In Vivo Delivery, Pharmacokinetic Analysis and Accelerated Stability Study Of A Dry-Powder Inhalable Formulation of Nanocrystalline Camptothecin for the Treatment of Lung Cancer

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ABSTRACT SUMMARY

The focus of this work was to create a dry-powder camptothecin (CPT) formulation that could be inhaled and potentially developed into a lung cancer therapeutic. CPT and CPT analogs have been shown to be effective in treating lung cancers. However, the physical properties of CPT have made it difficult to formulate into a deliverable therapeutic, limiting its utility in the clinic. CPT nanocrystals with an approximate diameter of 120 nm ± 80 nm were spray-dried with 10,000-dalton molecular-weight dextran (Dex10), creating particles of the correct size for dispersal into the lungs upon inhalation. Impactation analysis of the powder revealed that the mass median aerodynamic diameter (MMAD) of the particles was 2.7 µm and the fine particle fraction (FPF) was 78%. The powder was aerosolized and delivered to rats using a nose-only inhalation chamber at Lovelace Respiratory Research Institute (LRRI). Rats received a high or low inhaled dose of powder or an intravascularly (IV)-delivered dose, to compare CPT exposure to the lungs through inhalation and IV dosing.

INTRODUCTION

CPT is a highly potent topoisomerase I inhibitor that has shown remarkable anticancer activity in preliminary clinical trials. However, the successful use of CPT in the clinic has been hampered by its low solubility and toxicity. The therapeutic index of CPT must be increased, because hematological and gastrointestinal toxicities that occur upon systemic exposure can be dose-limiting (1, 2).

An aerosolized liposomal formulation of 9-nitrocamptothecin has been shown to be efficacious in treating a metastatic cancer mouse model (3). However, toxicities associated with aerosolized liposomal CPT formulations have been identified. Modest efficacy of aerosolized liposomal preparations has also been shown in treating lung cancer patients in clinical trials (4, 5).

To overcome the technical and toxicological challenges associated with advancing a nebulized formulation of CPT, we created a dry-powder formulation of CPT nanocrystals imbedded in Dex10. The dry-powder microparticles have the correct aerodynamic diameter for efficient deposition to the deep lung, enabling effective targeting of CPT to the lung and minimizing toxic side effects caused by off-site deposition and ingestion.

EXPERIMENTAL METHODS

The CPT nanocrystals were formed by milling bulk CPT crystals in a McCrone micronizing mill. Bulk CPT was milled with sodium lauryl sulfate (SLS), polyvinyl pyrrolidone (PVP), and simethicone to create nanocrystalline CPT.

Dry-powder formulations were prepared by spray-drying a suspension of nanocrystalline CPT and dissolved Dex10 in water on a laboratory-scale spray dryer with a two-fluid atomizer.

Next-generation impaction (NGI) was used to determine the MMAD, the geometric standard deviation (GSD), and FPF of the particles. The powder was delivered to the NGI device in capsules using a monodose inhalation device, and the NGI device was operated at 60 L/min. The NGI stages were analyzed using absorbance spectroscopy to determine the mass of CPT in the powder impacted in each stage.

The particles were also tested in an in vitro cuvette dissolution test to measure the solubility enhancement provided by the nanocrystalline CPT relative to bulk crystals of CPT. A 1-mg sample of either the CPT/Dex10 formulation or bulk crystals was placed on the surface of 2.5 mL of deionized water in a cuvette. CPT absorbance was then monitored over 15 minutes at 370 nm.

RESULTS AND DISCUSSION

The results of this project demonstrated the feasibility of an inhalable dry-powder CPT formulation. Use of spray-drying and a stabilizing excipient offers an attractive route to manufacture respirable engineered CPT particles. Dextran is attractive as a stabilizing excipient, because it has unique properties that make it particularly well-suited for pulmonary use including a high glass-transition temperature (Tg), low hygroscopicity, and precedence as a safe excipient for parenteral delivery.

The goal of this study was to demonstrate that a dry-powder CPT formulation could be produced that was capable of being aerosolized, would likely deposit in the lungs, and would release CPT. Particles were engineered by spray-drying an aqueous solution of CPT and Dex10 to produce particles with a composition of 19.1% CPT, 3.8% PVP, 0.3% SLS, 0.3% simethicone, and 76.5% Dex10.

Figure 1 shows images of the CPT crystals before and after nanomilling and after spray-drying. Before milling, particle diameters were ~20 to 400 µm, whereas after milling, they were ~120 nm ± 80 nm in diameter. Crystals were imaged using scanning electron microscopy (SEM) before milling, and cryo-transmission electron microscopy (cryo-TEM) after milling. To form respirable particles from the CPT crystals, Dex10 was added to an aqueous suspension of the nanocrystals, using a CPT:Dex10 mass ratio of 1:4. This suspension was then spray-dried to form respirable particles. SEM images of the resulting CPT/Dex10 particles were used to roughly...
estimate the size of the particles, which were in the 0.5-µm to 2-µm range.

The MMAD of the particles, which dictates particle dispersion in the lung, was measured using a NGI device (Plastiap RS01). The NGI device was operated at 60 L/min, 90% of the dose was emitted during actuation, 68% of the emitted dose was of a size that would be delivered to the lungs, and 22% of the emitted dose would be delivered to the throat. Figure 2 shows the results of this test. Analysis of the results shows that the MMAD of the particles was about 2.7 µm, the GSD was 2.1 µm, and the FPF was 78% based on emitted dose. These characteristics are excellent for particle distribution in the lung, and these results demonstrate the robust, general nature of our particle engineering and formulation approach.

The particles were tested in an in vitro cuvette dissolution test to measure the solubility enhancement provided by the nanocrystalline CPT relative to bulk crystals of CPT. Samples (1 mg) of the CPT/Dex10 formulation or bulk crystals were placed on the surface of 2.5 mL of deionized water within a cuvette. The absorbance was then monitored at a wavelength of 370 nm over time. Figure 3 shows the results. The CPT/Dex10 particles released CPT into the aqueous solution, whereas the bulk crystalline CPT did not release CPT. The initial spike of CPT for the CPT/Dex10 particles was due to larger particles settling to the bottom of the cuvette before dissolution. The dashed line represents the anticipated true dissolution rate of the CPT/Dex10 particles without the larger particles settling. The bulk crystalline CPT never wet with water and did not dissolve.

**CONCLUSION**

We successfully developed and tested an aerosolizable dry-powder formulation of CPT that can be inhaled. A noninvasive delivery route to administer relevant concentrations of CPT to the lungs could be a potentially ground-breaking means of treating lung cancer, because the pharmacologic agent would be deposited near its site of action; thus limiting the amount of active required for dosing and limiting offsite toxicities. The dissolution study described here also suggests that we have successfully increased the free fraction of CPT in an aqueous solvent after particle dissolution. A higher free fraction of CPT would likely lead to an enhanced bioavailability of CPT in aqueous solutions such as lung fluid.

**REFERENCES**


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