Development of an inhalable pMDI solution formulation of theophylline for COPD

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Summary

Background: Theophylline (TP) is a drug used to treat Chronic Obstructive Pulmonary Disease (COPD) that has been associated with multiple side effects, tempering its present use. This study aims to improve COPD treatment by creating a low-dose pressurized metered dose inhaler (pMDI) formulation with theophylline (TP). **Methods:** Aerosol performance was assessed using Andersen Cascade Impaction (ACI) and morphology of the particles was analyzed with a Scanning Electron Microscope (SEM). Calu-3 cell viability assay and cell transport were conducted to study the impact of the formulation on lung epithelial cells. **Results:** The mass deposition profile of the formulation showed an emitted dose of 250.04 ± 14.48 µg per 5 actuations, achieving the designed nominal dose (50µg/dose). SEM showed that the emitted particles were spherical and hollow. **Conclusion:** A suitable solution pMDI formulation of TP was developed that could be used for the treatment of COPD.

Introduction

TP is a bronchodilator[1] used orally to treat COPD, asthma and other lung diseases. However, TP has been associated with several side effects and its efficiency is dependent on the hepatic metabolism of patients, resulting in its limited use[2]. It has recently been shown that TP might be able to restore corticosteroid responsiveness and improve the anti-inflammatory response[3]. Pressurized metered dose inhalers (pMDIs) are medical devices designed to deliver low-dose medications (~250 µg)[4], furthermore, they have certain advantages over dry powder inhalers, e.g. dose consistency over product lifetime and satisfactory aerosol performance[5]. In order to reduce the required TP dose and therefore decrease side effects, a low-dose TP pMDI solution formulation containing 50µg active medical ingredient per actuation was developed as local treatment to be used for COPD therapy.

Methods and Materials

Anhydrous TP was used as supplied (MP Biomedicals, France). Methanol (HPLC grade) and Ethanol (100%) were obtained from Biolab (Clayton, Victoria, Australia). The propellant 1,1,1,2 tetrafluoralkane (HFA 134a) was obtained from Solvay Chemicals (Bruxelles, Belgium). The water was purified through reverse osmosis (MiliQ, Millipore, France).

Formulation

Theophylline pMDI formulation was formulated to contain 15% (w/w) ethanol, due to TP solubility limit in the mixture of HFA 134a, to deliver 50µg of active medication per actuation. The dose chosen was not considered as therapeutic dose. Further investigation on the actual therapeutic dose is ongoing. The aerosol performance of the pMDI formulation (actuator orifice size 0.3 mm) was evaluated using an Andersen cascade impactor at 28.3 L/min, firing 5 doses in order to overcome the limit of detection of chemical quantification. After cascade impaction, the ACI was disassembled and each part was thoroughly rinsed with deionized water into 100 mL volumetric flasks, followed by sample collection for chemical quantification using High Performance Liquid Chromatography (HPLC), with a validated method. The samples were quantified using a reverse phase C18 column (4.6 x 150 mm and 5 µm, XBridge™ Shield, Waters, USA) with methanol aqueous solution (55%, v/v), at a flow rate of 1 mL/min, detection wavelength 275 nm and injection volume 100 µL. The standard solutions were prepared daily and the linearity of standard solutions in the concentration of 0.01~100 µg mL was confirmed with a R^2 value > 0.999.

Morphology study

The morphology of deposited TP was studied using scanning electron microscopy (SEM) (Zeiss Ultra Plus, Carl Zeiss NTS GmbH, Germany). The particles were collected on adhesive carbon tape from stage 5 of the ACI and sputter coated with 15 nm gold (Sputter coater S150B, Edwards High Vacuum, Sussex, UK) according to a previously reported method[4,5].

Calu-3 cell viability assay

Human airway Calu-3 cells is an immortalized cell line used as a respiratory model of human respiratory function, structure and inflammatory response. The toxicity of TP was evaluated by exposing Calu-3 cells to increasing concentrations of TP phosphate buffered saline (PBS) solution. The cell viability was measured in a liquid covered

culture (LCC) following a 72-hour drug incubation, according to the previously published method[6]. Briefly, Calu-3 cells were seeded in a volume of 100 µL into a 96 well plate and incubated overnight at 37°C in 5% CO_2 atmosphere. Increasing concentrations of TP were added to the wells and incubated for 3 hours. To assess the viability, 20 µL of the CellTiter 96® Aqueous assay (MTS reagent) (Promega, Madison, USA) was added to each well. The plates were incubated for 3 hours at 37°C in humidified 5% CO_2 atmosphere. The absorbance was measured at 490 nm using a Wallac 1420 VICTOR 2 Multilabel Counter (Wallac, Waltham, USA). The drug concentration that resulted in a decrease of 50% in cell viability compared to the untreated control was deemed as the half maximal inhibitory concentration (IC_{50}).

Transport of theophylline across Calu-3 epithelium

The modified ACI setup described by Haghi et al.[7], was used to deposit the TP pMDI formulation on Calu-3 epithelial cells. 17 days post seeding, the Snapwells (0.4 µm pore size polyester membrane, 1.12 cm^2 surface area) (Corning Costar, Lowell, MA, USA) with the Calu-3 cells grown at air-liquid interface, were placed on the ACI stage 5. Two to four doses of the TP pMDI were actuated into the modified ACI to confirm the dose consistency. Following TP deposition, the Snapwells® were transferred to a 6-well plate containing Hank's balanced salt solution (HBSS) (Gibco-Invitrogen, Sydney, Australia) with incubation of 2 hours. At 10, 30, 45 and 60-minute time points, 200 µl samples were taken from the basal compartment and was replaced with same volume of fresh HBSS. Prior to analysis with HPLC, the epithelium was washed with 125 µL HBSS and then collected for chemical quantification. Finally, the cells were lysed using cell lysis reagents (CelLytic®, Sigma-Aldrich, Sydney, Australia) and sheared using a 21-gauge needle as described previously by Haghi et al.[8]. The supernatant was collected after ultracentrifugation for analysis of the intracellular drug component.

Results and discussion

The cascade impaction study showed TP aerosols emitted from the pMDI formulation had suitable aerodynamic characteristics, with an MMAD (mass median aerodynamic diameter) of 1.3 ± 0.01 µm, GSD (geometric standard deviation) of 2.1 ± 0.2 and fine particle fraction (FPF, defined as the fraction less than 4.7 micron) of 38.0 ± 1.1%. The total emitted dose from 5 actuations was 250.0 ± 14.5 µg, achieving the active dose of 50 µg/actuation, in line with pharmacopeia dose requirement (i.e. ± 25% of nominal dose)[9]. Figure 1 shows the mass deposition profile of the TP pMDI formulation. Approximately 10% emitted particles were deposited in the actuator, which is consistent with results from previous studies, due to the ballistic nature of this type of inhalers[10]. In addition, results showed that up to 50% of the produced aerosols were captured in the USP induction port, possibly due to the high ethanol concentration in the formulation, tampering the formulation vapour pressure[10]. However, particles in the size range < 3.3 µm (i.e. from stage 4 to filter, 40.8% of the emitted dose) showed a deposition profile suitable for inhalation delivery.

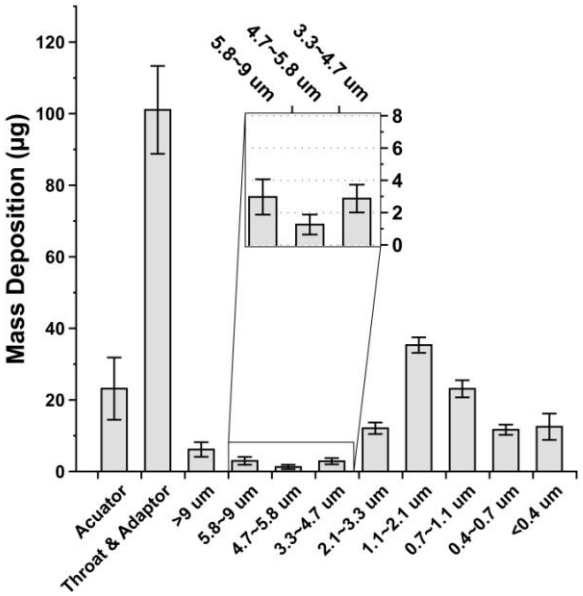

Figure 1 Mass deposition profile of TP pMDI formulation from 5 actuations (n=3 ± SD)

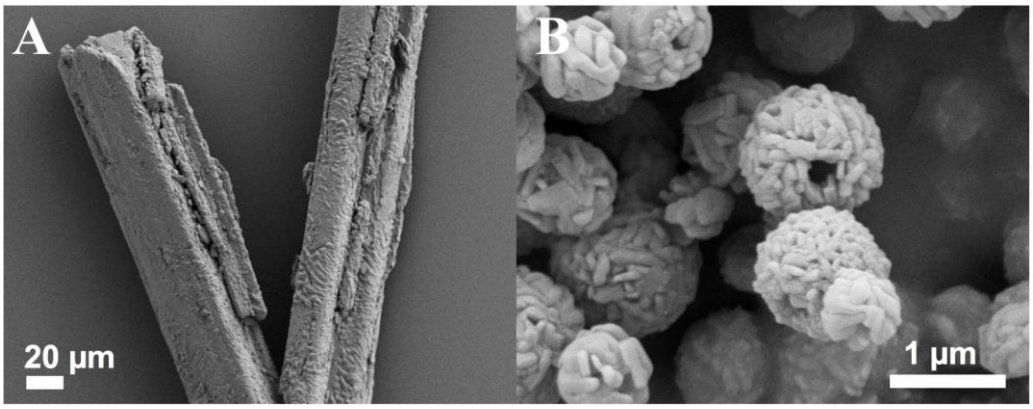

Figure 2 Representative electron micrographs of (A) raw TP material and, (B) TP particles collected after pMDI deposition on ACI stage 5

Figure 2 shows the morphology of the aerosolised TP particles collected on the ACI stage 5 and imaged by SEM. These particles appear to have a spherical morphology with a hollow core.

The viability of Calu-3 cells when exposed to TP solutions with different concentrations (19 nM~2.5 mM) is presented in Figure 3. Solutions with TP concentration below 0.32 mM did not show any effect on the viability of Calu-3 cells; however, the cell viability decreased from approximately 98% to 76.8% when the TP concentration increased from 0.63 to 2.5 mM, indicating the low toxicity profile of TP in the concentration range studied. Subsequently, this observation suggests the developed low-dose TP pMDI formulation to be non-toxic on Calu-3.

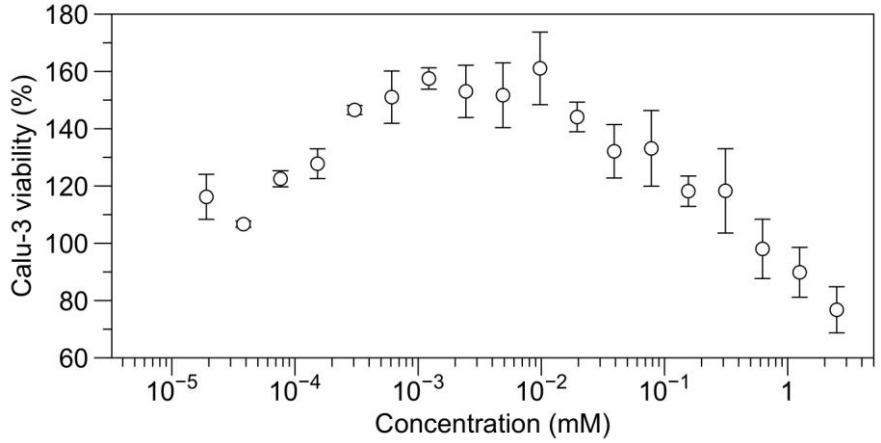

Figure 3 Cell viability of Calu-3 cells after incubation with TP (n= 3 ± SD).

Transport studies showed that 98.2% of the deposited drug was transported across Calu-3 cells over 180 minutes. This indicates that TP was able to be transported across the epithelial cell barrier and reach the A3 Adenosine receptors (TP site of action) located on the smooth muscle cells after 3 hours.

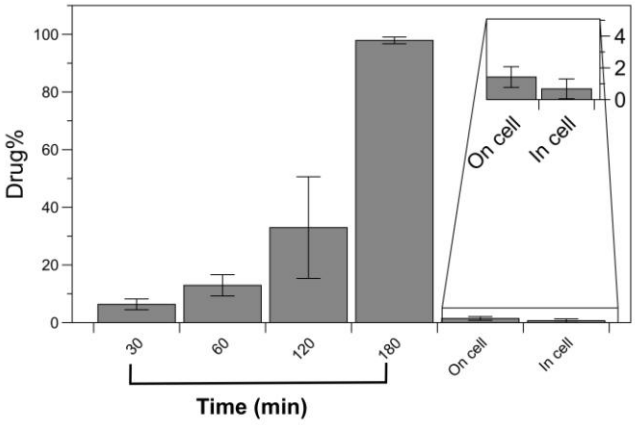

Figure 4 Transport of TP across Calu-3 cells grown in the air-liquid interface (n= 3 ± SD).

Conclusions

In this investigation, a pMDI solution formulation of TP was successfully developed. The formulation showed suitable physiochemical and aerodynamic characteristics for lung delivery, as confirmed by the morphology studies and aerosol performance investigations. Cell studies confirmed TP delivered on Calu-3 cell to be safe at the concentrations investigated and able to be transported across epithelial cells to reach its primary site of action on the smooth muscles. Further studies investigating TP anti-inflammatory properties and its effective dose *in vivo* are ongoing.

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