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Development and evaluation of well-tolerated and tumor-penetrating polymeric micelle-based dry powders for inhaled anti-cancer chemotherapy

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ABSTRACT

Despite the direct access to the lung offered by the inhalation route, drug penetration into lung tumors could remain an important issue. In this study, folate-polyethylene glycol-hydrophobically-modified dextran (F-PEG-HMD) micelles were developed as an effective pulmonary drug delivery system to reach and penetrate lung tumors and cancer cells. The F-PEG-HMD micelles were able to enter HeLa and M109-HiFR, two folate receptor-expressing cancer cell lines, *in vitro*, and *in vivo* after administration by inhalation to orthotopic M109-HiFR lung tumor grafted mice. Paclitaxel-loaded F-PEG-HMD micelles characterized in PBS by a Z-average diameter of ~50 nm and a zeta potential of ~−4 mV were prepared with an encapsulation efficiency of ~100%. The loaded micelles reduced HeLa and M109-HiFR cell growth, with half maximal inhibitory concentrations of 37 and 150 nM, respectively. Dry powders embedding the paclitaxel-loaded F-PEG-HMD micelles were developed by spray-drying. *In vitro*, good deposition profiles were obtained, with a fine particle fraction of up to 50% and good ability to re-disperse the micelles in physiological buffer. A polymeric micelle-based dry powder without paclitaxel was well-tolerated *in vivo*, as assessed in healthy mice by determination of total protein content, cell count, and cytokine IL-1β, IL-6, and TNF-α concentrations in bronchoalveolar lavage fluids.

Abbreviations: DiO, 3,3′-dioctadecyloxacarbocyanine perchlorate; MTT, 3-[4,5-dimethylthiazol-2-yl] diphenyltetrazolium bromide; AM, alveolar macrophages; BALF, bronchoalveolar lavage fluid; CB, carbon black; DPI, dry powder for inhalation; DMSO, dimethyl sulfoxide; DLS, dynamic light scattering; EE, encapsulation efficiency; ESEM, environmental scanning electron microscopy; FBS, fetal bovine serum; FPF, fine particle fraction; F-PEG-HMD, folate-polyethylene glycol-hydrophobically-modified dextran; FR, folate receptor; IC50, half maximum inhibitory concentration; LDH, lactate dehydrogenase; LPS, lipopolysaccharide; MMAD, mass median aerodynamic diameter; Mw, molecular weight; MWCO, molecular weight cut-off; PTX, paclitaxel; PSD, particle size distribution; PBS, phosphate buffer saline; PB5, phosphate buffer at pH5; PdI, polydispersity index; PMN, polymorphonuclear neutrophil; RT-qPCR, reverse transcription quantitative polymerase chain reaction; RPMI, Roswell Park Memorial Institute medium; SLF, simulated lung fluid; TEM, transmission electron microscopy; Z-average, Z-average mean diameter value.

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1. Introduction

Pulmonary delivery for lung cancer therapy has been partially explored over recent decades as an interesting adjuvant treatment to some conventional systemic chemotherapy (Zarogoulidis et al., 2012). Inhaled chemotherapy allows the administration of high doses of cytotoxic agents directly to the lung where the tumor is growing and spreading. It also reduces systemic distribution and toxicity to enhance the therapeutic ratio significantly (Gagnadoux et al., 2008). In the pulmonary drug delivery field, dry powder for inhalation (DPI) offers many benefits compared with nebulization. These benefits include the administration of high doses of poorly soluble drugs, inhaler activation by the patient's inspiratory flow, dry formulation stability, availability of disposable devices, limitation of environmental contamination, etc. DPI could therefore represent a technique well-adapted for an anti-cancer treatment (Wauthoz et al., 2011).

However, despite the direct access to the lung tumor(s), drug penetration into the lung tumor remains as an important issue as it is for systemic routes (Bertrand et al., 2014; Khawar et al., 2014). Moreover, most conventional cytotoxic anti-neoplastic agents need to reach their intracellular target(s) to exert their anti-cancer activity (Jordan and Wilson, 2004; Ndolo et al., 2010). Once deposited into the respiratory tract, reaching the intracellular site of action can still represent a real challenge (Taratula et al., 2013; Zarogoulidis et al., 2012). Among other objectives, many nanoscale drug delivery systems have been widely developed in the past decade to increase drug penetration into the tumor tissues and cells using the systemic (Bertrand et al., 2014) or inhalation routes (Garbuzenko et al., 2014; Zarogoulidis et al., 2012). In this context, we developed DPI formulations composed of a nanoscale drug delivery system, i.e. a nanocarrier, to improve drug penetration into lung tumor tissues and cells. The drug delivery system consists of folate-polyethylene glycol-hydrophobically-modified dextran (F-PEG-HMD) micelles that are potentially able to bind to folate receptors (FRs). These receptors are overexpressed in many lung cancers (Leamon and Reddy, 2004; Sudimack and Lee, 2000), especially adenocarcinomas (Bremer et al., 2013; Cagle et al., 2013; Christoph et al., 2014; O'shannessy et al., 2012), compared with healthy tissues. F-PEG-HMD is a self-assembled copolymer that spontaneously aggregates as micelles in aqueous media (Rosière et al., 2015).

Compared with the oral or parenteral routes, the availability of excipients approved for the pulmonary route is very limited (Pilcer and Amighi, 2010). Safety issues are a major concern in developing new drug delivery systems for the inhalation route (Kumar et al., 2014). Excipients entering the composition of an inhaled formulation have to be well-tolerated by the respiratory tract to be administered locally by inhalation. Reported local toxicities consecutive to pulmonary administration of polymeric delivery systems are mainly inflammation and cytotoxicity, depending on the polymer constituent and the nanoparticle properties (Barar et al., 2015). Because of a lack of information concerning the local toxicity induced by excipients for pulmonary use, stringent determination of the local tolerance profile for each new candidate excipient (i.e. for pulmonary application) is highly recommended (Pilcer and Amighi, 2010).

In this study, F-PEG-HMD micelles entering into the composition of DPI formulations were designed as an effective drug delivery system to reach and penetrate lung tumors and cancer cells. The poorly water-soluble drug paclitaxel (PTX) was chosen as the model drug. PTX is an effective anti-cancer drug currently used in lung cancer therapy. However, it causes severe systemic toxicities such as myelosuppression and neurotoxicity (dose-limiting toxicities) when delivered by the conventional intravenous route (Ramalingam and Belani, 2005; Socinski, 2014). In addition, the commercial PTX formulation (i.e. Taxol®) is composed of Cremophor EL, which is responsible for serious toxicities (such as hypersensitivity reactions and neurological toxicities) and modification of the PTX pharmacokinetic profile (Gelderblom et al., 2001). Developing new PTX-based aerosol therapy therefore involves the development of better-adapted PTX vehicles (Gautam and Koshkina, 2003; Hureaux et al., 2009). PTX has already been the subject of several preclinical developments using new vehicles in both aerosol chemotherapy (Gautam and Koshkina, 2003; Gill et al., 2011; Hureaux et al., 2009; Meenach et al., 2014) and targeted delivery (Feng and Mumper, 2013; Poon et al., 2011; Videira et al., 2012). Overall, the aims of this study were (i) to prepare PTX-loaded F-PEG-HMD micelles, (ii) to characterize them in terms of formulation and anti-cancer activity, (iii) to evaluate the effective incorporation of the F-PEG-HMD micelles into FR-expressing cancer cells and tissues, *in vitro* and *in vivo*, respectively, (iv) to prepare dry powders embedding the PTX-loaded F-PEG-HMD micelles and (v) to characterize them in terms of DPI formulation, and (vi) to evaluate local tolerance of a F-PEG-HMD-based DPI formulation without PTX administered to the lungs in healthy mice.

2. Materials and methods

2.1. Materials

3,3′-Dioctadecyloxacarbocyanine perchlorate (DiO), 3-[4,5-dimethylthiazol-2-yl] diphenyltetrazolium bromide (MTT), dimethyl sulfoxide (DMSO), dichloromethane, and ethanol were purchased from Sigma-Aldrich (Diegem, Belgium). Folate-free Roswell Park Memorial Institute medium (RPMI), fetal bovine serum (FBS), penicillin-streptomycin solution, gentamycin solution, phosphate buffer saline (PBS), and trypsin-EDTA solution were purchased from Life Technologies (Merelbeke, Belgium). L-Leucine was purchased from Merck Chemicals (Overijse, Belgium). Mannitol (Pearlitol® 200 SD) was purchased from Roquette (Brussels, Belgium). Matrigel® basement membrane matrix was purchased from Corning (Lasne, Belgium). PTX was purchased from Carbosynth Limited (Berkshire, United Kingdom). All solvents were purchased in analytical grade from Merck (Darmstadt, Germany). Ultrapure water was obtained from a Purelab-Ultra device (Elga, Lane End, UK).

2.2. Synthesis and characterization of folate-polyethylene glycol-hydrophobically-modified dextran (F-PEG-HMD)

F-PEG-HMD was synthesized using carbodiimide-mediated coupling chemistry in three main steps: (i) synthesis of HMD-succinate from dextran and stearic acid; (ii) synthesis of F-PEG-NH_2 from PEG and folic acid, and (iii) grafting of F-PEG NH_2 onto HMD-succinate. All the reaction steps and characterization of F-PEG-HMD have been described in our previous work (Rosière et al., 2015).

2.3. Preparation and physicochemical characterization of F-PEG-HMD micelles

2.3.1. Preparation

Empty F-PEG-HMD micelles were prepared by dissolving F-PEG-HMD in PBS at a concentration of 1 mg/mL. The resulting polymeric micelle solution was centrifuged (2 000*g* for 5 min) using a Heraeus multifuge X1R centrifuge (Thermo Fisher Scientific, Waltham, mA, USA) before characterization.

PTX-loaded F-PEG-HMD micelles and fluorescent F-PEG-HMD micelles were prepared using an adapted dialysis method (Lukyanov and Torchilin, 2004). Briefly, 100 mg F-PEG-HMD and