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Platinum pharmacokinetics in mice following inhalation of cisplatin dry powders with different release and lung retention properties

Vincent Levet*, Romain Merlos, Rémi Rosière, Karim Amighi, Nathalie Wauthoz

Laboratory of Pharmaceutics and Biopharmaceutics, Faculty of Pharmacy, Université libre de Bruxelles (ULB), Bd. du Triomphe CP 207, Campus de la Plaine, 1050 Brussels, Belgium

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Alpha-tocopheryl polyethylene glycol 1000 succinate (TPGS, PubChem CID:71406)
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Distearoyl phosphoethanolamine polyethylene glycol 2000, sodium salt (DSPE-mPEG-2000, PubChem CID:86278270)
L-leucine (PubChem CID: 6106)
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ABSTRACT

Pharmacokinetics of cisplatin administered by the pulmonary route were established in mice using dry powders inhaler (DPI) formulations showing immediate (F1) and controlled release (CR, solid lipid microparticles) *in vitro*, without (F2) or with PEGylated excipients (F3, F4). Formulation administration was realized using dry powder blends (correspondingly named thereafter $F1_B$ to $F4_B$) able to reproducibly deliver particles *in vivo* using a DP-4M Dry Powder Insufflator™. Their platinum pharmacokinetics were established over 48 h in lungs, total blood and non-target organs *vs.* IV and endotracheal nebulization (EN). EN and $F1_B$ were rapidly distributed from the lungs ($t_{1/2}{}^{i}$ 2.6 and 5.0 min). $F2_B$ was eliminated in ~1 h ($t_{1/2}{}^{i}$ 9.0 min). $F3_B$ lung retention was sustained for ~7 h ($t_{1/2}{}^{i}$ 59.9 min), increasing lung AUC 11-, 4- and 3-fold *vs.* IV, $F1_B$ and $F2_B$. Total blood t_{max} were higher and AUC and C_{max} lower using the pulmonary route *vs.* IV. Kidney C_{max} was reduced 6-, 2- and 3-fold for $F1_B$, $F2_B$ and $F3_B$. AUC in kidneys were 2- to 3-fold lower for $F1_B$ and $F2_B$ *vs.* IV but comparable for IV *vs.* $F3_B$, probably because of kidney saturation. PEGylated solid lipid microparticles provided cisplatin particles with interesting lung retention and CR properties.

1. Introduction

Cisplatin is one of the most potent anticancer drugs. It is used against various cancers (*e.g.*, lung, bladder, brain, cervical, testicular, ovarian, esophageal, gastric, head and neck, osteosarcoma). Its activity lies in its ability to bind with sulfurs, DNA and proteins, causing cell apoptosis (Jamieson and Lippard, 1999). It is administered against small cell and non-small cell lung cancer (SCLC and NSCLC) as part of the "doublet chemotherapy" (*e.g.*, one platinum derivative agent associated with a non-platinum derivative such as taxanes, gemcitabine or pemetrexed for NSCLC). It is prescribed for delivery every 1 to 3 weeks by intravenous (IV) infusion at doses ranging from 30 to 100 mg/m^2 of body surface area (NCCN, 2014, 2015). Because of its particularly cumulative and irreversible renal toxicity, which is dose-limiting, nephroprotective action such as large water intakes with mannitol and

Abbreviations: AUC, area under the curve; bw, body weight; CI, Carr's index; C_{max}, peak concentration; CR, controlled release; d_{ae}, aerodynamic diameter; DD, dry diluent; DPB, dry powder blend; DPI, dry powder inhaler; DSPE-mPEG-2000, distearoyl phosphoethanolamine polyethylene glycol 2000, sodium salt; DSC, differential scanning calorimetry; EE_D, emission efficiency in dose; EE_M, emission efficiency in mass; EN, endotracheal nebulization; ETAAS, electrothermal atomic absorption spectrometry; FDA, Food and Drug Administration; IR, immediate release; IV, intravenous; $k_{el}{}^{i}$, initial elimination rate constant; $k_{el}{}^{t}$, terminal elimination rate constant; LLOQ, lower limit of quantification; LOD, limit of detection; MCC, mucociliary clearance; MPS, macrophage phagocytic system; NSCLC, non-small cell lung cancer; PK, pharmacokinetic; PSD, particle size distribution; SCLC, small cell lung cancer; SD, spray dried; SLM, solid lipid microparticles; SEM, scanning electron microscopy; $t_{1/2}{}^{i}$, initial half-life; $t_{1/2}{}^{t}$, terminal half-life; T_a, targeting advantage; T_e, targeting efficiency; TPGS, alpha-tocopheryl polyethylene glycol 1000 succinate; t_{max}, time at peak concentration (C_{max}); TS, tristearin; UD_D, uniformity of the delivered dose; UD_M, uniformity of the delivered mass; USP, U.S. Pharmacopeial Convention; XRPD, X-ray powder diffraction.

* Corresponding author.
E-mail addresses: vincent.levet@ulb.ac.be (V. Levet), nawautho@ulb.ac.be (N. Wauthoz).

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electrolytes is crucial before and after administration (NHS, 2012). Consequently, administration of cisplatin often requires up to 6 to 8 h and day care hospitalization, mobilizing healthcare personnel and increasing health costs and patient discomfort.

The pulmonary route has proven its usefulness in the treatment of various lung diseases (*e.g.*, asthma, chronic obstructive pulmonary disease, pulmonary infections). Inhaled chemotherapy against lung cancer as a local treatment could be proposed as a novel modality for treating patients (Gagnadoux et al., 2008; Zhou et al., 2015). It could be administered as a (neo)adjuvant treatment to surgery or radiotherapy at all stages in SCLC and NSCLC. This would increase drug concentration at the site of action while limiting systemic exposure and the adverse effects frequently observed through parenteral administration, resulting in a higher therapeutic index (Gagnadoux et al., 2008; Zarogoulidis et al., 2012). It could also increase drug concentration locally, in the tumor and its surroundings, particularly in loco-regional lymph nodes. This would help to prevent micrometastasis, the principal cause of cancer resurgence (Kaifi et al., 2010; Kelsey et al., 2009).

Regarding pulmonary drug delivery, dry powder inhalers (DPIs) provide many advantages over nebulizers. These include large inhalation doses of poorly water-soluble drugs in a short administration time, long-term storage stability of formulations and patient-activated inhaler devices that can easily be disposed of, helping to limit environmental and healthcare personnel contamination (de Boer and Hagedoorn, 2015; Friebel and Steckel, 2010). Once inhaled and deposited in the lungs, drug particles are progressively solubilized in lung fluids at a speed rate depending on many parameters. The dissolved part will be available for pharmacological action locally but will also be distributed into the systemic compartment through the blood in the case of permeable drugs. The undissolved part will be subjected to non-absorptive mechanisms and will be progressively eliminated by mucociliary clearance (MCC) and the macrophage phagocytic system (MPS) (Gill et al., 2011). Moreover, too high doses of chemotherapy could lead to high peak concentrations of solubilized drug in the lungs and cause acute pulmonary toxicity, especially with immediate-release (IR) formulations. These issues could be avoided by careful dosage and progressive increase in drug concentration locally through controlled release (CR) delivery systems designed to promote lung retention and avoid clearance mechanisms, especially the MPS (Loira-Pastoriza et al., 2014). The CR of DPI formulations is a challenge in itself: in order to be inhaled, the aerodynamic diameter (d_{ae}) must lie in the low micrometer-range (1–5 μm). This generally necessitates reducing the geometric particle size and can in turn increase the burst effect and dissolution rates. More importantly, avoidance of recognition of particles and ensuing phagocytosis by the MPS through surface modification is an even greater challenge. Most DPI formulations developed up to now have been unsuccessful in controlling the pharmacokinetic (PK) profiles of drugs after lung deposition because of low lung retention (Gill et al., 2011; Loira-Pastoriza et al., 2014). Many strategies have also been developed to try to control drug release from inhaled particles, using delivery systems from the nanometer to the micrometer range. Strategies have used liposomes, solid nanoparticles, micelles and polymeric or lipidic biodegradable microspheres (Loira-Pastoriza et al., 2014). Moreover, PEGylation of the particle surface is a well-known and effective approach to limiting macrophage uptake (Patel et al., 2015) and most PEGylated excipients or particles are recognized as safe for inhalation (Klonne et al., 1989).

DPI formulations were developed in a previous study (Levet et al., 2016) in the form of solid lipid microparticles (SLM) embedding cisplatin microcrystals into a biocompatible matrix. The matrix was composed of a highly hydrophobic triglyceride, tristearin (TS), alone or with PEGylated excipients such as alpha-tocopheryl polyethylene glycol 1000 succinate (TPGS) or distearoyl phosphoethanolamine polyethylene glycol 2000 (DSPE-mPEG-2000) to modify the particle surface properties. Interesting results were obtained *in vitro* (Table 1). The particles exhibited dissolution properties, established on the inhalable fraction of particles only and in simulated lung fluid (Son and McConville, 2009), with a low burst-effect and CR over more than 24 h. They also showed a high drug content ($\geq$ 50% w/w) and high fine particle fraction (FPF, expressed as the fraction of particles from the loaded dose with a $d_{ae} < 5$ μm) of up to 52%, which helped to deposit high doses in the lungs (Levet et al., 2016).

Confirmation of the CR abilities *in vivo* of the DPI formulations produced is essential. These abilities may vary greatly because particles deposited in the lungs are subjected to many other mechanisms than *in vitro*. For instance, the remaining undissolved particles will also be subjected to MCC and MPS, increasing their elimination rate from the lung.

Preclinical testing of respiratory drugs is often realized on rodents to assess PK, toxicity and efficacy. However, many precautions are required for their administration. First, when using high drug content DPI formulations initially developed for human use, a proper dilution must be realized to meet acceptable doses in rodents. Second, dose-dependent preclinical studies require administration methods and devices that are reliable to deposit said diluted formulations at accurate and reproducible doses in rodent lungs. The DP-4M Dry Powder Insufflator™ (Penn-Century, Inc., Wyndmoor PA, USA) used in this study is an endotracheal device. This necessitates prior anesthesia of the mouse but has many advantages over the other possible systems (*e.g.*, intratracheal devices, nose-only and full-body aerosols). The

Table 1
Theoretical and actual composition, aerodynamic properties – median-mass aerodynamic diameter (MMAD, mean ± SD, n = 3) and fine particle fraction (FPF, mean ± SD, n = 3) – and released fractions of the developed DPI formulations after 10 min, 6 h and 24 h (mean ± SD, n = 3).

	Theoretical composition (% w/w)		*Actual cisplatin content (% w/w)	*FPF (% w/w)	*MMAD (μm)	*Released fractions (%w/w)		
						10 min	6 h	24 h
F1	Cisplatin	95	95.6 ± 2.6	52 ± 3	2.0 ± 0.2	97 ± 5	100 ± 0	100 ± 0
	TPGS	5						
F2	Cisplatin	50	48.5 ± 2.2	46 ± 7	2.2 ± 0.3	16 ± 4	63 ± 3	79 ± 10
	TS	50						
F3	Cisplatin	50.0	48.9 ± 2.3	37 ± 2	2.4 ± 0.2	24 ± 3	44 ± 9	55 ± 11
	TS	49.5						
	TPGS	0.5						
F4	Cisplatin	50.00	49.9 ± 2.7	50 ± 6	2.3 ± 0.7	25 ± 7	37 ± 9	56 ± 13
	TS	49.75						
	DSPE-mPEG-2000	0.25						

*From Levet et al. (2016).