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Cyclodextrin Affects Distinct Tissue Drug Disposition as a Novel Drug Delivery Vehicle

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Abstract

Introduction: Cyclodextrin is a novel solubilizing agent for parenteral administration of drugs with poor aqueous solubility, and it is commercially available as drug formulation excipient from different manufacturers. Its ability to dissolve drugs into stable aqueous solution *in vitro* and *in vivo* is well-established. However, tissue dispositions of drugs encapsulated in Cyclodextrin have not been extensively studied, and it is assumed to follow the same pattern of simple co-solvents. This study aims to assess the plasma and tissue drug disposition of Cyclodextrin by two different manufactures, in comparison to a liquid co-solvent formulation.

Methods: Two Cyclodextrin formulations (Captisolor Dexolve) or a co-solvent formulation (free solution) of an investigational drug were subcutaneously or intravenously administered to 3 cohorts of normal mice. Following a single SQ/IV dose of 5 mg/kg of treatment, 10 organs as well as plasma and blood were collected at a serial of time points from 0.17 h to 24 h post dose. The total concentrations of investigational drug in all tissue specimens were measured with LC-MS/MS.

Results: Compared to SQ administration of free solution, both Cyclodextrin formulations produced distinct tissue concentration-time profiles, and exposure (C_{max} and AUC) of the investigational drug in most of measured tissues, except for blood and plasma, wherein all three treatments had similar concentration-time profiles. In addition the pattern of drug tissue exposure over time by Captisol was drastically different from that of Dexolve, especially in the bone and fat pat. Such difference was more pronounced following SQ administration than IV administration, suggesting a first-pass filtering effect of the Cyclodextrin formulations. Furthermore, our analysis also showed that predominate driver for drug tissue distribution is the Cyclodextrin-drug complex and free drug, for the two Cyclodextrin formulations and free solution, respectively.

Conclusion: In addition to improving the solubility of the poorly soluble drugs, the Cyclodextrin formulations alter the drug position in tissue, which is largely governed by the interplay between the Cyclodextrin and the drug, and subsequently the uptake of Cyclodextrin-drug complex in tissue. The distinct tissue distribution of Cyclodextrin drug complex, but similar plasma total drug exposure challenges the traditional bioequivalence concept based on similar plasma drug exposure, calling for direct drug measurement in target organs for equivalence assessment.

Keywords: Cyclodextrin; Parenteral formulation; Bioavailability; Bioequivalence; Drug carrier

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Introduction

Cyclodextrins (CDs) are novel complexing agents that have been used extensively in the pharmaceutical industry to enhance the aqueous solubility, bioavailability and stability of drugs. There are more than 30 different pharmaceutical products worldwide that are formulated in Cyclodextrin [1]. In addition to improving the solubility of the poorly soluble drugs, Cyclodextrins can be used to reduce gastrointestinal drug irritation, convert liquid drugs into microcrystalline or amorphous powder, and decrease drug to drug interactions [1-9]. Cyclodextrins are oligosaccharides formed by (α-1,4)-linked α-d-glucopyranose units, with a hydrophilic outer surface, and a hydrophobic central cavity. For drugs that are highly hydrophobic, they can easily get encapsulated within the hydrophobic cavity of Cyclodextrins, while the hydrophilic surface makes the whole complex water soluble. There are thousands of variations of cyclodextrins that have different ring size and functional groups, and the most common natural Cyclodextrins are the α-CD, β-CD and γ-CD, which contain 6, 7 and 8 glucopyranoside units, respectively (Figure

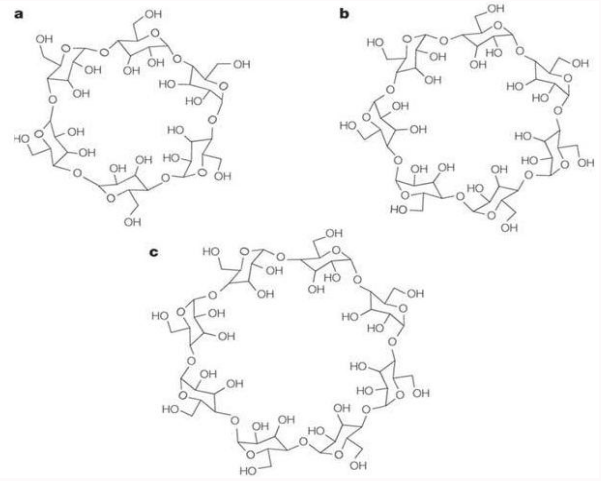

Figure 1: Schematic representation of cyclodextrins. (a) α-CD, (b) β-CD and (c) γ-CD [10].

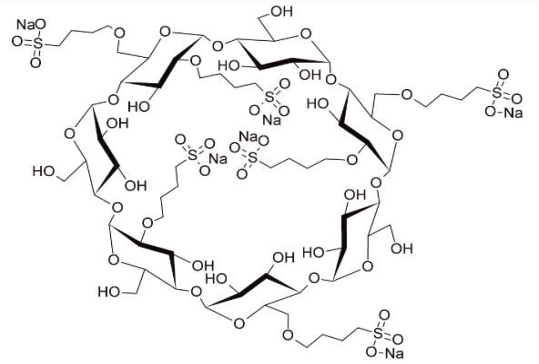

Figure 2: Structural overview of a representative species of Captisol® [11].

1) [10]. Although the natural Cyclodextrins and their complexes are hydrophilic, their aqueous solubility is rather limited, possibly due to the strong binding of the cyclodextrin molecule in the crystal state [1,4]. Hence, cyclodextrin derivatives have been developed to further enhance their aqueous solubility. A frequently used approach consists of partial methylation by substitution of any of the hydroxyl groups. Thus, many cyclodextrins derivatives display different degrees of substitution conferring unique biochemical and biological properties.

For instance, Captisol®, developed by Cydex, is a polyanionic β-CD derivative with a sodium sulfonate salt separated from the lipophilic cavity by a butyl ether spacer group, or Sulfobutylether (SBE) (Figure 2). Compared to the natural β-CD, Captisol provides higher interaction characteristics and can improve the water solubility by 50-fold. Moreover, it has been shown to have effective inclusion complexation potential and outstanding *in vivo* parenteral safety for biomedical uses [11]. Different generic versions of Captisol have been developed since 2011. Dexolve™, developed by Cyclolab Ltd, is the first generic USP-conform SBE-CD.

Numerous researches have been done to demonstrate the ability of cyclodextrins to dissolve drugs into stable aqueous solutions. However, tissue disposition of drugs encapsulated in cyclodextrins have not been well studied, and is often assumed to follow the same pattern of simple co-solvents, which do not contribute to drug disposition. Sun et al are one of the first to show that the β-CD drug complex, rather than just the free drug, widely distributed into various tissues in mice [12]. In addition, a few other studies showed that when a drug has an unusually high binding affinity for the cyclodextrins, incomplete and/or delayed dissociation of the free drug from cyclodextrins can occur, and this strong complexation would then result in altered pharmacokinetics and/or pharmacodynamic profile of the drug.

In our study, we aim to first investigate the plasma and tissue drug disposition of an investigational drug, encapsulated by either Captisol or Dexolve, in comparison to a liquid co-solvent formulation. Subsequently, we will characterize the key driving force that governs the drug exposure in circulation and peripheral tissues under each delivery system. The results from this study not only will help establish the role of cyclodextrins in the disposition of our investigational drug, but will also offer insight on assessing the bioequivalence, or clinical interchangeability of these two cyclodextrin delivery systems.

Methods

Chemicals and reagents

Investigational drug was produced at Carbogen AMCIS in Switzerland. The parenteral pH=6.8 formulation with Captisolor Dexolve was reconstituted from a lyophilized product, which was produced at Alcami Pharma at Charleston, SC. Free solution, or the Parenteral pH=4.5 formulation without cyclodextrins, was prepared at the University of Michigan, by adding investigational drug to citric acid solution, and titrating it using hydrochloric acid and sodium hydroxide solutions.

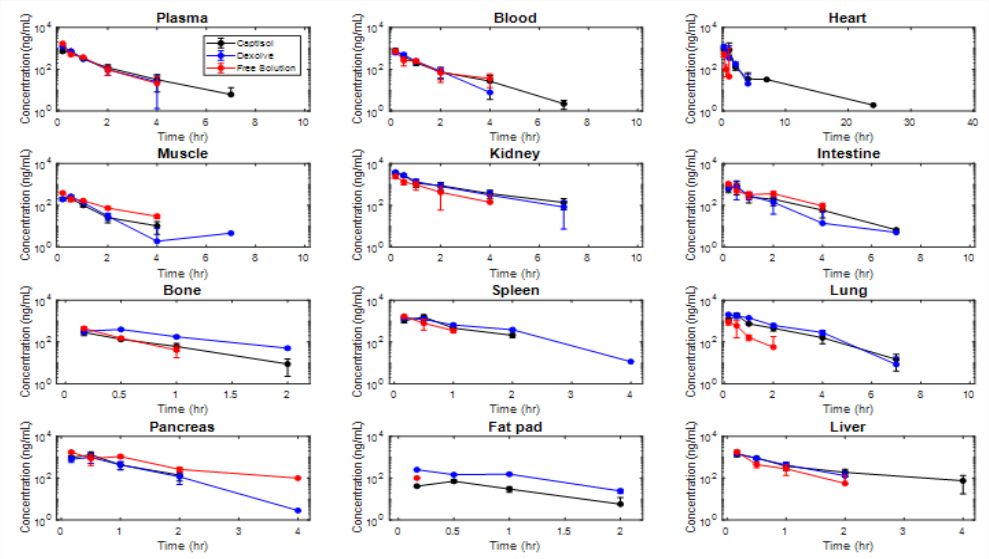

Figure 3: Total drug concentration to time profile in blood, plasma and tissues with free solution (red line), Captisol (black line) and Dexolve (blue line) SQ administration to mouse individually. N=3 for mean concentration and SD calculations.

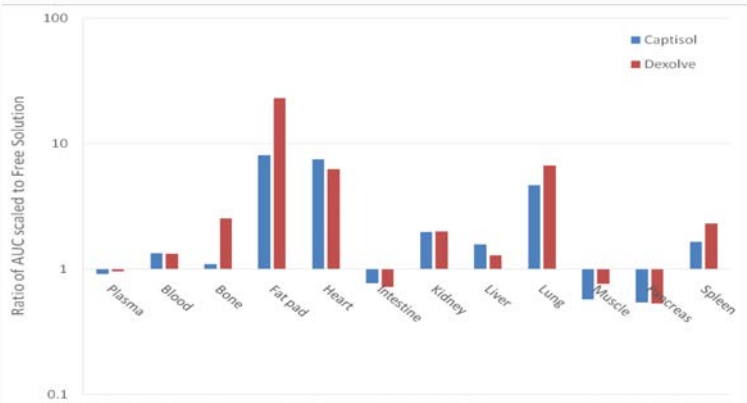

Figure 4: Ratio of AUC of Captisol or Dexolve versus those of free solution SQ administration in each type of tissues.

Animal experiments

Female CD-1 mice aged 6-8 weeks were purchased from Charles River Laboratories. Mice were administered one of two formulations of investigational drug, with each formulation having either Captisol or Dexolve as a carrier, via a single intravenous (IV) or Subcutaneous (SC) injection at 5 mg/kg. A control solution of investigational drug, or free solution containing no cyclodextrin carrier, was administered to another cohort of mice via IV or SC route at 5 mg/kg. Serial samples of blood, plasma, heart, lung, kidney, liver, spleen, pancreas, intestine, muscle, fat pad, and bone were collected at 0.167, 0.5, 1, 2, 4, 7, and 24 h post dosing with 3 mice used for each time point. At the given time points, blood samples were collected into heparinized calibrated pipettes from each mouse by terminal cardiac puncture. The blood samples were immediately centrifuged at 15,000 g for 10 min at 4°C and the plasma fraction was separated and stored at -80°C. Tissues were removed quickly, dissected, and placed in a sample tube for further processing (Precellys Lysing Kit, Bertin Instruments, CK28-R/7 mL). All samples were kept frozen until analysis.

Stock solution, working solution and quality control

Investigational drug was weighed and subsequently dissolved in DMSCO at a concentration of 5 mg/mL as stock solution. The solution was stored at -20°C. Quality control stock solutions were prepared at four different concentrations from separate weighing. Despropionyl para-Fluorofentanyl (Internal Standard, IS) stock solution was made by dissolving IS in DMSO at a concentration of 10 mM. The IS solution was further diluted in acetonitrile to a final concentration of 100 nM for sample preparation.

Sample preparation

Plasma and blood samples were prepared by dispensing 30 uL of plasma/blood from different time points into 30 uL acetonitrile followed by 200 uL of ice-cold acetonitrile containing 100 nM internal standards. The resulting mixture was vortexed for 10 min and then centrifuged at 3500 g for 10 min to precipitate protein. The supernatant was transferred and 2 uL was injected into LC-MS/MS for analysis. Tissue samples were thawed at room temperature. After weighing tissue samples, a solvent consisting of 20% acetonitrile and 80% water was added to each sample at a ratio of 5:1 (v/w). The samples were then subjected to tissue homogenization (Precellys, Bertin Instruments). Processing for the tissue suspension was the same as the plasma samples, however only 180 uL of the IS solution was added to each sample. Calibrated standards were prepared by diluted of the stock solution (5 mg/kg) to 1, 2.5, 5, 10, 25, 50, 100, 250,

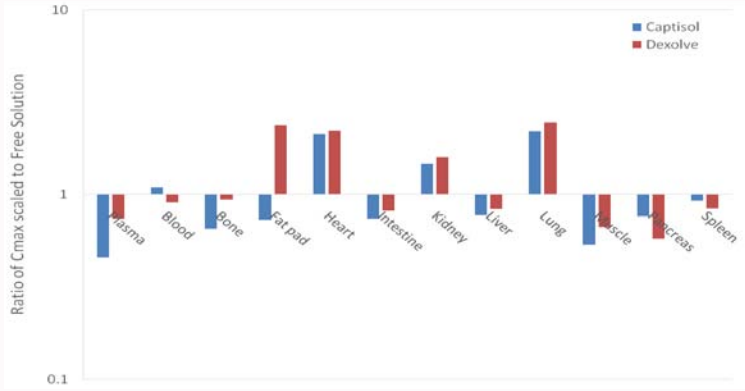

Figure 5: Ratio of Cmax of Captisol or Dexolve versus those of free solution SQ administration in each type of tissues.

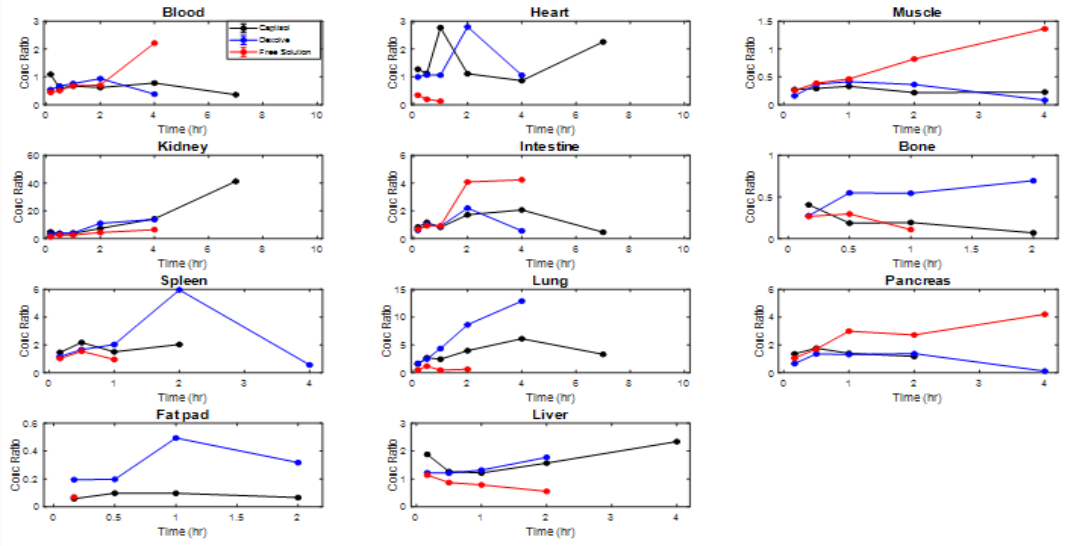

Figure 6: Ratio (y-axis) of total drug concentrations in tissues normalized by its corresponding plasma concentration at each time point for free solution (red), Captisol (black) and Dexolve (blue) administration, respectively.

500, 1000, 2500, 5000 ng/mL standard solution using acetonitrile.

Calibration curve

Calibration curves were constructed with eight nonzero standards by plotting the peak area ratios of investigational drug to the internal standard (Despropionyl para-Fluorofentanyl) versus the concentration using linear regression with a 1/concentration 2 (1/x2) weighting factor. A blank sample was used to exclude contamination or interference. The concentration range was evaluated from 1 to 5000 ng/mL. The Lower Limit of Quantification (LLOQ) for blood and plasma was between 1 and 5 ng/mL. The Lower Limit of Quantification (LLOQ) for tissues ranged from 1 to 50 ng/mL.

LC-MS/MS

Determination of investigational drug concentration in plasma, blood, and tissues using LC-MS/MS was performed on an ABI-4500 Qtrap (Sciex, Ontario, Canada) mass spectrometer with an electro spray ionization source coupled to a Shimadzu HPLC system. An X Bridge C18 column, 50 mm × 2.1 mm ID, 3.5 uM (Waters, Mild ford, MA, USA) operated at a 0.4 mL/min flow rate was used for separation. The mobile phase consisted of 0.1% formic acid in purified deionized water as A and 0.1% formic acid in acetonitrile as B. The gradient began at 5% B for 0.5 min, then increased linearly to 95% B in 1 min, and was kept at 95% B for 2 min. At 3.6 min, mobile phase B was decreased to 5% and kept at 5% for 2 min. Total run time for each sample was 5.7 min. The mass spectrometer was operated in ESI positive ion mode with Multiple Reaction Monitoring (MRM) for ion detection. The MRM transitions were monitored at m/z 459.1 to m/z 330.1 for investigational drug and m/z 299.1 to m/z 164.0 for the internal standard. For investigational drug, the Declustering Potential (DP), Entrance Potential (EP), and Collision Exit Potential (CXP) were 107.1, 5.91, and 5.93 V, respectively, and collision energy was -18 eV. Data acquisition and processing were completed using Analyst software version 1.6.2 (Applied Biosystems, MDS Sciex Toronto, and Canada).

Pharmacokinetic data analysis

The pharmacokinetics of investigational drug from free solution, Captisol, and Dexolve were compiled and calculated with Phoenix/Winnolin (Version 6.4, Pharsight Corp., and Mountain View, CA). The plasma/blood and tissue concentration–time data were compiled and plotted using MATLAB. The correlation was performed using ratio of Cmax and AUC between tissues and blood. The driving force analysis for tissue distribution was performed by comparing concentrations of plasma and tissues at each time points.

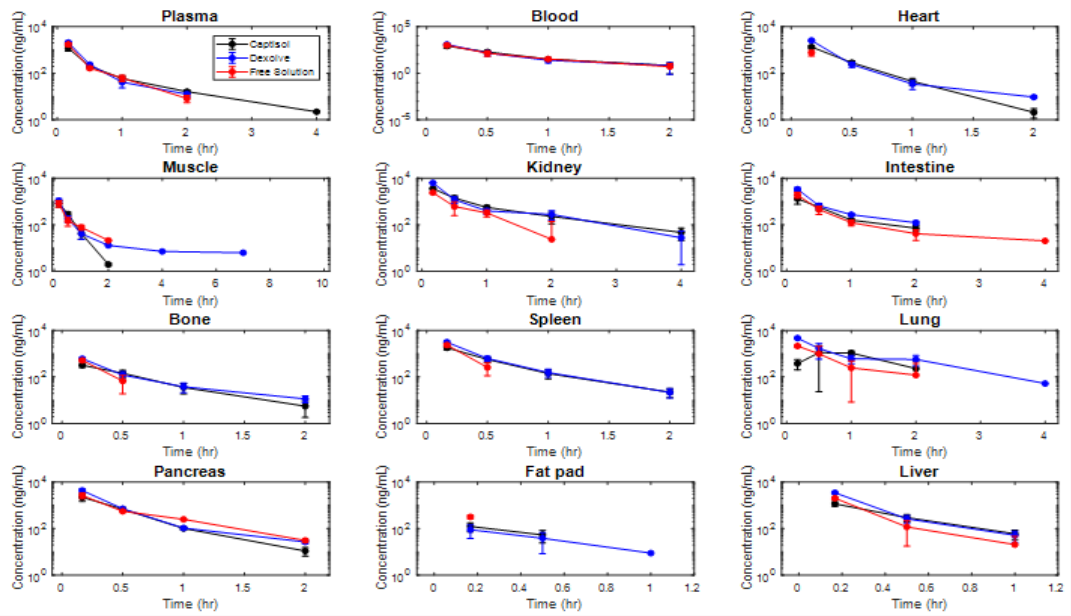

Figure 7: Total drug concentration to time profile in blood, plasma and tissues with free solution (red line), Captisol (black line) and Dexolve (blue line) IV administration to mouse individually. N = 3 for mean concentration and SD calculations.

Results

Accuracy and precision of analytical method assay

Calibration curves were generated with the blank of blood, plasma, or tissue and validated. Intra-day variability was evaluated using quality controls run in triplicate before, in the middle, and after running the samples. The intra-day accuracies were within 85% to 115% for QC. Intra-batch precision calculated and expressed as Relative Standard Deviation (RSD%), were also within the acceptable range. The calibration curves had correlation coefficient r square of larger than 0.9919 across all samples. The data indicated that the assay method was reliable and reproducible.

Subcutaneous administration

Investigational drug concentration–time profile in plasma, blood and tissues: Following the SQ administration of the 5 mg/kg dose of free investigational drug solution, Captisol and Dexolve, the concentration–time profiles of total drug in each type of tissue were determined and shown in Figure 3. In plasma, although free drug solution had the highest initial concentration (1710+692 ng/mL from free solution *vs.* 727+121 ng/mL from Captisol *vs.* 1220+156 ng/mL from Dexolve) at 10 min, all three formulations exhibited similar concentrations profile subsequently. Similar trend was also observed in blood.

In the heart, drug concentration was higher when encapsulated in cyclodextrins initially (927+138 ng/mL from Captisol *vs.* 1238+504 ng/mL from Dexolve *vs.* 531+4 ng/mL from free solution at 10 min). Free solution then decreased precipitously within 1 h, and became undetectable afterwards. Both Captisol and Dexolve decayed at a similar rate within the first 4 h, but Dexolve decreased below limit of quantification after 4 h. The lower drug exposure with free solution implies that free solution is less likely to cause cardio toxicity compared to Captisol or Dexolve.

In the muscle, investigational drug in free solution had a higher concentration (393+41 ng/mL) at the first time point of 10 min, compared to Captisol (199+37 ng/mL) and Dexolve (196+76 ng/mL). While both Captisol and Dexolve increased slightly within the first 30 min, they declined at a faster rate than free solution. Similar trend was also observed in the pancreas, in which free solution had a higher initial concentration, and decayed at a slower rate than the cyclodextrin formulations.

In the kidney, investigational drug formulated in cyclodextrins had similar concentration -time profile, and had slightly higher initial concentrations (3473+443 ng/mL from Captisol *vs.* 3850+1062 ng/mL from Dexolve) compared to that formulated in free solution (2400+835 ng/mL). Both Captisol and Dexolve remained detectable for the first 7 h, while free solution decreased below quantification of limit after 4 h.

In the small intestine, administration of free solution resulted in a concentration as high as 1057+196 ng/mL at the first time point of 10 min. This concentration started to decline and fluctuates slightly within the next 2 h. Free solution eventually became undetectable after 4 h. Both the cyclodextrin formulations had lower initial concentrations (616+214 ng/mL from Captisol *vs.* 726+77 from Dexolve), but the concentration levels increased for 30 min, before they started to decrease.

In the bone, drug concentration from free solution (449+171.13 ng/mL) was the highest at the first sampling time point of 10 min, compared to Captisol (290+86 ng/mL) and Dexolve (329+81 ng/mL). Captisol and free solution started to decline at a similar rate, with free solution decreased below the limit of quantification after 1 h. However, drug concentration with Dexolve administration remained high for the first 30 min, and then started to decline at a similar rate as Captisol. These data suggested the higher uptake of Dexolve in the bone, implying that investigational drug encapsulated in Dexolve may exhibit different bone specific efficacy and safety profiles, compared to the Captisol and free solution.

In the spleen, free solution had the highest initial concentration (1697+489 ng/mL), compared to Captisol (1059+246 ng/mL) and

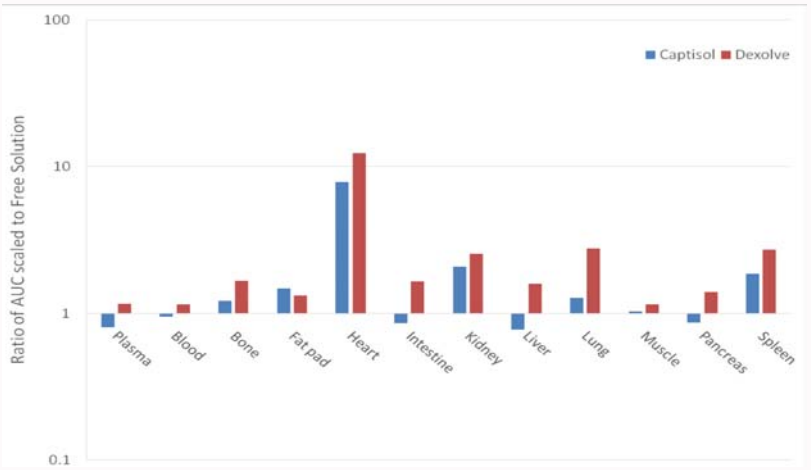

Figure 8: Ratio of AUC of Captisol or Dexolve versus those of free solution IV administration in each type of tissues.

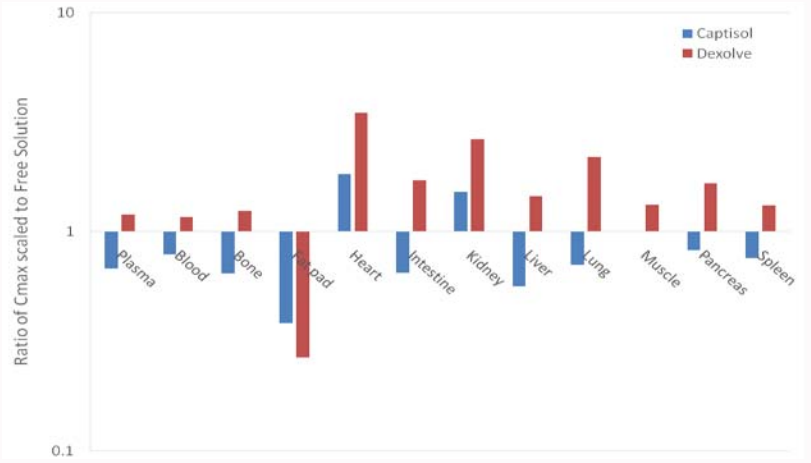

Figure 9: Ratio of C_{max} of Captisol or Dexolve versus those of free solution IV administration in each type of tissues.

Dexolve (1430+650 ng/mL). All three formulations decreased at a similar rate, with free solution and Dexolve became undetectable at 2 h and 4 h, respectively.

In the lung, Dexolve had the highest initial concentration (2057+663 ng/mL) at 10 min, compared to Captisol (1109+251 ng/mL) and free solution (901+393 ng/mL). However, Captisol increased to 1960+445 ng/mL at 30 min, before decaying at a similar rate to Dexolve. Compared to the Cyclodextrin formulations, free solution declined rapidly and became undetectable after 2 h. Like in the heart and kidney, investigational drug had a higher uptake in the lung when administered with Cyclodextrins.

In the fat, drug concentration from free solution was 101+35 ng/mL at the first sampling time point of 10 min, and decreased below the limit of quantification afterwards. However, both Captisol and Dexolve seemed to decrease at a similar rate, and remained within the limit of quantification for 2 h. These results suggested that Cyclodextrins significantly enhance the uptake of drug in the fat, compared to free solution. Like in the bone, a higher uptake of drug was seen with Dexolve administration compared to Captisol.

In the liver, free solution had a higher concentration (1800+260 ng/mL) at the first time point of 10 min, compared to Captisol (1381+336 ng/mL) and Dexolve (1487+411 ng/mL). While both Cyclodextrin formulations decayed at a similar rate, free solution decreased at a slightly faster rate and drop below the lower limit of quantification after 2 h. These data suggested the slightly higher uptake of drug in Cyclodextrins formulation in the liver.

Cmax and AUC exposure of investigational in different tissues from three different delivery systems: The ratio of Cmax and ratio of AUC of the two Cyclodextrin formulations versus those of free solution in each tissue were presented in Figure 4 and 5. In most tissues, especially fat pad, heart and lung, Captisol and Dexolve had distinct Cmax and AUC exposures from free solution, suggesting that the Cyclodextrins alter the tissue disposition of the investigational drug, and can potentially change the pharmacology of the drug *in vivo*. Furthermore, the different exposures between Captisol and Dexolve, especially in the bone, fat pad, liver, muscle, lung and spleen, indicated that the variation in the Cyclodextrins (brand *vs.* generic form) can also lead to a different distribution pattern in tissues, which can potentially lead to different clinical outcomes. Based on these results, these two formulations are not bioequivalent.

Driving force analysis for tissue distribution of free solution, captisol and dexolve: To analyze the driving force of drug distribution in each tissue from three different formulations, the total drug concentrations in tissue were normalized by their corresponding plasma concentration at all time points for free solution, Captisol and Dexolve, as shown in Figure 6. In the muscle, kidney and pancreas,

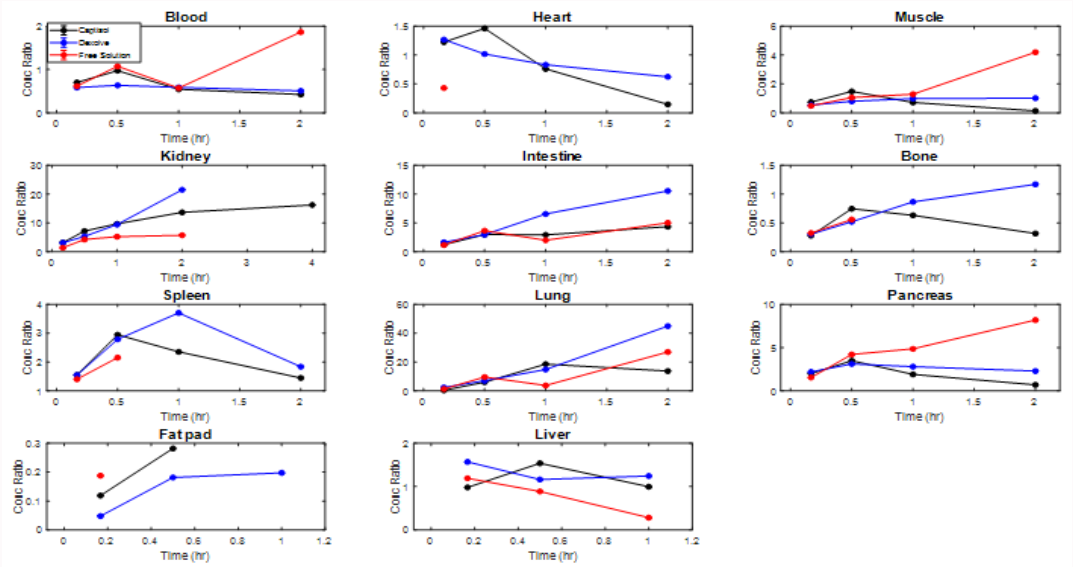

Figure 10: Ratio (y-axis) of total drug concentrations in tissues normalized by its corresponding plasma concentration at each time point for free solution (red), Captisol (black) and Dexolve (blue) administration, respectively.

all three formulations seemed to have similar concentration ratios initially. The Cyclodextrin formulations continued to have similar concentration ratios subsequently, but they deviated from free solution. This result suggested that while the initial tissue distribution of all three formulations is primarily governed by the free drug, the subsequent distribution is driven by the free drug for free solution and the Cyclodextrin-drug complexes for Captisol and Dexolve.

In the intestine, spleen and lung, while the three formulations exhibited similar concentration ratios initially, all of them deviated from each other subsequently. This result implied that the free drug is the initial driving force for all formulations. However, in the subsequent phase of distribution, the Cyclodextrin-drug complex is the driving force for both Captisol and Dexolve, and the free drug continue to be the driving force for free solution. In addition, the difference in concentration ratios between Captisol and Dexolve can probably be attributed to the variation between the two forms of Cyclodextrins.

In the liver and blood, Dexolve and free solution had similar initial concentration ratios, while deviated from each other subsequently, suggesting that the free drug is the driving force for both Dexolve and free solution at the early phase of distribution. While their initial concentration ratios were slightly different, both Captisol and Dexolve had similar concentration ratios during the later phase of distribution, suggesting the Cyclodextrin-drug complex is the main driving force for these two formulations, and the variation between Captisol and Dexovle does not have significant impact on the tissue distribution in these organs.

In the bone and fat pad, free solution exhibited similar initial concentration ratio compared to Dexolve and Captisol, respectively, suggesting free drug is the initial driving force for these formulations. All three formulations showed different concentration ratios subsequently, indicating that while free drug is the driving force for drug solution tissue distribution, the different Cyclodextrin-drug complex is the driving force for each of the Cyclodextrin formulations.

In the heart, all three formulations showed different concentration ratios throughout, suggesting that free drug is the driving force for free solution, and the different Cyclodextrin-complex is the driving force for the Cyclodextrin formulations.

Intravenous administration

Drug concentration-time profile in plasma, blood and tissues: Following the IV administration of the 5 mg/kg dose of free solution, Captisol and Dexolve, the concentration time profiles of total drug in each type of tissue were determined and shown in Figure 7. In plasma, all three formulations had similar concentration time profile for the first two hours. Both free solution and Dexolve decreased below the lower limit of quantification after 2 h, while Captisol remained detectable up through 4 h. Similarly, in blood, all three formulations exhibited the same concentration-time profile for the first 2 h, and decayed below the low limit of quantification afterwards.

In the bone, heart, kidney, fat pad and spleen, all three formulations exhibited similar concentration-time profile initially; however, free solution decayed at a much faster rate than the two Cyclodextrin formulations, and became undetectable after the first few time points. Comparing Captisol to Dexolve, the concentration-time profiles appeared to be similar, indicating that the variation between the two Cyclodextrins doesn't affect tissue the distribution.

In the intestine, Dexolve has the highest initial concentration and remained to be higher than Captisol and free solution, until it decreased below the lower limit of quantification after 2 h. Although free solution was higher than Captisol initially, it appeared to have similar profile to Captisol after the first time point and remained detectable for 4 h.

In the liver, although Dexolve had higher initial concentration than Captisol, both of them had similar concentration-time profile after the first time point. Free solution had moderate concentration initially, and appeared to decline at a similar rate to both the Cyclodextrin formulations. All three formulations decreased below the lower limit of quantification after 1 h.

In the lung, Dexolve had the highest initial concentration among the three formulations, and decreased below the limit of quantification after 4 h. Although both Captisol and Dexolve are Cyclodextrins,

Captisol had a 12-fold lower initial concentration compared to Dexolve. Concentration of drug in Captisol increased for the first hour before it decreased below the limit of quantification after 2 h. This result suggested that the different forms of Cyclodextrins can result in different drug distribution in the lung, which can potentially lead to different pharmacological properties. Free solution had moderate initial concentration and seemed to decay at a similar rate to Dexolve.

In the muscle, all three formulations had similar initial concentrations, but they started to deviate from each other after 30 min. Captisol had the fastest decay, while free solution had the slowest decay. Both Captisol and free solution decreased below the lower limit of quantification after 2 h. Dexolve had a moderate decay and plateaued after 2 h.

In the pancreas, Dexolve had the highest initial concentration, but it decayed rapidly, and both Captisol and Dexolve converged after the first time point. Free solution had a moderate initial concentration and decayed at a slower rate compared to Dexolve.

Cmax and AUC exposure of drug in different tissues from three different delivery systems: The ratio of Cmax and ratio of AUC of the two Cyclodextrins formulations versus those of drug solution in each tissue were presented in Figure 8 and 9. In most tissues, Captisol and Dexolve had distinct C_{max} and/or AUC exposures from free solution, suggesting that the Cyclodextrins alter the tissue disposition of investigational drug. Furthermore, the different exposures between Captisol and Dexolve, especially in bone, intestine, kidney, lung, heart, pancreas and spleen, indicated that the variation in the Cyclodextrins (brand *vs.* generic form) can also lead to a different distribution pattern in tissues.

Driving force analysis for tissue distribution of motolimod solution, captisol and dexolve: To analyze the driving force of drug distribution in each tissue from three different formulations, the total drug concentrations in tissue were normalized by their corresponding plasma concentration at all time points for free solution, Captisol and Dexolve, as shown in Figure 10. In the heart, both Captisol and Dexolve had similar initial concentration ratios, but distinct ratios from the free solution. This result indicated that the Cyclodextrin complex, rather than the free drug, is the driving force for Captisol and Dexolve. Captisol deviated from Dexolve subsequently, suggesting that the variation between the two Cyclodextrins drives the different distribution at this phase.

In the muscle, kidney, bone, spleen, lung, and pancreas, while all three formulations had similar concentration ratios initially, the two Cyclodextrin formulations started to deviate from the free solution, and from each other as time progressed. This result suggested that the free drug is the driving force for the initial tissue distribution for both Captisol and Dexolve, but each of the Cyclodextrin-drug complexes governs the subsequent disposition.

In the fat pad and liver, all three formulations showed different concentration ratios throughout, suggesting that free drug is the driving force for free solution and the different Cyclodextrin-complex is the driving force for the Cyclodextrin formulations.

In the blood, all three formulations seemed to have similar initial concentration ratios, suggesting that the free drug is the main driving force. The two Cyclodextrin formulations continued to show similar concentration ratios but deviated from free solution. This result implied that while the free drug is the driving force for free solution, the Cyclodextrin-drug complex is the main driving force for the Cyclodextrin formulation during this phase of distribution.

In the intestine, all three formulations had similar initial concentration ratios, but Dexolve deviated from both Captisol and free solution subsequently. This result suggested that free drug is the initial driving force for all three formulations, and continued to be the driving force for both free solution and Captisol. Dexolve, however, is driven by the Cyclodextrin complex during the later phase of distribution.

Discussion

Because of the poor solubility of our investigational drug, formulation vehicles, such as Cyclodextrins, are needed to serve as carriers to deliver the drug. When the drug enters into the cavities of Cyclodextrin, the hydrophobic groups of the drug are then embedded in the hydrophobic core, and the solubility of the drug can be improved by the hydrophilic surface of the Cyclodextrin-drug complex. In this study, we compared the pharmacokinetics behaviors between the two Cyclodextrin formulations with the free drug solution to decipher the role of Cyclodextrins in drug disposition. Furthermore, we also compared the drug exposures between Captisol and Dexolve, to assess the bioequivalence of the two β-CD formulations.

Cyclodextrins *vs.* free solution

Our experimental data showed that rather than acting as a co-solvent to simply increase the solubility of the investigational drug, the Cyclodextrins form unique drug-carrier complexes which alter the drug disposition in tissues. As shown in Figure 3, in subcutaneous administration, Cyclodextrins displayed different pharmacokinetics profile than the free solution in most measured tissues, besides blood and plasma in which all three treatments had similar profiles. Furthermore, this difference was also seen in exposure, as measured by AUC and Cmax, in majority of the tissues (Figure 4 and 5). Based on these observations, it is postulated that Cyclodextrins formed stable complexes with the investigational drug and deposited directly into the tissues. As a result, different tissue uptake and distribution is observed with Cyclodextrins formulations compared to free solution. Similar phenomenon was also observed in other studies, wherein Cyclodextrin formed a very strong interaction with the drug of interest, thus resulting in a slow dissociation of the complex *in vivo*, and altering the drug distribution and excretion profiles.

Our driving force analysis (Figure 6) further confirmed this hypothesis by showing that while the free drug drives the tissue disposition in free solution, the Cyclodextrins-drug complex is one of the main drivers of the eventual collective tissue levels of the investigational drug formulated in Captisol and Dexolve. Taken together, these results suggested that despite having the same active ingredient, different Cyclodextrin formulations produce different pharmacokinetics profiles, which can potentially lead to distinct pharmacological and therapeutic effects. As a result, the Cyclodextrins-drug complexes can be developed for lung indication, owing to their much higher exposure than the free solution. On the other hand, it is more likely to observe cardio toxicity with the Cyclodextrin-drug complexes than the free solution, because of their higher exposure in the heart. Hence, understanding the interplay between the formulation and drug of interest is critical in guiding drug development and understanding clinical outcomes.

While the different delivery systems resulted in distinct tissue

profiles, they exhibited similar pharmacokinetics behaviors in plasma and blood. Hence, measurement of blood or plasma concentration would not be adequate in characterizing the overall drug disposition, rendering it a poor surrogate for understanding and predicting clinical outcomes. In fact, for delivery vehicles that form stable complexes with drug of interest and drives the distribution of drug, a few recent studies also suggested that drug plasma level may not be a good indicator of drug tissue level. Moreover, the use of plasma concentration to predict efficacy may misinform decisions and result in poor clinical outcomes, especially when sufficient accumulation of drugs is needed at target tissues.

With intravenous administration, distinct pharmacokinetic profiles (Figures 7-9) were also observed between Cyclodextrins and free solution. However, the difference was less pronounced compared to that with subcutaneous administration, suggesting a first-pass filtering effect of the Cyclodextrins formulations.

Captisol *vs.* Dexolve

Captisol is a polyanionic β-CD derivative with a sodium sulfonate salt separated from the lipophilic cavity by a butyl ether spacer group, or Sulfobutylether (SBE), and Dexolve is a generic form of Captisol developed by a different manufacturer. Although Captisol and Dexolve presumably have similar chemical properties, the pharmacology of the two Cyclodextrin formulations can be very different.

For instance, in SQ administration, Dexolve exhibited different drug exposure than Captisol in some of the measured tissues, most noticeably in bone and fat pad (Figure 3). Since the Cyclodextrin-drug complex is the driving force for the drug disposition in these tissues (Figure 6), any slight variation between Captisol and Dexolve could potentially affect the interaction between Cyclodextrin and the drug, resulting in Cyclodextrin-drug complexes that confer distinct pharmacological properties. These findings suggested that comparing to Dexolve, Captisol act differently in delivering the investigational drug to certain tissues, and therefore, may not be equivalent. During drug development, the different pharmacological properties between the two Cyclodextrin formulations can be exploited to target a certain disease indication, or achieve a specific safety and efficacy profile.

While Captisol and Dexolve displayed different pharmacokinetics behaviors in some of the measured tissues, they exhibited comparable profiles in blood and plasma, in both SQ and IV injections. As our driving force analyses (Figure 6 and 10) suggest, all three treatments, Captisol, Dexolve and free solution, are predominantly driven by the free drug in blood and plasma. Therefore, the drug exposures were similar for all three treatments. Based on the blood and plasma concentration profiles, Captisol and Dexolve would be deemed bioequivalent and clinically interchangeable; however, the peripheral tissue distribution was significantly different between the two. These findings challenge the conventional practice for assessing bioequivalence using blood or plasma concentration, which would not be sufficient to fully characterize the pharmacology of Captisol and Dexolve.

In conclusion, the results from this study demonstrate that Cyclodextrin formulations alter the drug exposure in tissue distribution. Rather than acting as a cosolvent to help dissolving the drug in various tissues, Cyclodextrins form unique drug-carrier complexes with the investigational drug. As a result, this complex possesses its own specific delivery properties, which govern the distribution of Cyclodextrin-drug in tissues, and lead to different distribution pattern than free solution.

In addition, the distinct pharmacokinetics profiles between Captisol and Dexolve suggest that these two Cyclodextrin formulations act differently in delivering the investigational drug to tissues, except for blood and plasma, and therefore, may not be bioequivalent. These differences in tissue distribution may suggest different toxicity risk *vs.* benefit profile with each Cyclodextrin formulation. However, the different distribution pattern between Captisol and Dexolve cannot be deduced from the corresponding blood and plasma distribution, which appeared to be comparable for both Cyclodextrin formulations. Hence, the blood and plasma profile is a poor surrogate for assessing such Cyclodextrin-drug complex; rather, direct tissue measurement shall be studied to fully characterize its pharmacology, safety and efficacy.

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