0449 in vivo using bile-duct cannulated dogs, and in vitro using sandwich-cultured human hepatocytes. Both studies suggested that biliary clearance does not appear to be a primary determinant of the disposition of GDC-0449 following oral administration in dogs or humans. In addition, biliary clearance was incorporated into the oral PBPK model and tested (data not shown). The inclusion of biliary clearance had minimal to no effect on simulations generated by the model, and thus biliary clearance was subsequently removed from final model presented.

The clinical pharmacokinetics of GDC-0449 has also been recently described (Von Hoff et al., 2009). The compound showed high steady-state plasma concentrations with a median concentration of approximately 16.1 µM (inter-quartile range, 13.7 to 21.6), and an apparent lack of dose dependency from 150 to 540 mg. Data from healthy volunteers show that the half-life ($t_{1/2}$) of GDC-0449 in humans is very long, being in the order of approximately 12 days (Ding et al., 2010). Based upon the pharmacokinetic
characteristics, and systemic concentrations observed in humans thus far, the factors
influencing oral absorption of GDC-0449 in dogs may play a role in humans.

Since most drugs are delivered via the oral route, an understanding of the factors
influencing the rate and extent of oral absorption is important during the drug
development process. The dissolution of GDC-0449 is dependent on properties such as
particle size and compound solubility. Solubility also influences the permeation process;
however, others biological characteristics including metabolic intrinsic clearance (a
controlling factor of in vivo unbound concentrations) also contribute. As described
above, a unique feature of GDC-0449 distinguishing it from other Biopharmaceutics
Classification System II compounds was that incorporation of both the effects of
solubility rate-limited absorption and non-sink permeation on the intestinal membrane
permeation process was necessary to describe its pharmacokinetic behavior. Overall, the
oral disposition of GDC-0449 is dependent on the interplay between the dissolution and
permeation processes. Finally, PBPK modeling is an invaluable tool to understand
complex dynamic processes such as oral absorption.
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REFERENCES


FOOTNOTES

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LEGENDS FOR FIGURES

Figure 1  Chemical structure of GDC-0449.

Figure 2  Physiological Oral Absorption Model.

Figure 3  Plasma GDC-0449 Concentration-time Profile of GDC-0449 Following a Single 25 mg Intravenous (IV) Dose to Intact and Bile-Duct Cannulated Male Dogs (n = 4 per group). The Duration of Bile Collection for Bile-Duct Cannulated Dogs is Indicated on the Figure.

Figure 4  Mean GDC-0449 Plasma Concentrations Following a Single IV (25 mg/dog) or PO (25 mg capsule/dog) Dose of GDC-0449, With Or Without Administration of Activated Charcoal, in Male Beagle Dogs (n=4 per group). The Duration of Oral Charcoal Treatment is Indicated on the Figure. Mean Plasma Concentrations from Intact Male Dogs (IV) from the Bile-Duct Cannulated Dog Study Shown in Figure 3 is Presented Here for Comparative Purposes.

Figure 5  Mean Plasma Concentrations and Simulated Profiles on Day 1 (A) and Day 44 (B) for Male Dogs (n=5 per group) Given Twice Daily Doses of 7.5 mg/kg or 25 mg/kg GDC-0449. Corresponding Maximum Permeation Extraction Ratio are Presented for Day 1 (C) and Day 44 (D).
**Figure 6** Effect of Removal of Solubility Rate Limited Absorption (NO SRLA), Removal of Non-SINK Conditions (SINK) and Removal of Non-Sink Conditions and Solubility Rate Limited Absorption (SINK and NO SRLA) on Simulated Day 1 Plasma Concentration-time Profiles for Male Dogs (n=5 per group) Given Twice Daily Doses of 7.5 mg/kg (A) or 25 mg/kg (B) GDC-0449.

**Figure 7** Effect of Removal of Solubility Rate Limited Absorption (NO SRLA), Removal of Non-SINK Conditions (SINK) and Removal of Non-Sink Conditions and Solubility Rate Limited Absorption (SINK and NO SRLA) on Simulated Steady-State (Day 44) Plasma Concentration-time Profiles for Male Dogs (n=5 per group) Given Twice Daily Doses of 7.5 mg/kg (A) or 25 mg/kg (B) GDC-0449.

**Figure 8** Mean Plasma Concentrations and Simulated Profiles following PO Administration of Two Different Particle Sizes of GDC-0449 (150 mg Total Dose) to Male Dogs (n=6 per group).

**Figure 9** Simulations Examining the Impact of Dose and Dosing Regimen on Observed Particle Size Effects. Presented are Simulations for 600 mg QD on Day 1 (A) and at Steady-State (B) and, 150 mg QID on Day 1 (C) and at Steady-State (D).
Figure 10 Process of Oral Absorption
Table 1  Pharmacokinetics of GDC-0449 in Intact and Bile-Duct Cannulated Male Dogs following administration of a single 25 mg Intravenous Dose.

<table>
<thead>
<tr>
<th></th>
<th>Intact Dogs (n=4)</th>
<th>Bile-Duct Cannulated Dogs (n=4)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Dose</strong></td>
<td>25 mg IV</td>
<td>25 mg IV</td>
</tr>
<tr>
<td><strong>CL (mL/min/kg)</strong></td>
<td>0.132 ± 0.009</td>
<td>0.189 ± 0.009</td>
</tr>
<tr>
<td><strong>Vss (L/kg)</strong></td>
<td>0.940 ± 0.088</td>
<td>1.12 ± 0.0185</td>
</tr>
<tr>
<td><strong>t1/2 (hr)</strong></td>
<td>80.0 ± 13.8</td>
<td>68.3 ± 1.3</td>
</tr>
<tr>
<td><strong>AUC0-∞ (µM×hr)</strong></td>
<td>795 ± 80</td>
<td>636 ± 41</td>
</tr>
<tr>
<td><strong>CLbiliary (mL/min/kg)</strong></td>
<td>-</td>
<td>0.0367 ± 0.0073</td>
</tr>
</tbody>
</table>
Table 2  GDC-0449 Concentrations and Exposures of GDC-0449 Following a IV (25 mg/dog) or PO (25 mg/capsule) Dose, With or Without Administration of Activated Charcoal to Male Dogs.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>C&lt;sub&gt;24hr&lt;/sub&gt; (µM)</th>
<th>C&lt;sub&gt;96hr&lt;/sub&gt; (µM)</th>
<th>AUC&lt;sub&gt;0-168&lt;/sub&gt; (µM×hr)</th>
</tr>
</thead>
<tbody>
<tr>
<td>25 mg PO</td>
<td>0.650 ± 0.287</td>
<td>0.137 ± 0.093</td>
<td>48.4 ± 20.1</td>
</tr>
<tr>
<td>25 mg PO with Charcoal</td>
<td>0.847 ± 0.733</td>
<td>0.004 ± 0.005</td>
<td>25.9 ± 21.2</td>
</tr>
<tr>
<td>25 mg IV&lt;sup&gt;a&lt;/sup&gt;</td>
<td>5.07 ± 0.61</td>
<td>3.16 ± 0.52</td>
<td>603 ± 57</td>
</tr>
<tr>
<td>25 mg IV with Charcoal</td>
<td>4.49 ± 0.42</td>
<td>0.018 ± 0.016</td>
<td>173 ± 24</td>
</tr>
</tbody>
</table>

AUC<sub>0-168</sub> - area under the plasma concentration-time profile from time 0 to 168 hours post-dose; C<sub>24hr</sub> - plasma concentration at 24 hours post-dose; C<sub>96hr</sub> - plasma concentration at 96 hours post-dose.

<sup>a</sup> Data from intact male dogs from bile-duct cannulated dog study. Data presented here for comparative purposes.
Table 3  Oral Pharmacokinetics of GDC-0449 in Male Dogs (n=5 per group) on Day 1 and Day 44 Following Continuous Dosing of 7.5 mg/kg BID or 25 mg/kg BID GDC-0449.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Day</th>
<th>C&lt;sub&gt;max&lt;/sub&gt; (µM)</th>
<th>t&lt;sub&gt;max&lt;/sub&gt; (hr)</th>
<th>AUC&lt;sub&gt;0-24&lt;/sub&gt; (µM×hr)</th>
</tr>
</thead>
<tbody>
<tr>
<td>7.5 mg/kg BID</td>
<td>1</td>
<td>8.07 ± 0.59</td>
<td>10.0 (10.0-24.0)</td>
<td>146 ± 13</td>
</tr>
<tr>
<td></td>
<td>44</td>
<td>43.7 ± 3.9</td>
<td>0.0 (0.0-5.75)</td>
<td>869 ± 96</td>
</tr>
<tr>
<td>25 mg/kg BID</td>
<td>1</td>
<td>18.1 ± 1.9</td>
<td>24.0 (10.0-24.0)</td>
<td>316 ± 39</td>
</tr>
<tr>
<td></td>
<td>44</td>
<td>41.8 ± 5.9</td>
<td>1.0 (0.0-24.0)</td>
<td>883 ± 113</td>
</tr>
</tbody>
</table>

AUC<sub>0-24</sub> - area under the plasma concentration-time profile from time 0 to 24 hours post-dose (timed following the first daily dose); C<sub>max</sub> – highest observed plasma concentration; t<sub>max</sub> – time at which C<sub>max</sub> occurred (0.0 hr refers to the pre-dose sample).
Table 4  Effect of Particle Size On Oral Exposures of GDC-0449 following PO Administration of Capsules Containing a 150 mg Total Dose to Male Dogs (n=6 per group)

<table>
<thead>
<tr>
<th>Treatment</th>
<th>C_{max} (µM)</th>
<th>t_{max} (hr)</th>
<th>AUC_{0-168} (µM×hr)</th>
</tr>
</thead>
<tbody>
<tr>
<td>150 mg capsule (Small Particles)</td>
<td>8.12 ± 4.01</td>
<td>4.0 (4.0-4.0)</td>
<td>453 ± 271</td>
</tr>
<tr>
<td>25 + 125 mg capsules (Large Particles)</td>
<td>2.66 ± 1.56</td>
<td>3.0 (1.0-4.0)</td>
<td>150 ± 95</td>
</tr>
</tbody>
</table>

AUC_{0-168} - area under the plasma concentration-time profile from time 0 to 168 hours post-dose; C_{max} – highest observed plasma concentration; t_{max} – time at which C_{max} occurred.
Figure 2
Figure 3

The graph illustrates the plasma concentration (µM) over time (hr) for two groups: Intact Dogs (solid line) and Bile-Duct Cannulated Dogs (dashed line). The x-axis represents time in hours (0 to 192), while the y-axis represents plasma concentration in µM (from 0.1 to 10). The data points for Intact Dogs are indicated by filled squares, and those for Bile-Duct Cannulated Dogs are indicated by open triangles. Error bars are present for both groups, indicating variability in the data. The graph also includes a label for the bile collection duration.
Figure 4
Figure 5
Simulated Average Day 1
GDC-0449 Concentrations in Dogs
Dosed 7.5 mg/kg BID

- FULL MODEL: 7.6 μM
- NO SRLA: 8.3 μM
- SINK: 7.6 μM
- SINK and NO SRLA: 8.4 μM

Simulated Average Day 1
GDC-0449 Concentrations in Dogs
Dosed 25 mg/kg BID

- FULL MODEL: 13.9 μM
- NO SRLA: 25.7 μM
- SINK: 14.8 μM
- SINK and NO SRLA: 26.1 μM

Figure 6
Simulated Average Steady-State GDC-0449 Concentrations in Dogs Dosed 7.5 mg/kg BID

- FULL MODEL: 34.7 µM
- NO SRLA: 42.6 µM
- SINK: 41.9 µM
- SINK and NO SRLA: 45.1 µM

Simulated Average Steady-State GDC-0449 Concentrations in Dogs Dosed 25 mg/kg BID

- FULL MODEL: 42.8 µM
- NO SRLA: 124.3 µM
- SINK: 80.8 µM
- SINK and NO SRLA: 141.1 µM

Figure 7
Figure 8

This figure shows the plasma concentration (in μM) over time for two different types of particles: Large Particles and Small Particles. The graph includes error bars to indicate variability in the data. The x-axis represents time in hours (0 to 192), and the y-axis represents plasma concentration. The data points and lines are as follows:

- **Large Particles**: Open squares connected by a solid line.
- **Large Particles Simulated**: Dashed line.
- **Small Particles**: Filled diamonds connected by a solid line.
- **Small Particles Simulated**: Dashed line.

The graph illustrates how plasma concentration changes over time for both particle types, with separate lines for the actual and simulated data points.
Figure 9

A. Day 1 - 600 mg

B. Steady-State - 600 mg

C. Day 1 - 150 mg QID

D. Steady-State - 150 mg QID

Plasma Concentration (µM)

Time (hr)
Figure 10