Microencapsulated Controlled-Release Cisplatin Formulations for Oncology Applications

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BACKGROUND

Cisplatin is widely used as an effective drug against a number of cancer diseases. However, toxicity is a challenge, and this limits the dosage which Cisplatin can be administered systemically. In order to overcome this challenge, and to retain high drug efficacy at lower dosage, several attempts have been made in the past to modulate the release profile of Cisplatin. For this purpose, Cisplatin formulations have been developed in the past based on microencapsulation, liposomes, gel delivery, and other strategies. Our goal was to develop polymeric microparticulated based formulations of Cisplatin, with the specific goal of allowing for the use of the formulation with a variety of promoting therapeutic strategies. In addition to the stand-alone use of the polymeric microparticulated formulation for systemic administration, our goal includes (a) stand-alone local administration (b) local administration with the drug formulation incorporated into a medical device platform as a combination product, and (c) the above strategies in concert with other drug molecules (i.e., combination therapy, administered both systemically and locally). The overarching motivation was to create a Cisplatin formulation with relevance to all the above therapeutic strategies.

We have been successful in creating a range of microencapsulated Cisplatin formulations using polyglycolic acid (PLGA) polymers, with the employment of solid in oil (s/o) emulsion based solvent evaporation methods. This has resulted in particles that are uniformly spherical and a wide size range (from the sub-micron (sub) to polymer aggregate to 150 μm in diameter). Additionally, high drug loading levels were achieved, in the range of 20-30% (w/w), which is at the higher end for loading, compared to what is published in the literature. Most importantly, we have been successful in achieving a wide range of release profiles for Cisplatin, creating the range of release over a few hours to over several weeks. The characterization of the formulation provides information on the morphology of the microencapsulated particles, the distribution of drug within them, as well as the crystalline state and surface conformation of the drug molecule. These measurements provide the basis to optimize pharmacodynamic characteristics of the formulation by a combination of variables such as drug-loading, type of PLGA polymer, molecular weight of encapsulating polymer, as well as process variables such as shear rate, speed of organic phase addition, and others. Preliminary data generated in cell culture with the A549 lung cancer cell line has established a significantly lower IC50 value for the microencapsulated Cisplatin formulation compared to unencapsulated Cisplatin, thus emphasizing the increased potency of our formulation with the promise of better efficacy at lower drug dosage.

In addition, we have been successful in achieving double-encapsulation / multi-encapsulation for Cisplatin, which opens up significant new possibilities, such as (a) encapsulation of a few or more drugs, including Cisplatin and (b) multiple bursts in the kinetics of Cisplatin release profile. We have also been successful in crystallizing new combination product concepts, such as co-administration of drug delivery components into RF Ablation device through which the microencapsulated Cisplatin could be administrated locally into the tumor ablation margins. These preliminary results provide an encouraging basis for our ongoing work, namely further characterization and optimization of our formulation, followed by the evaluation of their efficacy in cell culture and preclinical models.

CHARACTERIZATION METHODS

In vitro release kinetics were measured by the application of experimental sink conditions (i.e., conditions allowing no more than 10% Cisplatin elution). Approximately twenty-five milligrams of each batch of Cisplatin microspheres were added to dialysis tubing with a molecular weight cut-off of 50,000 Da, and then submerged in phosphate buffered saline with Tween 20 at pH 7.4 at maintained at 37°C. The elution of the Cisplatin from the dialysis tubing into the dissolution media was then measured by the collection of samples at each of the following time points: 2, 4, 24, 48, 72, 120, 192 hours. The collected samples were subjected to HPLC analysis to determine Cisplatin concentration at different times, and ultimately as cumulative percent released vs. time. HPLC analysis was run on an Agilent 1100 system with a Waters Jupiter 18μm column using UV detection at 210nm. Particle size distribution was obtained using Malvern Mastersizer 2000. SEM images were obtained utilizing an FEI Quanta 600F environmental scanning electron microscope. The powder x-ray diffraction (XRD) patterns were collected using a Siemens/Bruker D8 diffractometer. HMR spectra were collected using a Bruker Avance T100 equipped with a 4mm 5/2 Proximal Magic Angle Spinning (CIRMAS) probe. A 1H, 13C, 2H, and 15N 2D-COSY experiment was used to analyze the Transformable spectrum. The effect of Cisplatin formulations was evaluated in terms of cytotoxicity towards the A549 lung cancer cell line over a wide range of concentrations. The cells were plated in 96-well plate at a density of 1,200 cells per well, between passage 2 and passage 3. Cell viability was evaluated at the day T+5 point using the Promega Substrate Cell Titer Glo Assay (One Solution Reagent Mixture (metabolism) assay), and absorbance reading at 490nm. The Inhibitory Concentration (IC50) was then determined for microencapsulated Cisplatin, as well as un-encapsulated, regular Cisplatin.

DISCUSSION

We have developed a range of Cisplatin formulations, which cover diverse Cisplatin release profile kinetics ranging from release over a few hours to over several weeks. Drug loadings ranging from 18-30% were achieved, and at loadings significantly higher compared to previously published work. Further characterization methods demonstrated the robustness of our microencapsulation process because a narrow size fraction was achieved from 100-200 μm and the pH and pHR spectra suggest improved design does not affect the crystalline structure of Cisplatin. Preclinical efficacy data has been encouraging, and this is being formed from the basis for ongoing and planned cell culture and preclinical experimentation - with the specific purpose of optimizing the Cisplatin formulation for maximal efficacy. As we further pursue cell culture and preclinical experimentation, we anticipate being able to achieve an optimized Cisplatin formulation with an adequate level of potency as well as a longer (IC50) to address the drug/solubility requirements. Additionally, we have developed a novel localized drug delivery based Cisplatin formulation in conjugation with medical devices (e.g., RF Ablation devices) which offer the synergistic combination of therapeutic strategies for pre-metastatic cancer treatment.

REFERENCES