Local and systemic pharmacokinetic evaluation of immediate-release and controlled-release cisplatin dry powders for inhalation against lung cancer

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Summary

Immediate-release and controlled-release cisplatin dry powder formulations based on solid-lipid microparticles (SLMs) with high drug-content (≥ 50%) and exhibiting high deposition abilities in vitro (fine particle fractions (FPF) between 37% and 52%), were produced by high-pressure homogenization and spray-drying using tristearin and polyethylene glycol (1000) tocopheryl-vitamin E succinate[1]. Dry powder blends (DPB) were realized using a spray-dried Mannitol:L-Leucine (9:1) diluent and mixed with the formulations for accurate and reliable administration to mice. DPB were characterized by their drug-content, uniformity of content, recovered mass, recovered cisplatin and particle size distribution by laser diffraction with a Malvern Spraytec® through the actuation of a Penn-Century Dry Powder Insufflator™ model DP-4M for mouse. All but one of the DPB were able to be reliably delivered in vitro. The local and systemic pharmacokinetic distributions of cisplatin from formulations were then evaluated in vivo in CD-1 mice vs. IV and vs. a nebulized cisplatin aqueous solution. Quantification of platinum (Pt) content in organs was realized using validated methods by electrothermal atomic absorption spectrometry (ETAAS) in lungs, kidneys, liver, spleen, mediastinum and total blood of mice after a single 1.25 mg/kg administration and dosed over 48 hours. It showed (1) that the inhaled route could effectively lower systemic exposure while increasing lung exposure when compared to IV, (2) that immediate-release formulations were very quickly absorbed in the lungs and (3) that controlled-release formulations promoted higher total exposure in the lungs, but that the presence of PEGylated excipient was needed to avoid active and fast elimination of particles from the lungs.

Introduction

Non-small cell lung cancer (NSCLC) accounts for 1.6 million deaths per year and has a very poor prognosis at advanced stages, with one of the lowest 5-year survival rates of all cancers[2]. Treatment includes combinations of surgery, radiotherapy and chemotherapy, depending on the stage of the disease. Cisplatin is one of the most potent and the most employed anti-cancer drug against various cancers and is the principal constituent of doublet chemotherapy against NSCLC[3]. It is currently only administered by IV infusion in concomitant hydration with nephroprotective agents because of its acute and chronic dose-limiting renal toxicity[4]. Local administration of cytotoxic agents against lung cancer has been investigated in the past using various agents, mostly by aerosol therapy, but lacked the ability to reach efficacious doses within a suitable timeframe compatible with clinical practice[5]. Dry powder formulations for inhalation are able to deliver large doses of active agent to the deeper lung in a few minutes and are patient actuated, which could help limit environmental and healthcare personnel exposure. They could also help lower systemic exposure while increasing exposure in the lungs. Controlled-release (CR) formulations could also help lower acute exposure locally in order to limit lung toxicity and diminish the strain of repeated administrations. However, inhaled particles undergo many challenges as they are confronted by elimination processes such as mucociliary clearance and uptake by alveolar macrophages in the lungs, which could hinder their CR properties. Potential stealth properties of inhaled particles, provided by the addition of PEGylated excipients to formulations, are very much needed in order to promote their local residence[6]. These aspects have to be fully evaluated through preclinical studies using animal models such as rodents, along with adapted endotracheal administration devices[7]. Formulation behavior using those devices has to be fully characterized in vitro in order to ensure the reliability of in vivo results. This study focuses on the development and in vitro characterization of suitable dry powder blends (DPB) for the administration of cisplatin to mice and on the comparative pharmacokinetic (PK) results obtained in vivo with different immediate-release (IR) and CR formulation strategies for pulmonary nebulization and the IV route.

Previous work

Cisplatin formulations for human use have been previously produced and characterized[8]. Briefly, cisplatin at 5% w/v was micronized in an isopropanol suspension through high-pressure-homogenization for 40 cycles up to 20 000 psi. The resulting microcrystals, with a d(0.5) of 0.89 ± 0.01 μm, were then spray-dried with polyethylene glycol (1000) tocopheryl-vitamin E succinate (TPGS, F1) or with solubilized tristearin (TS) only (F2) or with TS and a PEGylated excipient using TPGS (F3) or distearoyl phosphoethanolamine polyethylene glycol 2000 (DSPE-mPEG-2000, F4), all at 2% w/v in solids content (Table 1). These formulations exhibited a high fine particle fractions (FPF) based on the nominal dose and a high fine particle dose (FPD). The inhalable fraction of these formulations also displayed IR for the carrier-free formulation F1, CR over more than 24 h for the 50% TS-comprising formulations (F2, F3) and a low burst-effect in vitro in modified simulated lung fluid (mSLF)[9].
Table 1. Theoretical compositions, FPFs and FPDs related to a 20 mg-filled capsule of cisplatin formulations obtained using a multistage liquid impinger operated at 100 L/min for 2.4s (n = 3) using an Axahaler© dry powder inhaler (SMB S.A., Belgium), and the in vitro drug released fractions after 10 min and 24 h from the inhalable fraction (particles below 5 µm) of formulations.

<table>
<thead>
<tr>
<th>Formulation</th>
<th>Theoretical composition (% w/w)</th>
<th>FPF (% w/w)</th>
<th>FPD (mg)</th>
<th>Released fractions (%/w/w)</th>
</tr>
</thead>
<tbody>
<tr>
<td>F1</td>
<td>Cisplatin 95% TPGS 5%</td>
<td>52 ± 3</td>
<td>9.8 ± 0.6</td>
<td>97 ± 5  100 ± 0</td>
</tr>
<tr>
<td>F2</td>
<td>Cisplatin 50% TS 50%</td>
<td>46 ± 7</td>
<td>4.5 ± 0.7</td>
<td>16 ± 4  79 ± 10</td>
</tr>
<tr>
<td>F3</td>
<td>TS 49.5% TPGS 0.5% Cisplatin 50.0%</td>
<td>37 ± 2</td>
<td>3.6 ± 0.2</td>
<td>24 ± 3  55 ± 11</td>
</tr>
<tr>
<td>F4</td>
<td>TS 49.75% DSPE-mPEG-2000 0.25%</td>
<td>50 ± 6</td>
<td>5.0 ± 0.6</td>
<td>25 ± 7  56 ± 13</td>
</tr>
</tbody>
</table>

Materials and methods

1. Production and characterization of dry blends

In order to administer dry powder formulations to mice in vivo at a 1.25 mg/kg dose, DPB of formulations had to be realized using a suitable diluent for a repeatable and accurate administration with an inhalation device, a Dry Powder Insufflator™ model DP-4M for mouse (Penn-Century Inc., USA). The diluent was obtained by spray drying an aqueous solution of 1.0% w/v Pearlitol 25C mannitol (Roquette Frères, France) and 0.1% w/v L-Leucine (Merck-Millipore, Germany) with a Mini Spray Dryer B-290 (Büchi, Switzerland). The spray-drying parameters were as follows: solution feed rate 3 g/min, inlet temperature 130°C, 0.7 mm nozzle, 1.5 mm nozzle-cap, spraying air flow 800 L/min, drying air flow 35 m³/h and the use of a B-296 dehumidifier (Büchi, Switzerland). The actual outlet temperature was 54°C and the process yield was around 71%. DPB was then prepared by mixing the formulations with diluent using a Turbula 2C three-dimensional motion mixer (Bachofern AG, Switzerland) for 4 hours in a 2-mL glass vial, aiming at a target concentration of 2.0% w/w cisplatin in DPB. F1 was therefore diluted 50-fold, while F2, F3 and F4 were diluted 25-fold. DPB were then characterized by their drug content (mean and uniformity of content) by ETAAS[1] with a 2.0 mg mass for each DPB (n = 10). They were then characterized for their ability to be administered with the DP-4M. The in vitro delivery from the DP-4M was assessed by its efficiency and repeatability of deliverance for each DPB by weighing the device before and after activating the device 5 times (mean ± SD, n = 15). The in vitro accuracy of delivery was established between the theoretical dose calculated from the delivered mass of DPB and the actual emitted dose of DPB, which was measured by activating the device 5 times into an empty 5-mL glass vial, and quantifying the deposited Pt content with ETAAS (mean ± SD, n = 10). Particle size distributions (PSD) of DPB were measured in the plume from the DP-4M (n = 5) using a Spraytec laser diffraction meter[7] (Malvern Instruments Ltd., UK) and expressed as d(0.5), d(0.9) and percentage particle volume undersize (particles below 5 µm).

2. Administration protocol

200 CD-1 (ICR) female mice aged between 5 and 15 weeks were administered cisplatin at a 1.25 mg/kg dose either in solution via the intravenous route (IV) or by nebulization or with a DPB via inhalation. Inhaled formulations were administered to mice via the endotracheal route using a MicroSprayer® Aerosolizer - Model 1A-1C-M for mouse (Penn-Century Inc., USA) or the DP-4M (i.e. for solution or for DPB, respectively), using the protocols described elsewhere[5]. Briefly, the MicroSprayer and the DP-4M are comprised of a reservoir (syringe or sample chamber, respectively) that are connected to a hollow stainless-steel tip. They can deliver nebulized solutions or powders into a plume directly into the lungs of anesthetized mice through endotracheal insufflation using the syringe or a pump, respectively. Mice were then euthanized with sodium pentobarbital (Nembutal®, Ceva Animal Health, Belgium) at 10 min, 30 min, 1 h, 2 h, 4 h, 8 h, 24 h and 48 h (n = 5). Blood was sampled by retro-orbital bleeding in a lithium-heparin tube (Sarstedt, Germany) and organs (i.e. lungs, kidneys, liver, spleen and mediastinum) were removed, washed in saline and weighed and frozen until analysis using ETAAS. All animal experiments were approved by the ethical committee of the Faculty of Medicine, ULB (CEBEA) under the agreement number 585N.

3. Quantification of the platinum content in organs and in total blood

Organs were thawed and digested in 69% v/v Suprapur® nitric acid (Merck-Millipore, Germany) under ultrasonication at 65°C for 3 hours to obtain completely clear yellow solutions. Pt content in total blood was established by digesting 50 µL of heparinized blood using 50 µL Suprapur nitric acid and 100 µL 0.5% v/v Triton X-100 (Merck-Millipore, Germany). Measurements were then realized with a SpectrAA 300 atomic absorption spectrometer using adapted[11] and validated methods consistent with the requirements of the FDA[10]. Calibration was realized by the autosampler from freshly prepared matrix-matched 800 ng/mL cisplatin standards and blank matrix for each organ. Results were all adjusted to the same drug dose based on the emitted mass and drug content of DPB. AUC was calculated using the trapezoidal rule between measured data points.
Results and discussion

The IR formulation F1 and CR formulations F2, F3 and F4 were diluted to obtain the DPBs (Table 2), showing a smaller PSD for DPB1 and a uniformity of drug content comprised between 7.3 and 8.9% for all formulations except for DPB4, for which a higher variability of 13.4% w/w was observed. In vitro results showed that delivery from the DP-4M endotracheal device was more efficient and more repeatable with DPB1. This is probably attributable to the greater quantity of excipients present in this blend (50-fold dilution vs. 25-fold dilution for all the others formulations). Moreover, DPB1 was the only formulation without lipids, which could have impaired delivery. This is because it could have increased interparticle interactions with the diluent during mixing and within the device, causing more particle aggregates, especially as the initial formulation exhibit larger PSD\(^{[5]}\). Finally, the accuracy of delivery showed that emitted cisplatin levels were in accordance with the expected values calculated from the weighed device before and after actuations for DPB1, DPB2 and DPB3. These values were later on used in vivo. In contrast, a great variability in accuracy was observed for DPB4, probably due to its lesser uniform content and by particle demixing during actuation of the device. DPB4 was therefore discarded for the in vivo part of the study. In vivo, the DPB were administered to mice with lower delivery efficiency and a greater variability than in vitro. This could be caused by a blockage of the device tip or by powder sticking caused by the damper environment in mouse trachea.

Table 2. Measured parameters for the DPBs (PSD, drug-content, delivery with the DP-4M device).

<table>
<thead>
<tr>
<th>Dry powder blend</th>
<th>Laser diffraction</th>
<th>Drug content</th>
<th>In vitro delivery</th>
<th>In vivo delivery to the lung</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>d(0.5) (µm)</td>
<td>Undersize (%) below</td>
<td>Cisplatin content (% w/w, n = 10)</td>
<td>Efficiency and repeatability (% w/w, mean ± SD, n = 15)</td>
</tr>
<tr>
<td>D1</td>
<td>2.4 ± 0.8</td>
<td>86.5 ± 6.7</td>
<td>2.37 ± 0.21</td>
<td>8.9</td>
</tr>
<tr>
<td>D2</td>
<td>3.7 ± 0.8</td>
<td>69.1 ± 14.0</td>
<td>1.67 ± 0.17</td>
<td>9.9</td>
</tr>
<tr>
<td>D3</td>
<td>3.8 ± 0.5</td>
<td>69.2 ± 10.3</td>
<td>1.64 ± 0.12</td>
<td>7.3</td>
</tr>
<tr>
<td>D4</td>
<td>3.4 ± 0.5</td>
<td>73.8 ± 9.0</td>
<td>1.60 ± 0.21</td>
<td>13.4</td>
</tr>
</tbody>
</table>

PK results (Figure 1) showed that lung exposure was relatively higher with all inhaled experiments compared to IV. It also showed that nebulization and IR DPB1 had similar PK profiles, and that Pt levels fell very rapidly, already before the first dosing time (10 min). PK profiles of CR formulations in vitro (DPB2 and DPB3) showed that DPB2, despite being preserved in larger amounts at the first dosing times, was rapidly eliminated within the first hour. This suggests an active elimination process, probably by alveolar macrophages uptake. However, the PEGylated excipient-comprising formulation F3, showed high and sustained levels of platinum for up to 24 hours, confirming the well-described abilities of PEG chains to delay macrophage recognition and capture of particles\(^{[6]}\). Blood platinum levels, were higher with IV than with the pulmonary route and showed absorption profiles compatible with the slow distribution from the lungs to the systemic compartment for all inhaled experiments.

![Figure 1](image1.png)

**Figure 1.** Local and systemic pharmacokinetic profiles showing Pt content in lungs and in total blood after a single 1.25 mg/kg administration (Mean ± SEM, n = 5 for each dosing time).

Pt C\(_{\text{max}}\) which is more representative of the acute toxicity linked to cisplatin exposure, was also reduced in total blood (Figure 2a) for all the inhaled experiments as compared to IV. This was especially the case for CR formulation DPB3 (161 ± 57 ng/mL vs. 791 ± 181 ng/mL for IV). The reduced exposure was also particularly observable in kidneys (Figure 2b) for IR formulation DPB1 and nebulization (0.6 ± 0.2 ng/mg and 1.1 ± 0.5 ng/mg vs. 3.0 ± 0.7 ng/mg for IV). Observed C\(_{\text{max}}\) at 10 min was, however, significantly increased in lungs for DPB2 and DPB3 (Figure 2c), which does not reflect CR properties of these formulations as ETAAS does not differentiate between available Pt, dissolved Pt and undissolved Pt from particles in the lung parenchyma (Kruskall-Wallis test with Dunn’s multiple comparison test, p < 0.01).
It also showed that IR formulations were quickly absorbed (less than 10 min). Analysis of the area under the curve (AUC) for all PK profiles (Figure 2d) showed that the global exposure of lungs to Pt was increased for inhaled formulations compared to IV injection (very significantly with DPB3 with a more than 10-fold increase, \( p < 0.001 \)). Interestingly, AUC in kidneys was lowered for all inhaled formulations, as compared with IV (for instance, AUC in kidneys of 475 ± 187 ng.min/mL vs. 2168 ± 149 ng.min/mL for DPB2 and IV, respectively). An exception was observed for DPB3, for which AUC in kidneys was closer to IV with 1625 ± 806 ng.min/mL (mean ± SEM, \( n = 5 \) per group per dosing time, 8 times). Further experiments are needed to explain this phenomenon. Exposure was also lowered in liver for all inhaled formulations, as compared with IV.

![Figure 2](image)

**Figure 2.** \( C_{\text{max}} \) in total blood (a), kidneys (b) and lungs (c) (mean ± SEM, \( n = 5 \) per group) and total exposure of organs (d) expressed as the AUC in organs for all considered routes and formulations (mean ± SEM, \( n = 5 \) per group per dosing time, 8 times). Significance of the difference with the IV control group or the mentioned group was calculated using a Kruskall-Walls test with Dunn’s multiple comparison test (c) or a two-way ANOVA with Bonferroni’s multiple comparison test (d) and is expressed as follow: ** = \( p < 0.01 \), *** = \( p < 0.001 \).

### Conclusion

The PK of cisplatin is of tremendous interest in order to assess the outcome of cisplatin or its reactive species after lung administration. We showed that a formulation comprised of TS and PEGylated excipients greatly improves Pt exposure in the lungs for more than 8 hours compared with (i) an IV-delivered cisplatin solution, (ii) a nebulized cisplatin solution or (iii) IR formulations. The use of PEGylated excipients seems crucial in order to prolong residency of inhaled SLMs in the respiratory tract. The increased Pt exposure observed with the CR formulation in the kidneys will have to be further assessed using more specific renal parameters for renal toxicity such as blood urea nitrogen or creatinine levels. Lung toxicity will also have to be assessed using broncho-alveolar lavage fluid analysis

### References