Cisplatin Microspheres Demonstrate Improved Cytotoxicity Profile against 6 Cancer Cell Lines

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ABSTRACT

Cisplatin has been classified as one of the most potent anticancer agents for many solid tumours, including liver tumours. However, the efficacy of cisplatin is limited due to serious side effects and resistance phenomena associated with its administration [1, 2]. We have developed a microsphere based sustained-release formulation of cisplatin, with the goal of preserving its efficacy, while mitigating side-effects and drug-resistance.

Our lead microsphere based formulation, called "Cis-MS-30", contains cisplatin at 27% (w/w) and was prepared with PLGA as the encapsulating polymer [PLGA i.e. poly (D,L-lactide-co-glycolic acid with a 75:25 ratio between lactide and glycolide], and with a size range of 105 µm – 150 µm in diameter. The in-vitro release profile achieved with this formulation in ideal sink conditions is represented in Figure 1. The anti-proliferative potential of Cis-MS-30 microspheres was evaluated using a panel of six human cancer cell lines for different durations of treatment ranging from 5 h – 168 h, over a wide concentration range (1nM - 100µM), and was directly compared with the effects of un-encapsulated, free cisplatin using the same cell-lines, durations of treatment and concentrations. The human tumor cell-lines used in this evaluation were those corresponding to hepatocellular carcinoma (Hep G2), urinary bladder carcinoma (5637), cervix squamous cell carcinoma (SiHa), lung carcinoma (A549), ovary teratocarcinoma (PA-1) and pancreas adenocarcinoma (AsPc-1).

As summarized in Figure 2, the Cis-MS-30 microsphere formulation for cisplatin demonstrated decreased *in vitro* cytotoxicity profile as compared to free cisplatin for the same dosage, as evident by 1.5-3.0 log higher IC_{50} values in target cancer cell lines for shorter durations of exposure. At the same time, the data suggests that by later time-points, the efficacy achieved with the Cisplatin microspheres is similar to that of free Cisplatin given that the IC_{50} values achieved with Cisplatin microspheres are at the same order of magnitude compared to those achieved with free cisplatin at later time points, reflecting the gradual and sustained release of cisplatin from PLGA microspheres. Hence there exists a proportional and cumulative correlation between the release of cisplatin and the cytotoxic activity exerted by the cisplatin microspheres. The data suggests that the Cis-MS-30 formulation of cisplatin microspheres is capable, over-time, of demonstrating anti-tumor efficacy similar to a single administration of free cisplatin at the same dose. At the same time, the cisplatin microspheres offer the potential of reduced side-effects and drug-resistance by virtue of the slow-release at a lower concentration of the cisplatin for the same dose spread over a longer period of time, rather than a high concentration from the dose that would result with free cisplatin administration. Significant advantages could therefore be offered for interventional oncology applications.

BACKGROUND and METHODS

Cisplatin is one of the most effective chemotherapeutic agents used against various forms of cancer. However, its administration is associated with serious side-effects and resistance phenomenon, both of which are a function of drug dosage and both of which represents limitations on its therapeutic applications. Previous studies have demonstrated that induction of apoptosis is more effective with Cisplatin upon intermittent administration of even sub-therapeutic doses rather than after a single high dose [3].

We sought to develop a longer-lasting, sustained-release formulation for Cisplatin with a focus on localized administration such as TACE (Trans Arterial Chemo-Embolism). One of our key long-term goals is the development of microsphere formulations which can simultaneously deliver more than one therapeutic agent (e.g. Cisplatin and Doxorubicin together) for better patient outcomes using localized interventional oncology techniques, such as TACE.

In terms of methodology, our approach was based on the measurement of half maximal inhibitory concentration (IC-50) of the sustained release Cisplatin microsphere formulation - as measured by the cytotoxic effects on human tumor cell-lines i.e. hepatocellular carcinoma (Hep G2), urinary bladder carcinoma (5637), cervix squamous cell carcinoma (SiHa), lung carcinoma (A549), ovary teratocarcinoma (PA-1) and pancreas adenocarcinoma (AsPc-1). The human cancer cell lines tested were plated in 96-well plates over a wide range of seeding cell densities (ranging from 30,000 per well for the 5 h time-point to 3,000 per well for the 168 h time point). A suspension of the Cisplatin microsphere formulation ("MS-30") was prepared in methyl cellulose and then exposed to the plated cells over a wide range of drug concentration (1 nM – 100 µM). Regular, un-encapsulated Cisplatin was used as the positive control over the same concentration range, and non-drug bland microspheres were used as negative controls. The MTT assay was used for estimation of cytotoxicity, a colorimetric assay system which measures the reduction of a tetrazolium salt (MTT) into a blue / purple colored formazan product by the mitochondria of viable cells, which is then read spectrophotometrically (at 540 nm). A comparison between treated and untreated cells provided a value for percentage cytotoxicity. The time-points which were measured are: 5 h, 24 h, 48 h, 72 h, 96 h, 120 h, 144 h and 168 h, and the IC-50 value was determined at each time-point based on the cytotoxicity profile as a function of drug-concentration.

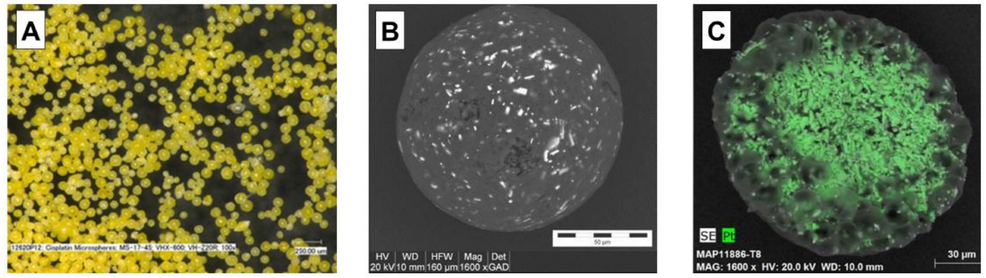


Figure 1: Images of 35% (w/w) Cisplatin loaded 75:25 PLGA microspheres. (A) Optical image (B) Scanning Electron Micrograph (SEM) image of a single microsphere (C) SEM / EDS map of a cross-sectioned microsphere demonstrating a high concentration of Cisplatin

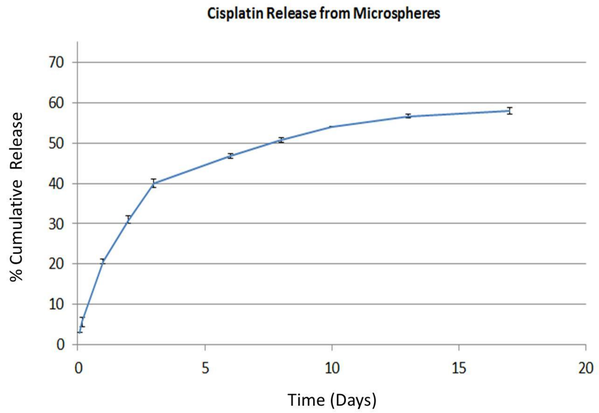


Figure 2: Cisplatin release profile from microsphere formulation, which was designed for release over 2 – 3 weeks. Error bars represent Standard Deviation.

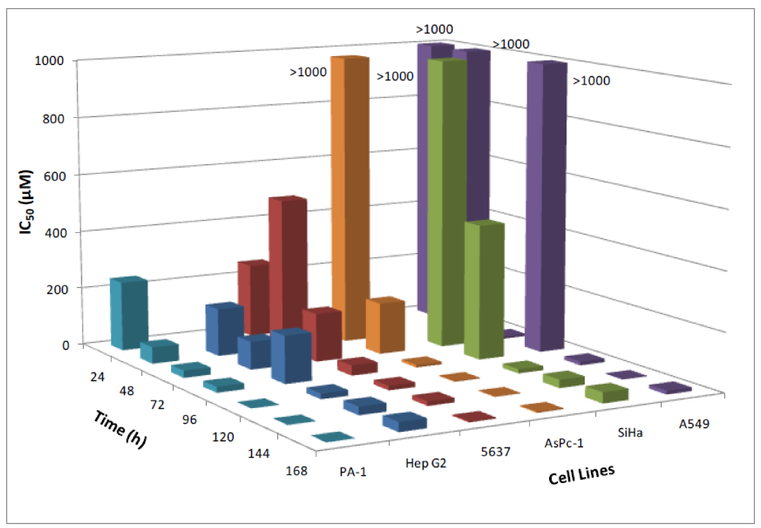


Figure 3: IC_{50} values (µM) of Bup-MS-30 in 6 cancer cell lines after 24 h – 168 h of treatment, i.e. through a one week period. For all 6 cancer cell-lines, the IC_{50} values for the sustained release formulation of Cisplatin consistently decrease over the course of the week, i.e. the cytotoxic potency increases over time to match that of regular Cisplatin

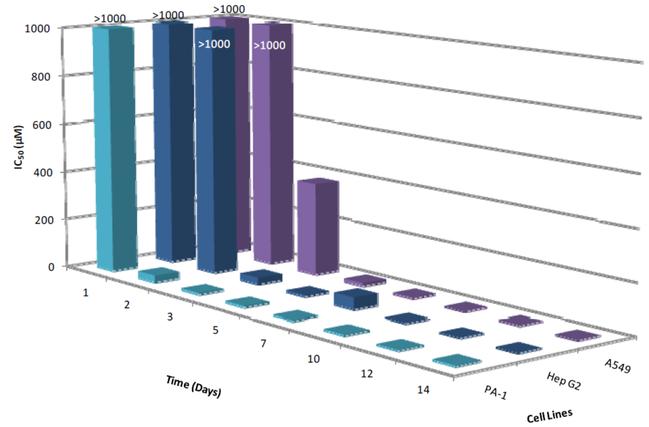
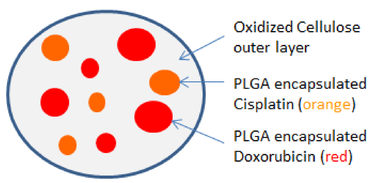
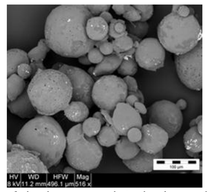


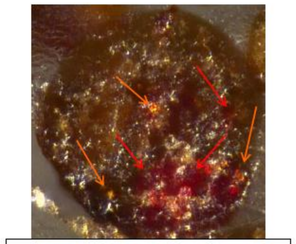
Figure 4: The trend with the IC_{50} values (µM) of Bup-MS-30 was consistent also over a 2-week period when tested against 3 of the six cell lines, i.e. PA-1 (ovarian), Hep G2 (liver) and A549 (lung). Testing in the other 3 cell lines was not pursued

Next Generation Dual-Encapsulated Microspheres with Cisplatin and Doxorubicin

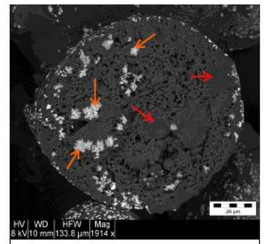




SEM of dual-encapsulated microspheres



Light microscopy of a single cross-sectioned dual-encapsulated microsphere



SEM of a single cross-sectioned dual-encapsulated microsphere

→ Inferred as Cisplatin particles (in orange)

→ Inferred as Doxorubicin particles (in red)

→ Higher concentration of Platinum (Pt) (signature of Cisplatin) in some inner spheres – confirmed by Energy Dispersive (EDS). Pt was also diffusely distributed

→ No Pt signature in other inner spheres (inferred loaded with Doxorubicin) – confirmed by Energy Dispersive (EDS)

DISCUSSION

The key motivation behind the work reported here was the pursuit of a sustained-release formulation of Cisplatin for use in interventional oncology, as the first step in developing a two-drug microsphere formulation with both Cisplatin and Doxorubicin for TACE (Trans Arterial Chemo Embolism) applications.

The *in vitro* cell-culture results obtained with 6 different human cancer cell lines as summarized in this poster are encouraging as the data demonstrates a consistent reduction in the IC-50 values over the time-course of 1-2 weeks, in a clear reflection of the corresponding *in vitro* release profile measured for Cisplatin. The achievement (over time) of similar IC-50 values as un-encapsulated Cisplatin by the Cisplatin slowly released from microspheres suggests improved cytotoxicity against healthy cells as well as a potential for reduced resistance behavior, without compromising effectiveness. A confirmation of this experimental outcome in preclinical studies is planned next.

ACKNOWLEDGMENTS

For the work presented in this article, the authors wish to acknowledge the counsel and support received from the following individuals at Covidien: Daniel Costa, Lan Pham, Gary Nichols, William Hildebrand, and Cliff Herman.

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