Aerosol Performance and Stability Characteristics of Spray-Dried Tobramycin Excipient Enhanced Growth Inhalation Powder Formulations

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Summary

Despite advances in aerosol delivery with devices such as the TOBI™ Podhaler™, there is still room to improve the antibiotic dose delivered to the lungs, especially if powder inhalers are to be used for pediatric patients. The excipient enhanced growth (EEG) technology enables high efficiency delivery of powder aerosols even at low inhalation flow rates. This study sought to characterize the aerosol performance and stability of new tobramycin EEG (TOB-EEG) formulations. TOB-EEG powders were spray dried and their chemical stability evaluated following storage at ambient temperature and low humidity conditions for 10 months. TOB-EEG formulations were aerosolized following exposure to ambient temperature and humidity for 15 minutes and following storage at ambient temperature and low humidity for 6 months. The primary particle size of TOB-EEG powders was assessed following storage at ambient temperature and low humidity for up to 15 months. There was no significant change in the tobramycin content (98.8% of the initial content) for a TOB-EEG mannitol formulation following 10 months storage. Results also showed that 15 minute exposure to ambient conditions for a TOB-EEG sodium chloride formulation did not affect its aerosolization characteristics. A small improvement in the dispersion properties was observed following 6 months storage, perhaps indicating it is important to remove residual moisture before packaging. The geometric primary particle size of TOB-EEG powders were similar (x_{50}~1 µm) when tested following storage, suggesting the powders are stable over long-term when maintained in low humidity environments, as could be achieved with foil-wrapped capsules or other packaging methods.

Introduction

Chronic bacterial infection in the airways of cystic fibrosis (CF) patients is believed to cause progressive injury of their respiratory tissues and loss of lung function [1, 2]. Repeated and intensive antibiotic treatment targeting the major bacterial pathogen *P. aeruginosa* is recommended and has shown significant clinical benefits by increasing the median survival of CF patients in the last decades [1, 3, 4]. Inhaled antibiotics are desired as they better target drugs to the site of pulmonary infection and minimize their systemic exposure and side effects. Among the existing antibiotics, tobramycin has long post-antibiotic effect and low systemic toxicity, which makes it suitable to be developed into high-dose inhalable antibiotic products [5].

Presently, tobramycin is commercially available as both inhalation solution (e.g. TOBI®, Novartis AG, Switzerland) and inhalation powder (TOBI™ Podhaler™, Novartis AG, Switzerland). While the powder formulation uses PulmoSphere™ technology to improve aerosol dispersion, which increases drug deposition in adults' lung to 34% (three times higher than the inhalation solution) [6], TOBI Podhaler requires CF patients to inhale at least 30 L/min and is not recommended for children under 6 years of age [7]. As *P. aeruginosa* infection may occur early in CF patient's life [2], a high efficiency tobramycin formulation - aerosol delivery device combination designed particularly for paediatric CF patients aged between 2-10 years may be beneficial for early treatment of *P. aeruginosa* infection and prevent pulmonary exacerbation.

In this study, the initial development of new tobramycin powder formulations using the excipient enhanced growth (EEG) technique is described [8]. This EEG technique allows drug aerosols to be delivered with an initial small particle size to minimize device and upper airway deposition, while aerosol size increases in the lung through condensational growth to facilitate aerosol deposition and lung retention. The chemical and physical stability together with the aerosol performance of the tobramycin EEG (TOB-EEG) formulations were assessed to evaluate the robustness of the new formulations.

Materials and Methods

Preparation of TOB-EEG Powder

TOB-EEG inhalation powders were prepared using the spray drying technique described in Son et al. [9]. A spray drying solution was prepared by dissolving approximately 200 mg tobramycin (Spectrum Chemicals, New Brunswick, NJ), 190 mg sodium chloride (Fisher Scientific, Waltham, MA) or Pearlitol® PF-Mannitol (Roquette Pharma, Lestrem, France), 100 mg L-leucine (Sigma-Aldrich, St Louis, MO) and 10 mg poloxamer 188 (BASF Corporation, Florham Park, NJ) in 100 mL 20% ethanol / 80% deionized water (v/v). The solution was spray dried using Buchi Nano Spray Dryer B-90 (BUCHI Corporation, New Castle, DE) with a 4 µm vibrating mesh spray nozzle. Liquid feed rate was set to 100%, drying airflow rate was controlled at 120 L/min and inlet temperature at 70 °C. After spray drying, the formulation was collected from the electrostatic precipitator and stored in a sealed amber vial inside a desiccator (22±2 °C and <10% RH).

Assessment of Chemical Stability of TOB-EEG Powder

A previously established LC-MS method [10] was used to quantify tobramycin content and screen for additional degradation peaks in the TOB-EEG formulations immediately following spray drying and after storage at ambient temperature and low humidity conditions (22±2 °C and <10% RH) for 10 months. 1 mg TOB-EEG powder was dissolved in 1 mL deionized water and injected into the LC-MS. For reference purposes, a freshly prepared solution containing a mixture of the drug and excipients was prepared at a similar concentration and used as a control. Analyses was performed using Alliance e2695 HPLC (Waters, Milford, MA) coupled with Micromass Quattro micro API Mass Spectrometer (Waters, Milford, MA). Chromatographic separation was performed using a Hypersil Gold C18 column (150×4.6mm, 3µm; Thermo Scientific, Waltham, MA) and mobile phase consisting acetonitrile:0.1% trifluoroacetic acid (TFA) (30:70). Isocratic elution was used at a flow rate of 0.3 mL/min and sample injection volume was set at 10 µL. Electrospray ionization was used with a positive mode. An ion scan was performed at a range from 100 to 900 for each sample. For tobramycin quantification, single ion monitoring at 468.5 daltons was employed. Other parameters were: capillary voltage 3.78 kV; cone voltage 20 V; extractor 6 V; Rf Lens 0.1 V; source block temperature 130 °C; desolvation temperature 350 °C; nitrogen desolvation flow 600 L/min. Mass Lynx 4.0 (Waters, Milford, MA) was used for HPLC-MS control and data processing.

Aerosol Characterization and Assessment of Physical Stability of TOB-EEG Powder

Physical stability of TOB-EEG powder was first evaluated by characterizing its aerodynamic particle size distribution (APSD) delivered from Handihaler® device (Boehringer Ingelheim, Ingelheim, Germany) using Next Generation Impactor (NGI; MSP Corporation, Shoreview, MN). Experiments were designed to assess (a) stability of TOB-EEG powder inside the capsule after piercing and being exposed to ambient temperature and humidity (22±2 °C and 40±5%RH) for 15 minutes; this was designed to evaluate the robustness of the TOB-EEG formulation when used in a practical scenario; and (b) stability of TOB-EEG formulations after being stored at room temperature in a desiccator for 6 months (22±2 °C and <10% RH; similar conditions to aluminium-sealed packaging). Prior to each experiment, the pre-separator and NGI collection plates were coated twice using Molykote® 316 Silicone Release Spray (Dow Corning, Midland, MI) to avoid particle re-entrainment. Approximately 2 mg TOB-EEG powder was filled into a size 3 hydroxypropylmethyl cellulose (HPMC) capsule (Capsugel, Peapack, NJ) and placed into the Handihaler device. The 2 mg dose was selected for these initial development and stability studies with the Handihaler, although it is recognized that higher doses are required for therapeutic use. The capsule was then pierced and device inserted into a mouth-piece adapter designed to ensure air tightness between the inhaler and pre-separator/NGI. The USP inlet was omitted in all cases to determine the size distribution of the total emitted aerosol. Airflow rate was controlled at 45 L/min (equivalent to 4 kPa pressure drop across the inhaler) and pulled for 5.3 sec following procedures described in the United States Pharmacopeia (USP) [11]. Tobramycin deposition on the device, capsule, mouth-piece adapter, pre-separator and NGI plates was collected using known volumes of acetonitrile:0.4% trifluoroacetic acid (TFA) (80:20) and assayed using a HPLC-MS method described above. Single doses were used and each protocol was performed for at least three replicates. Emitted dose (ED) was calculated by subtracting drug mass remaining inside the device and capsule from the total recovered mass. Fine particle fraction ($FPF_{5\mu m/ED}$) and submicrometer fine particle fraction ($FPF_{1\mu m/ED}$) were calculated for the fraction of particles smaller than 5 µm and 1 µm, respectively, using linear interpolation from the cumulative percent under size vs. NGI stage cut-off diameter profiles and normalized to ED. Mass median aerodynamic diameter (MMAD) was also calculated using the linear interpolation approach.

Geometric particle size distributions of TOB-EEG formulations were also determined using Sympatec laser diffraction instrument installed with ASPIROS/RODOS dry dispersing unit and HELOS laser diffraction sensor (Sympatec GmbH, Clausthal-Zellerfeld, Germany). The powders were sized immediately following spray drying or stored at room temperature in a desiccator for 6 or 15 months (22±2 °C and <10 %RH). This technique was used to evaluate the long-term stability of the TOB-EEG formulations. The lens and measuring range was selected as R1:0.1 / 0.18-35 µm, and the disperser was initially set at 4 bar with a feed rate of 60 mm/s. Background was removed by conducting a reference run each time and three measurements were performed for each powder. WINDOX 5 software (Sympatec GmbH) was used for instrument control and data evaluation.

Results and Discussion

Chemical Stability of TOB-EEG Powder

There was no significant change in the tobramycin content uniformity for a TOB-EEG mannitol formulation following 10 month storage, with the mean (SD) content of 98.8 (9.3) % of the initial content. However, it was observed that there was greater variability in the content at the 10-month time point compared to the initial time point. For comparison, a similar study with two albuterol-EEG formulations with mannitol as the hygroscopic excipient showed mean (SD) drug contents (% of initial) of 97.8 (1.6) % after three years and 100.6 (0.5) % after two years storage. Examination of the mass spectral chromatographic profile following 6 months storage of the TOB-EEG formulation with sodium chloride as the hygroscopic excipient revealed no additional peaks when searching for $[M+H]^+$ ions at m/z 484, 527, 323 and 307 corresponding to known tobramycin potential degradation products kanamycin B, 6''-O-carbamoylkanamycin B, neamine and nebramine [12], respectively. While the present HPLC-MS method is sufficient for quantifying tobramycin (and possibly its potential degradation products), the formulation contains excipients including sodium chloride or mannitol, L-leucine and poloxamer 188. More robust assay methods will be needed in the future to characterize both tobramycin and the excipients to fully evaluate the chemical stability of TOB-EEG powder over shelf-life.

Aerosol characterization of TOB-EEG Powder

The aerodynamic properties of a TOB-EEG formulation with sodium chloride as the hygroscopic excipient were evaluated. The results for the three testing conditions are summarized in Figure 1 and Table 1:

(a) 5 days storage at ambient temperature and low humidity (22±2 °C and <10 %RH; Control);
(b) Exposure to ambient temperature and RH for 15 minutes (22±2 °C and 40±5%RH) immediately prior to aerosolization following storage under control conditions (a) for 5 days (to evaluate the effect of ambient humidity during inhaler use);
(c) 6 months storage at ambient temperature and low humidity (22±2 °C and < 10 %RH; to evaluate the effect of 6 months storage);

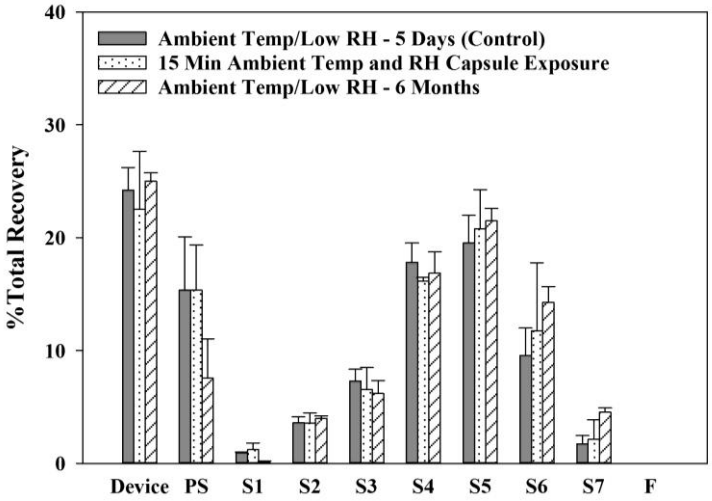

Figure 1. Effects of storage conditions on the aerosolization characteristics of TOB-EEG sodium chloride formulation, shown as the % tobramycin (% total recovered dose) when tested using Handihaler. Data presented as mean and standard deviation (n≥3). "Device" includes Handihaler and capsule; "PS", pre-separator, includes mouth-piece adapter and pre-separator; "S1-S7", NGI stages 1-7; "F", filter.

All three testing conditions produced highly dispersed particles with relatively good device emptying (ED > 75%) and notably large fine particle fractions ($FPF_{5\mu m/ED}$ > 72%) as shown in Table 1. Statistical analyses using Student's t test showed that exposure to ambient conditions for 15 minutes had no effect on aerodynamic properties of TOB-EEG powder; the distribution of tobramycin on Handihaler device, pre-separator and NGI stages produced no significant differences compared to the control group (Figure 1; $p>0.05$). Surprisingly, the TOB-EEG powder stored for 6 months produced slightly smaller particles than the control group (MMAD = 1.7 µm and 1.9 µm, respectively; Table 1), and statistically significant differences were observed for tobramycin distribution on the pre-separator and NGI stages 1, 6 and 7 between the 6 months group and control group (Figure 1; Student's t-test, $p<0.05$). It is hypothesised that the freshly spray-dried TOB-EEG formulation may contain residual moisture that slightly affects the powder dispersity. Moisture content in the spray-dried formulations and its potential effects on powder dispersity may need to be quantified in future experiments. In addition, residual moisture may need to be removed from formulation powders before storage.

Table 1. Effects of storage conditions on aerodynamic properties of TOB-EEG sodium chloride powders. ED, emitted dose (% total recovered dose); $FPF_{5\mu m/ED}$, fine particle fraction of drugs smaller than 5 µm (% ED); $FPF_{1\mu m/ED}$, fine particle fraction of drugs smaller than 1 µm (% ED); MMAD, mass median aerodynamic diameter. Data are presented as mean and standard deviation (n≥3).

Storage Conditions	Exposure to Ambient Conditions	ED (%)	$FPF_{5\mu m/ED}$ (%)	$FPF_{1\mu m/ED}$ (%)	MMAD (µm)
Ambient temp/low RH – 5 days	No	75.8 (2.0)	72.8 (6.7)	12.2 (3.4)	1.9 (0.2)
Ambient temp/low RH - 5 days	15 min	77.5 (5.1)	73.0 (8.0)	14.4 (7.1)	1.8 (0.3)
Ambient temp/low RH - 6 month	No	75.0 (0.7)	83.7 (5.0)	21.0 (2.1)	1.7 (0.1)

Geometric particle size distributions of TOB-EEG powders initially and after 6 months and 15 months storage are summarized in Table 2. While these powders are from mixed batches and contain different hygroscopic excipients (mannitol or sodium chloride), data shows that the powders produced consistently small particles with the volume (mass) median diameters between 1.0 to 1.1 µm and similar polydispersity. This suggests that the spray drying techniques used for produce TOB-EEG powders were robust and the formulations appear to be stable over long-term storage.

Table 2. Geometry diameter of TOB-EEG powders after storage at ambient temperature and low humidity (<10% RH) for 6 or 15 months. Data are presented as mean and standard deviation (n=3).

	Storage Period	X_{10} (µm)	X_{50} (µm)	X_{90} (µm)
TOB-EEG mannitol	Initial size	0.5 (0.0)	1.1 (0.0)	2.4 (0.0)
TOB-EEG sodium chloride	6 Months	0.5 (0.0)	1.0 (0.0)	2.0 (0.0)
TOB-EEG mannitol	15 Months	0.5 (0.0)	1.0 (0.0)	2.1 (0.0)

Conclusions

The preliminary data showed TOB-EEG powders are well dispersed and generally stable when exposed to 15 minutes ambient conditions and after long-term storage in the desiccator. The powder has potential to be used for treating paediatric CF patients aged between 2-10 years, and further studies will be needed to optimize the formulation and storage conditions. More robust HPLC-MS methods will also be needed to fully characterize the formulation and facilitate detection of potential degradation products from both tobramycin and the excipients.

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