Sustained Release Cisplatin from a Microsphere Formulation Demonstrates Improved Safety and Efficacy in a Xenograft Bladder Cancer Rodent Model

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ABSTRACT

Cisplatin is one of the most potent anticancer agents against many kinds of cancer, including liver and bladder tumours. However, the serious side effects and resistance phenomena associated with cisplatin administration limits its overall efficacy potential [1, 2]. We have developed a microsphere based sustained-release formulation of cisplatin, with the goal of mitigating the side effects of cisplatin while enhancing its efficacy on the basis of its slower sustained-release and bioavailability.

Our cisplatin microsphere 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. Figure 2 is representative of the in-vitro release profile achieved with this formulation over about a 2 week period in ideal sink conditions. The anti-tumor potential and safety-profile of Cis-MS-30 microspheres was evaluated in a xenograft tumor model in athymic nude mice which underwent subcutaneous inoculation with the human 5637 urinary bladder cancer cell-line. Following the growth of subcutaneous solid tumors to a mean volume of ~ 150 mm^3, Cis-MS-30 microspheres and un-encapsulated Cisplatin were administered through intra-tumoral injections at a dosage of 4.05 mg Cisplatin normalized per kg of animal weight, the frequency of the intra-tumoral administration being once every 12 days. Using vernier caliper measurements of length and width, tumor volume was monitored as a function of time, with tumor volume measurements made twice a week.

Following 4 cycles of intra-tumoral administration (i.e. every 12 days), a reduction in tumor-volume by 88.7% was observed by the day-69 time-point for the Cis-MS-30 microsphere formulation (16.8 mm^3) in comparison to free cisplatin (148 mm^3), which was statistically significant ($p<0.001$). In addition, 3 animals out of 8 in the group of animal administered with Cis-MS-30 microspheres achieved full tumor regression (defined as no measurable tumor for 3 consecutive time-points). In comparison, none of the group of animals administered with un-encapsulated cisplatin achieved full tumor regression. Additionally, there was greater mortality of animals undergoing administration of un-encapsulated Cisplatin compared to Cis-MS-30 microspheres , and also a greater loss of weight (10% compared to none).

The data suggests that the Cis-MS-30 formulation of cisplatin microspheres is capable, over-time, of demonstrating better anti-tumor efficacy compared to the intra-tumoral administration of free cisplatin at the same dose. At the same time, the cisplatin microspheres offer the potential of reduced side-effects 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. For preclinical studies, 100µl of the cell suspension of the human bladder cancer cell-line (5637) containing 5 $X10^6$ cells were subcutaneously injected into the flank region of experimental animals, and eventually 24 mice were selected from the experimental animals and grouped on basis of tumor size of ~260mm^3 into three groups containing 8 animals in each group. The animals were dosed intra-tumorally with the dosing repeated once every twelve days, with the following dosage: 10.95 mg/kg (placebo microspheres), 4.05 mg/kg (Cisplatin) and 15 mg/kg (cisplatin microspheres, cisplatin MS-30). Such dosing ensured (a) equal amounts of cisplatin drug administered when comparing the cisplatin group with the cisplatin microspheres group, and (b) equal amounts of PLGA polymer administered when comparing the placebo group with the cisplatin microspheres group. Daily cage-side observation was performed to check clinical signs and mortality if any. Twice a week body weight & tumor volume was recorded throughout the experiment. Tumor volume measurements were determined using vernier caliper measurements.

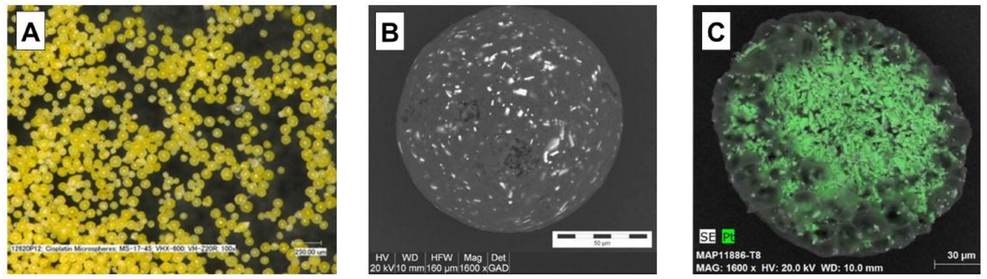


Figure 1: Images of 35% (w/w) Cisplatin loaded 75:25 PLGA microspheres. (A) Optical image (B) Scanning Electron Micrograph (SEM) image of a single microsphere (C) SEM / EDS map of a cross-sectioned microsphere demonstrating a high concentration of Cisplatin

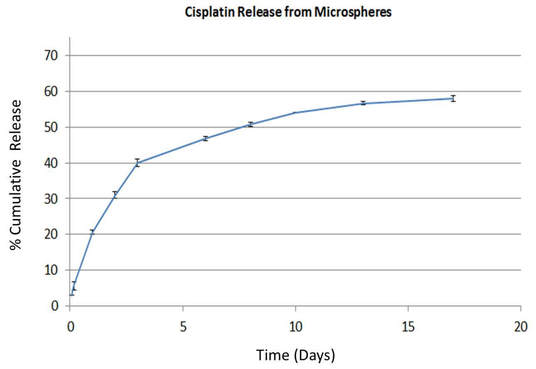


Figure 2: Cisplatin release profile from microsphere formulation, which was designed for release over 2 – 3 weeks. Error bars represent Standard Deviation.

IC_{50} (µM) of Cisplatin (unencapsulated) and Cis-MS-30 in 6 cell lines

Cell Line	Time (h)	24	48	72	96	120	144	168
PA-1	Cisplatin	3.79	1.87	0.88	0.68	0.21	0.21	0.01
	Cis-MS-30	238.7	56.97	24.73	19.99	0.5	0.16	0.02
HepG2	Cisplatin	203.5	69.72	23.7	7.96	0.95	3.38	5.75
	Cis-MS-30	NA	163.5	95.26	161.9	18.87	27.81	31.74
5637	Cisplatin	36.92	5.56	2.52	2.31	0.67	0.65	0.45
	Cis-MS-30	252.4	519.2	164.9	34.93	15.27	14.42	3.45
AsPc-1	Cisplatin	> 1000	43.22	16.57	2.93	1.59	4.22	1.47
	Cis-MS-30	NA	>1000	176.5	8.96	2.51	1.71	3.17
SiHa	Cisplatin	93.09	29.45	15.03	5.71	2.68	3.23	11.84
	Cis-MS-30	NA	NA	>1000	463.6	14.46	28.12	36.51
A549	Cisplatin	158.6	49.03	1.08	24.42	9.12	0.88	26.37
	Cis-MS-30	>1000	>1000	8.17	>1000	12.54	1.82	12.35

Table 1: IC_{50} values as a function of time

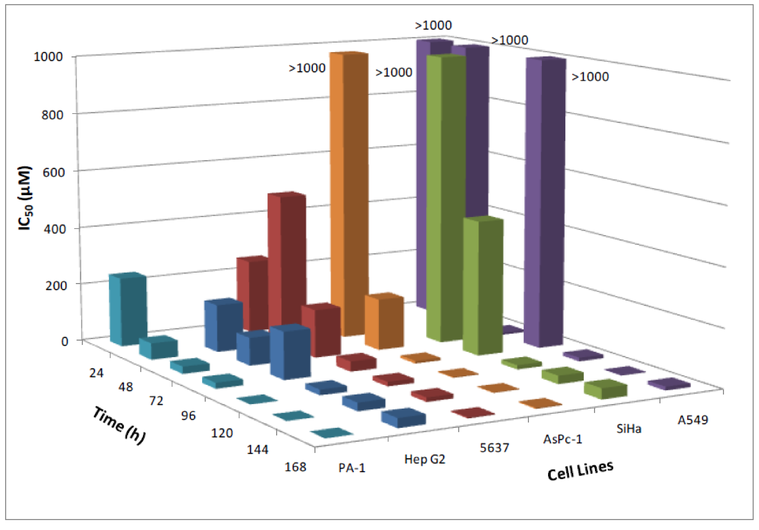
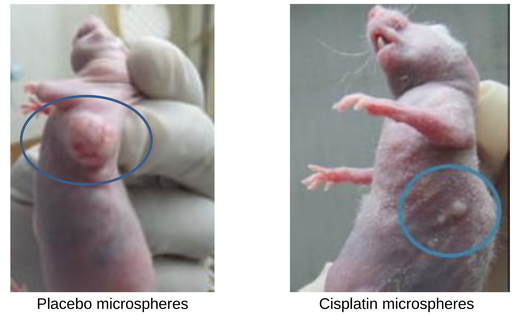


Figure 3: IC_{50} values (µM) of Cis-MS-30 in 6 cancer cell lines after 24 h – 168 h of treatment, i.e. through a one week period. For all 6 human cancer cell-lines [PA-1 (ovarian cancer), Hep G2 (liver cancer), 5637 (bladder cancer), AsPc-1 (pancreatic cancer), SiHa (cervical cancer), A549 (lung cancer)]. The IC_{50} values for the sustained release formulation of Cisplatin consistently decrease over the course of the one week (168 hours), i.e. the cytotoxic potency increases over time to match that of regular Cisplatin. Three of the cell-lines were taken out to 2 weeks (PA-1, Hep G2 and A549), where the trend with the decreasing IC_{50} values was found to be consistent over the 2 week period (the other 3 cell lines, 5637, AsPc-1 and SiHa were tested only through one week).

Pre-clinical Studies in Xenograft Model in Nude Mice



Placebo microspheres Day 34 | Cisplatin microspheres Day 62

Figure 4: Representative images of tumor sizes for the mice receiving placebo and Cisplatin microsphere administrations through intra-tumoral injections.

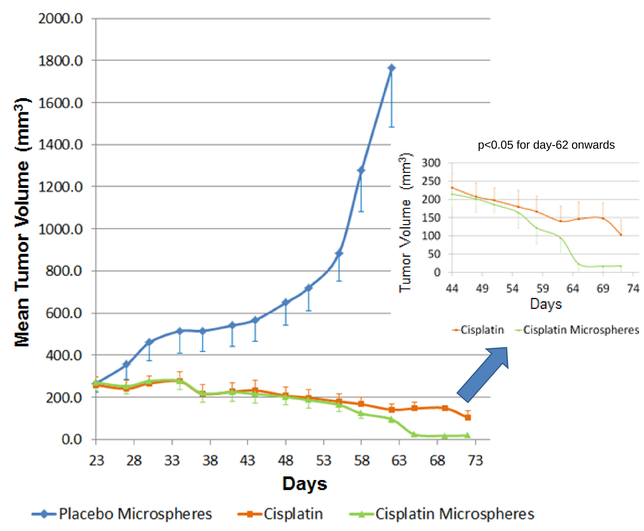


Figure 5: Mean Tumor Volumes (mm^3) as a function of time for microsphere administrations through intra-tumoral injections in a xenograft tumor model in nude mice. The administrations were carried out every 12 days starting with day-23, following inoculation with the human bladder cancer cell-line 5637. The administered Cisplatin microspheres resulted in statistically lower tumor volumes compared to administered un-encapsulated Cisplatin at day-62 and higher ($p<0.05$). The Bars indicate Standard Error of Mean.

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. In pre-clinical testing, greater effectiveness was achieved with intra-tumoral injections of Cisplatin microspheres compared to administered un-encapsulated Cisplatin at day-62 and higher time-points ($p<0.05$).

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