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Minimal amounts of dipalmitoylphosphatidylcholine improve aerosol performance of spray-dried temocillin powders for inhalation

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ABSTRACT

Administration of antibiotics by inhalation can greatly improve drug targeting to the site of respiratory infections. In addition, dry powder inhalers are particularly convenient for the patients. The purposes of this study were to demonstrate the interest of pulmonary temocillin delivery to reach high temocillin concentrations locally in the lungs as well as to prepare a spray-dried temocillin powder for inhalation using a minimal amount of generally recognized as safe excipients. Intratracheal instillation of a temocillin solution allowed to reach higher and more sustained drug concentrations in the lungs than intravenous injection in mice, although a 10-fold lower temocillin dose was delivered intratracheally than systemically. A spray-dried powder of pure temocillin presented a fine particle fraction of 9% of the dose loaded in the inhaler. However, the incorporation of 0.5% to 20% of dipalmitoylphosphatidylcholine (DPPC) in the powder increased the fine particle fraction 4- to 5-fold. X-ray photoelectron spectroscopy and X-ray diffraction revealed that DPPC concentrated at the particle surface with its aliphatic chains laterally packed. The minimal amount of DPPC needed to improve the aerosol performance of temocillin supports the use of this excipient in the formulation of cohesive antibiotic powders for inhalation.

1. Introduction

Temocillin is a semi-synthetic 6-alpha-methoxy derivative of ticarcillin. It is non-toxic and well-tolerated. Unlike several other β-lactams, temocillin is highly stable to most bacterial beta-lactamases. Therefore, it is considered a useful alternative to carbapenems in infections caused by several resistant Gram-negative pathogens. Indications include septicaemia, urinary tract infections and lower respiratory tract infections where susceptible Gram-negative bacteria are suspected or confirmed. In 2004, temocillin was granted orphan drug status by the European Commission for the treatment of the Gram-negative *Burkholderia cepacia* complex lung infection in cystic fibrosis. However, temocillin lacks activity against the Gram-negative organism *Pseudomonas aeruginosa*, although it might be useful for *P. aeruginosa* strains with impaired efflux (Buyck et al., 2012; Livermore and Tulkens, 2009; Van Acker et al., 2010).

Temocillin is currently administered by intramuscular or intravenous injection or by continuous intravenous infusion. However, only a small proportion of antibiotic can access the airways and lung parenchyma after parenteral administration and respiratory infections are therefore difficult to treat. Moreover, bacterial biofilms develop in the bronchial tree of patients and the minimal inhibitory concentration of antibiotics to biofilm-growing bacteria may be 100–1000 fold higher than to planktonic bacteria (Ciofu et al., 2015; Van Acker et al., 2010). Consequently, high doses of drugs are required to maintain drug levels above the minimal inhibitory concentration at infection sites. Delivery of antibiotics directly to the respiratory tract offers an attractive solution for the treatment of respiratory infections because it allows higher drug concentrations to be achieved at the target site with lower systemic exposure than parenteral administration (Weers, 2015). At this stage, dry powders of tobramycin and colistin and solutions of aztreonam are the most widely used formulations for inhalation

Abbreviations: BAL, bronchoalveolar lavage; d, primary geometric particle diameter; d_{aer}, primary aerodynamic diameter of the particles; DPI, dry powder inhaler; DPPC, dipalmitoylphosphatidylcholine; ED, emitted dose; FPF, fine particle fraction; GRAS, generally recognized as safe; HBSS, Hank's balanced salt solution; HPLC, high performance liquid chromatography; MMAD, mass median aerodynamic diameter; MSLI, Multi-Stage Liquid Impinger; PBS, phosphate buffer saline; ρ, powder density; XPS, X-ray photoelectron spectroscopy; XRD, X-ray diffraction.

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(Quon et al., 2014); dry powders of fluoroquinolones are under development (Stass et al., 2015).

Dry powder inhalers (DPIs) are particularly convenient for the patients. In fact, the use of DPIs greatly reduces daily treatment burden compared with nebulization and low daily treatment burden is particularly critical to attain good patient compliance (Weers, 2015). Because DPIs are portable and do not require a power source to function, they do not restrict administration to the home setting. In addition, drugs are more stable in the dry state than in the liquid state and dry powders for inhalation may avoid the refrigeration needed during storage.

A drawback of inhalation dry powders is that the mass loads that can be inhaled each time are limited. This is a particularly relevant drawback for antibiotics because antibiotic doses are high. Therefore, antibiotic dry powders free of lactose carrier particles are developed and no or a limited amount of excipient is incorporated in the antibiotic formulation (Healy et al., 2014). For instance, each dose in TOBI Podhaler consists in inhaling the content of four capsules, each containing 50 mg of spray-dried tobramycin powder—the tobramycin powder being made of 85% w/w tobramycin and 15% w/w distearoylphosphatidylcholine and $CaCl_2$. Each dose of Colobreathe consists in inhaling the content of one capsule containing 125 mg of neat micronized colistin (Weers, 2015).

The objectives of this study were (i) to show the interest of pulmonary temocillin delivery to reach high temocillin concentrations locally in the lungs compared with intravenous injection of the drug, (ii) to prepare an inhalation dry powder of temocillin presenting good aerosolization properties using a minimal amount of generally recognized as safe (GRAS) excipients. Pulmonary temocillin delivery was carried out by intratracheal instillation of a temocillin solution in mice. The temocillin dry powder was prepared by spray-drying using a small proportion of lactose, alanine or DPPC. The powders were characterized for particle size, particle density and aerodynamic behavior in the Multi-Stage Liquid Impinger (MSLI). The most interesting formulations were further characterized in terms of particle surface composition using X-ray photoelectron spectroscopy (XPS) and powder crystallinity using X-ray diffraction (XRD), and they were visualized by scanning electron microscopy.

2. Materials and methods

2.1. Materials

Temocillin (Negaban®) was from Eumedica (Brussels, Belgium). Ticarcillin and sulforhodamine 101 were obtained from Sigma–Aldrich (St. Louis, MO, USA). Dipalmitoylphosphatidylcholine was purchased from Lipoid (Lipoid GMBH, Ludwigshafen, Germany) and L-alanine from Acros Organics (Geel, Belgium). Acetonitrile HPLC grade and chloroform were purchased from VWR Chemicals (Fontenay sous Bois, France). Unless otherwise stated, chemicals and reagents were from Sigma–Aldrich.

2.2. In vivo disposition of temocillin following pulmonary delivery and intravenous injection

Female NMRI mice (7 to 9 week-old; Elevage Janvier, Le Genest-St-Isle, France) were anaesthetized by ketamine/xylazine (90/10 mg/kg) intraperitoneal injection. Temocillin (1 mg in 25 μl phosphate buffer saline, PBS) was then administered intratracheally. The solution was instilled in the trachea of the mouse positioned with a 45° angle of tilt using a curved high-precision microsyringe (100-μL precision Hamilton syringe; Sigma–Aldrich). Intratracheal instillation was immediately followed by intratracheal insufflation of a 200 μl air bolus (Todoroff et al., 2013). Temocillin (10 mg in 100 μl PBS) was also administered intravenously to another group of mice. A 10-times higher dose was delivered intravenously versus intratracheally in order to attain similar temocillin concentrations in the lungs by both routes of drug administration. The unanesthetized mouse was placed in a restraining cage and the solution was injected in a lateral tail vein. At various pre-determined times (0, 15, 30 or 60 min) following temocillin administration, mice were slightly anaesthetized to collect blood samples by orbital bleeding. The mice were then sacrificed by a lethal injection of pentobarbital. A bronchoalveolar lavage (BAL) was performed. One ml of Hanks' balanced salt solution (HBSS; Gibco, Life Technologies; Gent, Belgium) was injected into the trachea and left for 30 s. This was followed by withdrawal and re-injection of 0.5 ml of the fluid, and then all the BAL liquid was removed from the lungs. This procedure was repeated twice until a total volume of 3 ml was injected. Afterwards, the lungs were removed and ground to release temocillin in 2 ml of HBSS using a tissue grinder (Potter, Merck Eurolab, Leuven, Belgium) for 2 min, and the tissue grinder was rinsed with 1 ml of HBSS. The time between temocillin administration and the collection of all biological samples was approximately 5 min. Sera, BAL and tissue homogenate samples were centrifuged at 3000 × *g* at 4 °C for 10 min. The supernatants were stored at −20 °C until they were assayed for temocillin content by HPLC. Three mice per time point were used.

The experimental protocols were approved by the Institutional Animal Care and Use Committee of the Université catholique de Louvain (Permit number: 2011-2/UCL/MD/028P). All studies were performed under anesthesia and all efforts were made to minimize animal suffering.

2.3. HPLC

Temocillin content in BAL, tissue homogenate and serum extracts were measured by reverse-phase high performance liquid chromatography (HPLC) with the Hewlett Packard series 1100 system (Agilent Technologies, Palo Alto, CA) using a C_{18} Jupiter column (250 × 4.60 mm i.d., 5 μm, 300 Å, Phenomenex, Torrance, CA) (Shull and Dick, 1985). Isocratic separation was achieved using a 0.1 M CH_3COONa pH 7/acetonitrile (95/5) solvent system at a flow rate of 1 ml/min. The column effluent was monitored at 242 nm. All measurements were performed at room temperature. Temocillin was extracted from the biological samples as follows: 100 μl of BAL supernatant, lung tissue homogenate supernatant, serum or PBS were pipetted into eppendorfs. 20 μl of internal standard solution (200 μg/mL ticarcillin disodium in PBS), 850 μl of chloroform-*n*-amyl alcohol and 50 μl of 0.4 M hydrochloric acid were then added. After vortexing for 2 min, the eppendorfs were centrifuged at 320 × *g* and 4 °C for 5 min. The aqueous phase was transferred into a new eppendorf and the organic phase was discarded. 100 μl of PBS were then added to the aqueous phase and the eppendorfs were vortexed and again centrifuged at 320 × *g* and 4 °C for 5 min. 50 μl of the aqueous phase supernatant were injected into the HPLC column. The standard curve was constructed by plotting the ratios of temocillin to ticarcillin areas against temocillin concentrations. The fraction of temocillin and ticarcillin extracted from the serum was 71% and 69%, respectively. These values were respectively 74% and 76% for the extraction from PBS. The process of the extraction did not affect the area ratios of temocillin to ticarcillin because the extraction of temocillin or ticarcillin was identical ($p > 0.05$, Student *t*-test). Therefore, samples of the standard curve were simple solutions of temocillin and ticarcillin in PBS, without extraction. The limits of temocillin detection and quantification were 4 μg/ml and 18 μg/ml, respectively. The method was linear between 10 and 250 μg/ml and the intra-assay relative standard deviation (RSD) was 1–2% over 25–250 μg/ml and 14% at 10 μg/ml.