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. 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 known sustained-release and bioavailability.

Our cisplatin microsphere formulation, called “Cis MS-30”, contains cisplatin at 27% (w/w) and was prepared with PGLA as the encapsulating polymer (PGA i.e. poly(D,L-lactide-co-glycolide) acid with a 75:25 ratio between lactide and glycolide), and with a size range of 105 – 150 μm in diameter (Figure 2). The release profile of the microsphere formulation achieved with the formulation over about a 2 week period in in vitro conditions. The anti-tumor potential and safety profile of Cis MS-30 microspheres was evaluated in a xenograft model in human bladder cancer xeno-grafted mice. Following the growth of subcutaneously established tumors to a mean volume of ~ 150 mm3, Cis MS-30 microspheres and un-encapsulated Cisplatin were administered through intra-tumoral injections at a dosage of 4.05 mg/kg Cisplatin normalized per kg of animal weight, the frequency of the intra-tumoral administration being once every 12 days. Using volumer caliber 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 administrations (i.e. every 12 days), a reduction in tumor-volume by 88.7% was observed by the day-60 time-point for the Cis MS-30 microsphere formulation (16.8 μm3) in comparison to free cisplatin (148 μm3), which was statistically significant (p<0.0001). In addition, 3 animals out of 8 in the group of animals 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 an encapsulated Cisplatin compared to Cis MS-30 microspheres and also a greater loss of weight (10% compared to control).

The data suggests that the Cis MS-30 formulation of cisplatin microspheres is capable, over-time, of delivering better anti-tumor efficacy compared to the intra-tumoral administration of free cisplatin at the same dose. At the same time, the microsphere formulations 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 phenomena, 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 therapeutic dosages rather than after a single high-dose.

We sought to develop a longer-lasting, sustained-release formulation for Cisplatin with a focussed central localization such as TACE (Trans Arterial Chemo-Emboilment). One of the key long-term goals is the development of microsphere formulations which can simultaneously deliver more than one therapeutic agent (e.g. Cisplatin and Bevacizumab) 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 (IC50) of the sustained release Cisplatin microsphere formulation - as measured by the cytotoxic effects on human tumor cell-lines. For practical studies, 100μl of cell suspension of the human bladder cancer cell-line 5637 containing 5×10^5 cells were subcutaneously injected into the flank region of experimental animals, and eventually 24 mice were selected from the experimental tumor size (~0.8mm) 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: 10.05 mg/kg (plaque microsphere), 4.05 mg/kg (Cisplatin) and 15 mg/kg (Cisplatin microspheres, Cis MS-30). Such dosing ensured (i) equal amounts of cisplatin drug administered when comparing the plaque group with the microsphere group, (ii) dose-to-volume measurements. In the plaque group with the microsphere group, daily cage-side observation was performed to check clinical signs and mortality if any. Twice a week body weight and tumor volume were recorded throughout the experiment. Tumor volume measurements were determined using the following formula:

\[ V = \frac{4}{3} \pi r^3 \]

where r is the tumor radius. A series of images were taken using a 3D scanner. Tumor growth was measured weekly and recorded throughout the experiment.

REFERENCES


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Figure 1: Images of 35%/w/w Cisplatin loaded 75:25 PGLA 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: IC50 values (μM) of Cis MS-30 in 6 cancer cell lines after 24 h – 8 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), AsH-1 (pancreatic cancer), SiHa (cervical cancer), A549 (lung cancer)). The IC50 values for the sustained release formulation of Cisplatin consistently decrease over the course of the one (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 IC50 values was found to be consistent over the 2 week period (the other 3 cell lines, 5637, AsH-1 and SiHa were tested only through one week).

Table 1: IC50 values (μM) of Cisplatin (encapsulated and Cis MS-30 in 6 cell lines

<table>
<thead>
<tr>
<th>Cell Line</th>
<th>PA-1</th>
<th>497</th>
<th>0.08</th>
<th>0.48</th>
<th>0.15</th>
<th>0.21</th>
<th>0.03</th>
<th>0.22</th>
<th>0.01</th>
<th>0.02</th>
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<tr>
<td>Cisplatin</td>
<td>20.3</td>
<td>80.7</td>
<td>38.7</td>
<td>7.94</td>
<td>3.96</td>
<td>3.8</td>
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<td></td>
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<tr>
<td>Cis MS-30</td>
<td>10.05</td>
<td>3.22</td>
<td>2.13</td>
<td>0.87</td>
<td>0.65</td>
<td>0.64</td>
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<tr>
<td>IC50 (a)</td>
<td>24.02</td>
<td>0.92</td>
<td>1.72</td>
<td>0.19</td>
<td>0.69</td>
<td>0.62</td>
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<tr>
<td>IC50 (b)</td>
<td>10.05</td>
<td>3.22</td>
<td>2.13</td>
<td>0.87</td>
<td>0.65</td>
<td>0.64</td>
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Figure 4: Representative images of tumor sizes for the mice receiving placebo and Cisplatin microsphere administrations through intra-tumoral injections.