Volume 4, Issue 1, 2015, 241-246 ISSN 2284-6808

Letters in Applied NanoBioScience

www.NanoBioLetters.com

Original Research Article Open Access Journal

Received: 22.10.2014 / Revised: 20.12.2014 / Accepted: 10.01.2015 / Published on-line: 30.03.2015

Investigation of intrinsic dissolution rate based absorption model to predict exposure enhancement via nanosuspensionformulation of 1, 3-dicyclohexylurea in rats

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ABSTRACT

Hypertension is a common medical condition in the general population. Researchers are constantly looking for new medications to control this disease. Recently, soluble epoxide hydrolase (sEH) was identified as possible new drug target for hypertension. 1, 3-dicyclohexylurea (1,3-DCU) was reported to inhibit the sEH at nM range. However, due to the poor oral bioavailability (BA) of 1,3-DCU (even at low dose), utilizing1,3 –DCU in this target research become problematic. It is believed that the key ADME issues of 1,3-DCU are poor aqueous solubility, poor dissolution rate, and high systemic clearance. Formerly, ananosuspension/crystalline nanoparticle formulation has been used in the rats and observed higherBA. Despite the improvement, the mechanism ofhow crystalline nanoparticleimproves the BA has not been fully understood. The prediction of howcrystalline nanoparticleperforms *in vivo*remains uncertain. Therefore, a tool needs to be developed to better understand the enhanced BA of 1,3-DCUcrystalline nanoparticle. In this study, the limitation of the oral delivery of 1, 3-DCU was assessed by a dynamic model combines measured intrinsic dissolution rate, particle size, gastrointestinal transit (GI), and diffusion through unstirred water layer to estimate the amount of drug absorbed and then followed by multi-compartmental pharmacokinetic analysis. It was found that this modeling approach adequately captures the effect of a crystalline nanoparticle formulation and mimicking the oral BA. The ultimate goal for this study is to assess the suitability of using a new dynamic modeling approach to predict *in vivo* performance of the crystalline nanoparticle. This work may aid in future investigations of similar compounds.

Keywords: *Crystalline nanoparticle, 1,3-DCU, BA, sEH, intrinsic dissolution rate, dynamic, modeling.*

1. INTRODUCTION

In the exploratory phase of drug discovery, compounds with sub-optimal potency and non ideal *ADME* properties are often used in efficacy and toxicology studies to demonstrate the new target validation (i.e. PK/PD or efficacy) and the target safety. Due to those tool compounds frequently bearnon ideal ADME properties, they often present challenges to formulation scientists when high systemic concentrations are desired in animal studies. Despite the use of enabling formulations, increases in exposure following oral delivery are often not achieved.

IT has been studied that (EETs) can affect vasodilatation and vasoconstriction in several vascular beds [1-4]. According to their research, anti-inflammatory, anti-hypertensive, and other therapeutic benefitscan be achieved with increasing the levels of EETs [5-11]. In order to take the full benefit of thetherapeutic effects, the level of EETs in the circulation needs to be at high level. It was found that EETs are hydrolyzed by the enzyme soluble epoxide hydrolase (sEH) to metabolites DHETs(dihydroxyeicosatrienoic acids) with lost of potency.Therefore, inhibition of soluble sEH (hence, increase the EETs in the circulation system) is a potential drug target to treat cardio disease such as hypertension. It has been reported by researchers that 1,3-dicyclohexylurea (also known as N,N'-dicyclohexylurea and hereafter refer as 1,3-DCU) inhibits sEH with an*in vitro* IC50 of 2 nM [12]. Because of its favorable potency, 1,3-DCU was believed to be a great tool sEH inhibitor to further examine this drug target in vivo efficacy models [2,14]. However, in spite of having high *in vitro* potency, its poor *ADME* propertieslimit the usage of 1,3-DCU. The above, makes oral delivery of 1,3-DCU and sustain exposure at high level very difficult[13-15]. Unfortunately, this problem is not specific to 1,3-DCU. Nowadays, similar issues are encountered everywhere in the pharmaceutical industry. In order to address these issues, special formulations have been developed to better deliver molecules with ADME issues [12-23]. Amount those formulations; crystalline nanoparticle has been broadly explored in the health industry as a tool to address exposure related issues [13-15]. A crystalline nanoparticlebased formulations were used to in rats to deliver 1,3-DCU and observed much improved exposures [12,14]. It is reported, withmuch improved exposure, a dose/exposure depended efficacy was established in the diseased animal model and inhibition of sEH as drug target was demonstrated first time pre-clinically [12]. However, despite utilizing crystalline nanoparticleof 1, 3-DCU demonstrating efficacy in a disease rat model, understanding of how a crystalline nanoparticle improves exposure was lacking.Therefore, limits further utilization of 1, 3-DCUcrystalline nanoparticle to further appraise the Pharmacokinetic and Pharmacodynamic relationships in other models [24]. In general, in order to take full advantage of the crystalline nanoparticle for drug delivery, better comprehension is needed. One approach is to conduct more in vivo studies for every compound (i.e. dose escalation studies). However, in vivo resources are often very limited and of running in vivo studies are high. Hence, a prediction tool to better predict crystalline nanoparticlebecomes essential. This effort is aiming to assess the

suitability of using this new approach to predict *in vivo* performance of the nano and serve as the foundation work for future investigation on similar compounds. We chose 1,3-DCU as model compound since a large number of drugs and drug candidates have similar challenges as 1,3-DCU. In this research, an *in vivo* absorption model based on the intrinsic dissolution rateand drug surface area (hereafter refer as SA) was established to evaluate *in vivo* exposure with regular suspension. It is to our best knowledge that integrating the measured intrinsic dissolution rate constant into a dynamic drug absorption model is novel. The parameters that were generated from this model were used to fit with a multi-compartment PK model to predict the exposure of 1,3-DCUcrystalline nanoparticle in the rats.

2. EXPERIMENTAL SECTION

2.1. Materials.

Glass beads were purchased from Glen Mill (NJ) and wet washed and dried in heated vacuum oven (80C, in house vacuum) for 5 days before used. 1, 3-DCU, Polysobrate 80 and other regents were purchased from Sigma-Aldrich or Fluka (St. Louis, MO). Acetonitrile (LC grad) was purchased from Burdick & Jackson (Muskegon, MI).

Mictrotrac®S3500 (PA, USA) instrument was used for measuring the particle size distribution (PSD) of each sample. For each individual sample, triplicates measurement were made and the average value was used for the PSD. The solid form assessment was done by the PXRD at ambient temperature with a Rigaku PXRD(Texas, USA). The water purification system used was a Milli-Q system.

2.2. Intrinsic Dissolution Study.

For the intrinsic dissolution test, a paddle over stationary disk system (assembled in house) was used. Weighed material (50 mg) was added to a stainless steel die (8 mm) with plungers and then compressed into a flat surface by using a Carver Press (5000 lb for 10 seconds). The open end of the die was placed into a 400 mL beaker facing up. An exact amount of dissolution media (200 mL) was then added into the beaker (submerging the die) with an overhead paddle stirring at 50 rpm. Dissolution media used in our study is 50 mM pH 7.4 Na Phosphate buffer. Analytical samples (0.5 mL) were taken at 0, 1, 2, 3, 4, 5, 10, and 15, 30, 45, 60, 90,120 minutes, and replenished with fresh dissolution media each sampling. Griseofulvin was used as model compound to calibrate the system [25]. Samples were analyzed by LC/MS/MS. The SA was assumed constant during this short period of time. Dissolution rate constant was calculated based on the total amount of drug dissolved at each time point using the Noyes-Whitney equation:

$$dM/dt = K * S\ (C_s - C_t(t))$$

Where: S: surface areas of the sample disk ($\pi r^2 = 0.5026\ cm^2$); K: dissolution rate constant (cm/min); C_s: Saturation Solubility of Solute in the Media; $C_t(t)$: Bulk Solute Concentration; dM = delta amount drug dissolved in the media between t_1 and t_2; dt = delta time ($t_1 - t_2$)

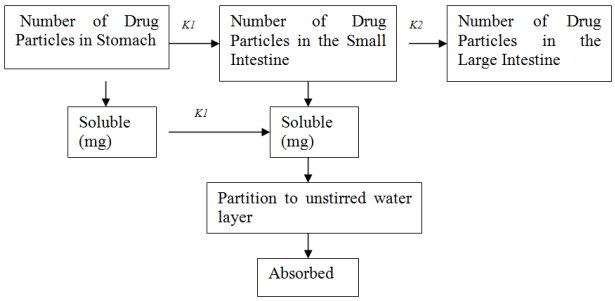
2.3. Pharmacokinetic Study of 1,3-DCU

Pharmacokinetic parameters were calculated using WinNonlin Professional® (version 4.1, Pharsight Corporation, Mountain View, CA). For the in vivo, Male Sprague-Dawley (SD) rats weighing between 250-300g were obtained from Charles River Laboratories (Wilmington, MA),were housed in a room with an ambient temperature on a 12 hour light/dark cycle. Rats were allowed for around one week to adopt the environmentwith free assess of standard rat chow and water as previously described [2, 13, 14]. The current study was conducted in comply with the guidelines for humane treatment of animals and was approved by the IACUC.

One day prior the study, animals were anesthetized with isoflurane and implanted with vascular catheters in the carotid artery and jugular vein. Rats were then placed in Culex cages overnight prior to dosingto adopt the environment. For the oral arm (regular suspension 3 mg/kg, crystalline nanoparticle 10 mg/kg) were dosed orally via gavage needle. For IV bolus arm (3.0 mg/kg) animals were dosed by the use of the jugular vein catheter. For the IV bolus arm, the injection volume was controlled at 1 mL per Kg of body their weight. A total of ten blood samples were collected from each rat by the Culex at 0, 0.083, 0.25, 0.5, 1, 2, 4, 6, 8, and 12 h time points for the both IV bolus and oral doses.

2.4. Absorption Modeling.

In order to understand the limit of 1,3-DCU crystalline crystalline nanoparticle oral dose, a three compartment absorption model was used to mimic the dynamic of GI transit and it is illustrated as Figure 1.



K1: Transit rate constant from stomach to small intestine
K2: Transit rate constant from to small intestine to large intestine

Figure 1. Absorption Model

This dynamic model assumes that 1) the small intestine as the main absorption site and that 2) the compound that reaches the large intestine does not contribute to absorption. The stomach empting time was taken into consideration. Non-homogeneous linear ordinary differential equation was applied on the model (Figure 1) to calculate the amount of particles presented in each compartment.

$$AL = AS_{(0)}\ (1+(1/(K_1-K_2) * (K_2 e^{-K1t} - K_1 e^{-K2t})$$

$$AI = (AS_{(0)} * K_1 / (K_1-K_2) * (e^{-K1t} - K_1 e^{-K2t})$$

$$AS_{(t)} = AS_{(0)} - (AL+AI)$$

Where: AL: Number of particles in large intestine at time t; AI: Number of particles in small intestine at time t; $AS_{(0)}$: Number of particles in stomach (dosed); $AS_{(t)}$:Number of particles in stomach at time t; K_1: Transit rate constant from stomach to small intestine; K_2: Transit rate constant from small intestine to large intestine.

The amount of drug solubilized in stomach and small intestine were calculated by the following equation under the sink condition:

$$Xd\ (t) = \gamma * K * Cs * A\ (t)$$

Where: Xd (t): Amount of drug dissolved at time t; K: measured dissolution rate constant (cm/min); γ: correction factor (from in vitro to *in vivo* assuming =1); Cs: saturated solubility of compound in each compartment; A(t): Total surface are of the drug particles at time t in each compartment.

For the particle number and SA calculation, all particles were assumed to be sphere.Initial particle radius ($r_{(0)}$) , initial volume (Vo) , weight of each particle (Wp) and total particle number (Nu) were calculated by usingequation 1, 2 and 3 respectively. The available SA of the 1,3-DCU at time t in each compartment was calculated by the using the equation (4). The new particle radius at time t was calculated based on the amount of drug release after dissolution taking place between t and t0 and assign in each compartment as r(t).

$$Vo = 3/4\ \pi\ r_{(0)}^3 \quad (1)$$
$$Wp = Vo \times 1.30\ cm3/gm \quad (2)$$
$$Nu = ((amount\ dosed)/Wp) \quad (3)$$
$$A(t) = (4\pi r(t)^2) \times Nu\ (t)\ (i.e.\ Nu = AS\ at\ t0) \quad (4)$$

The amount of drug absorbed was estimated by using the equationsreported (27-29). For high permeability compound, diffusion through the unstirred water layer was considered the rate limiting step and the amount absorbed was calculated by the following the equations.

$$dAt/dt = Puwl * S\ (Xd/vf)$$
$$F = Total\ abs\ (A)/Dose$$

Where, *Puwl*: Diffusion coefficient; *MW*: Molecular Weight; *Xd*: Soluble drug in the small intestine at time t; *S*: Effective surface are of small intestine; *dAt*= The amount drug absorbed at time t; VfEffective fluid volume in the small intestine at time t; Fraction absorbed (F) ,*A*=Total drug absorbed (total from t0 to t)

2.5. Pharmacokinetic Modeling.

3. RESULTS SECTION

Nano and micro particles are well practiced formulation tools in industry to increase *in vivo* exposure for poorly soluble drugs. TheSA increased by reducing particle size often translated to an increase overall apparent drug dissolution. For oral dose, with a fixed GI transit time (i.e 4 hrs), improving dissolution rate of the compound help improve oral BA. This approach is very useful to address drugs with dissolution rate limited absorption. However, similar to other technology, crystalline nanoparticle has its own limit. Theoretically, the best usage of crystalline nanoparticles to improve oral exposure is to use(at the dose)where absorption is within the dissolution rate control range (ideally, not the solubility control range). In an oral setting, it is more useful for compound with good permeability such as 1,3–DCU [12]. Since the oral absorption of compound with higher permeability is more likely of achieving sink condition upon dosing [32-37]. In which largerSA of the crystalline nanoparticles formulation will provide faster dissolution and translated into higher *in vivo* exposure. However, despite the understanding of SA impact on the drug dissolution, question such as how to estimate crystalline nanoparticles impact on exposure (without conduct the in vivo experiment) remain unclear [25]. For example , also it has been reported that crystalline nanoparticleimprove the exposure of 1,3-DCU [12], estimate crystalline nanoparticles impact on exposure

In order to understand differences in absorption between SD materials generated by the different manufacturing processes, pharmacokinetic analysis was performed by using an in-house built 2 –compartment model [29].

2.6. Formulation.

For the crystalline nanoparticle preparation, an in house wet milling devise was used as described previously [2,13]. A crystalline nanoparticle stock formulation (50 mg/mL) was prepared and harvested as previously described [2, 13]. For regular suspension, formulation was made by addition of bulk 1,3-DCU in the vehicle and the mixture was sonciated for a period of 5 minutes and visually checked to ensure formulation is free of large particle and all particles are uniformly dispersed. Formulation concentrations were determined by LC/MS/MS (MRM). The stability and PSD of the 1,3-DCU formulations (both regular suspension and crystalline nanoparticle) was assessed and found no issue [13-15]. PSD of the regular suspensionand crystalline nanoparticleare 20.2 μm and 0.8 μm respectively (asD_{50}).

2.8. LC/MS/MS Analysis

1,3-DCU plasma concentrations were quantified by using LC/MS/MS (MRM) with internal standard method as previously described [2,]. Briefly, fix amount of internal standard was first added to each plasma samples and then protein was first precipitation with ACN and removed with centrifugation. Samples was then directly applied to HPLC (no further purification) coupled withan AB Sciex QTRAP 5500 Mass spectrometer.The atmospheric pressure electrospray ionization source was used for detection with the positive ion mode. The m/z transition for 1,3-DCU was 225.4 (parents) to 100.2 (daughter). The lower limit of quantitation (LOQ) was 0.013 μM with a signal to noise ratio of 6 in plasma.

(dose) hence PK curve are not fully understood [14]. To better understand the degree of improvement of crystalline nanoparticle formulation oral performance in general, simulations were performed using a dynamic absorption model (established in house) that incorporate in vitro data including intrinsic dissolution rate, particle size, gastrointestinal transit, and diffusion through unstirred water layer to assess the suitability of prediction of fraction absorbed (F). This tree compartment absorption model was used to mimic the GI transit and an example is illustrated as Figure 2.

This dynamic model considers small intestine is the main absorption site and compound reaching large intestine is considered no longer available for absorption. Non-homogeneous linear ordinary differential equation was applied on the model (Figure 1) to calculate the amount of particles presented in each compartment. The stomach transit time (0.2 hr) and small intestine transit time (4 hr) were used. The particle size and surface are in each compartment were calculated as described in the method section. For example, the 10 mg/Kg crystalline crystalline nanoparticle dose of 1,3-DCU (2.5 mg total dose for 0.25 Kg rat) give an individual particle volume of 2.68 $x10^{-13}$ cm^3, particle weight of $3.48x10^{-13}$g, and a total particle number (TPN) of 7.18 x 10^9 (2.5 mg of total dose). The total SA at t_0 was estimated to be around144 cm^2. The particle number distribution in each compartment verse time is illustrated as Figure 2.

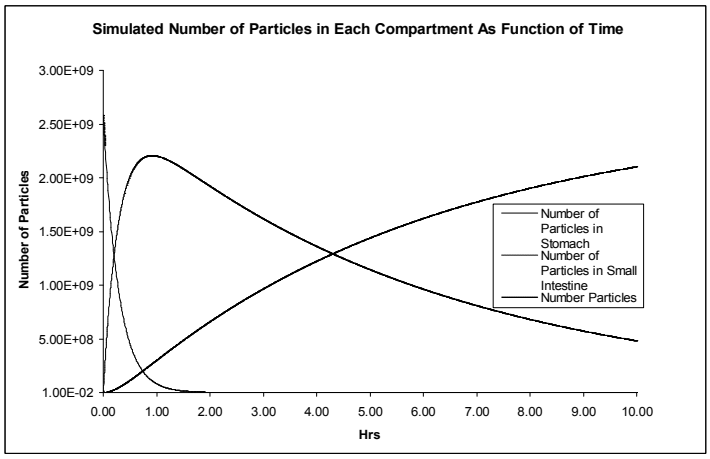


Figure 2. Example of Model Simulation Number of Particles in Each Compartment as Function of Time (10 mg/Kg Nano Suspension Dose)

With the measured intrinsic dissolution rate constant K of 0.14 cm/min and solubility of 4 µg/mL (measured in the simulated intestine fluid at 37C), the predicted fraction absorbed for 3 mg/Kg dose with regular suspension (D_{50} of 20.2 um) is approximately 0.25 and for the 10 mg/Kg crystalline crystalline nanoparticle (D_{50} of 0.8 um) is 1. These values have good agreement with the *in vivo* results. The relative BA of each formulation was calculated based on the comparison with the IV dose. For each dose, the AUC (area under the curve)/Dose calculated by using PK solution software (SummitPK, Montrose, CO) is 1.4 ug*hr/mL/mg (IV), 0.4 ug*hr/mL/mg (regular suspension) and 1.2 ug*hr/mL/mg (crystalline nanoparticle).

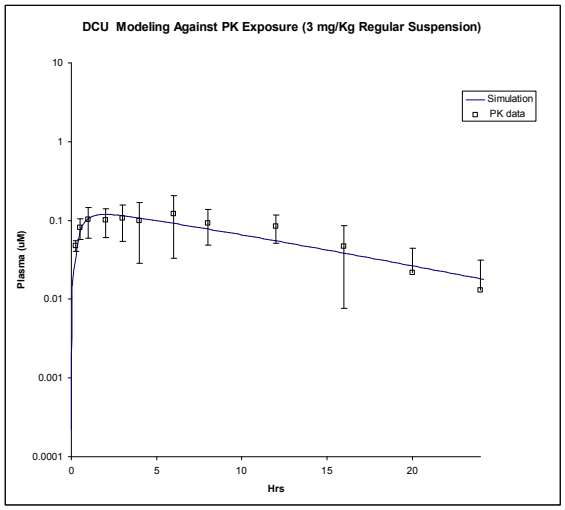


Figure 3.1,3-DCU model predict against *in vivo* data (3mg/Kg dose with Regular Suspension).

This translated to an oral BA is 28% for the regular suspension and 86 % for the Nano suspension. An absorption rate constant Ka for each formulation can be estimated by the approximation of take the ratio $dM_{abs}/\delta t/M$ (delta drug absorbed/ delta time/ total drug available) and shown a 10-fold improvement by using the crystalline nanoparticle orally deliver the 1,3-DCU.

The resulting Ka forthe regular suspension andthe crystalline nanoparticle is 0.1 and 1.0 hr-1 respectively. Other methodswere used to estimate Ka (such as the residual method) gave comparable outcomes [2,29].

Pharmacokinetic (PK) parameters were calculated using WinNonlin Professional® (version 4.1, Pharsight Corporation, Mountain View, CA). The two-compartment, first order model was found to give the best fit for pharmacokinetic modeling. Calculated PK parameters of V_1 (central volume of distribution) is about 1290 mL/Kg, k_{10} (systemic elimination rate) is 1.62 h^{-1}, terminal half life of 4.7 hr, and CL_{total} of 2 L/hr/Kg, Vdss of 2800 mL/Kg. The inter-compartmental rate from central to peripheral compartmentk_{12} is 0.22 h^{-1}, and inter-compartmental rate from peripheral to central compartment$k_{21}$is 0.17 h^{-1}. The above PK parameters were used to simulated exposure curve for both the regular and nano suspension and found to have good agreement with the *in vivo* data and results are illustrated as Figure 3 and Figure 4.It is worth notice this model (approach) not only successfully predicted the oral BA of 1,3-DCUwith both regular and crystalline nanoparticle, it also captures the overalloral exposure curve in rats.

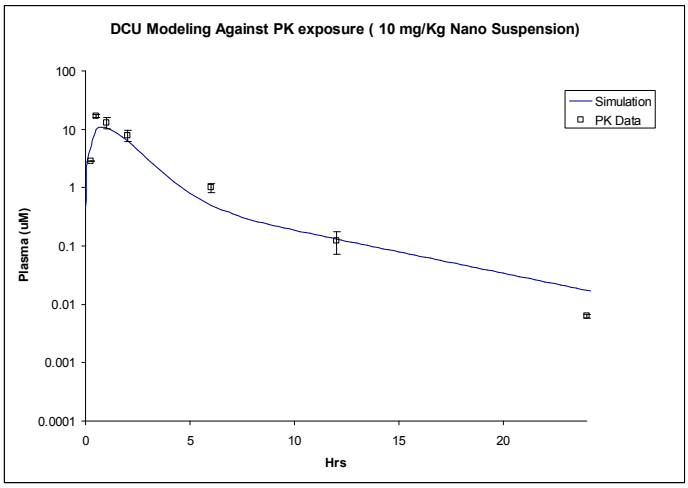


Figure 4.1,3-DCU model predict against *in vivo* data (10 mg/Kg dose with crystalline nanoparticle).

The successful of capturing the fast on set of the crystalline nanoparticle which reflected on both the C_{max} and T_{max} demonstrated the validity of this model. This model should work where absorption is dissolution rate limited (amount drug absorbed is affect by dissolution hence; increase SA improve oral BA). Otherwise, should show deviations when absorption reaching solubility-rate limited range. This model was found sufficient to predict the exposure for dissolution rate limited absorption. It worth mentioning that sometime, when enabling formulation was used (such as crystalline nanoparticle) more than dose linear increase in exposure can be observed due to the saturation of clearance mechanism [29,33]. Since it is out of the scope of this article, it will not be discussed here.

4. CONCLUSIONS

Oral BA is often influenced by factors such as the physicochemical properties, intestinal permeability and metabolism of drugs. It also known that one of the major concerns of any new drug candidate is low oral BA. Unable to fully understand the limiting factor of oral BA of drug candidates in the early discovery phase can leads to catastrophic failure in the clinic. Amount those, dissolution rate was foundto be one of the key limiting factor for oral BA (i.e. compound with poor

solubility/dissolution rate and good permeability). In order to better address this issue, the industry has spend significant amount of resources to better resolve the dissolution rate limited oral absorption. The goal is to come up with better tools for drug delivery and minimize the risk in moving forward [30-38]. Reducing drug particle size has become one of the major approaches to overcome the dissolution rate limited absorption. In this study, we initiate a dynamic model that incorporate in vitro data of intrinsic dissolution rate, SA, gastrointestinal transit, and free drug diffusion through unstirred water layer to prediction of fraction absorbed (F). With F in hand, we further utilized the multi-compartmental pharmacokinetic modeling to study the impact of crystalline nanoparticle on oral exposure of a model compound 1, 3-DCU. It was found that this dynamic approach successfully captured the effect of crystalline nanoparticle improve oral BA*in vivo* in rats. Furthermore, it successfullycaptures the faster on set of crystalline nanoparticle which reflected on both the C_{max} and T_{max}.This dynamic modeling approach(from *in vitro* to *in vivo*) helped us to better understand the advantage of crystalline nanoparticle. The goal is to demonstrate using this new dynamic modeling approach to better predict *in vivo* performance of the crystalline nanoparticle. We believed this work can serve as the foundation for future investigation on compounds that bear similar dissolution rate limited exposure issues.

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6. ACKNOWLEDGEMENTS

Special thanks to Genentech Inc. for supporting this research.