European Journal of Pharmaceutical Sciences 76 (2015) 68–72

Contents lists available at ScienceDirect

European Journal of Pharmaceutical Sciences

journal homepage: www.elsevier.com/locate/ejps

The formulation of a pressurized metered dose inhaler containing theophylline for inhalation

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ARTICLE INFO

Article history:
Received 16 February 2015
Received in revised form 20 April 2015
Accepted 20 April 2015
Available online 6 May 2015

Keywords:
Theophylline
Pressurized metered dose inhaler
Chronic obstructive pulmonary disease
Toxicity

ABSTRACT

Background: Theophylline (TP) is a bronchodilator used orally 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) inhalable formulation of TP.
Methods: Aerosol performance was assessed using Andersen Cascade Impaction (ACI). Solubility of TP in HFA 134/ethanol mixture was measured and morphology of the particles analyzed with a scanning electron microscope (SEM). Calu-3 cell viability, epithelial cell transport and inflammatory-response assays 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 (50 μg/dose). SEM showed that the emitted particles were hollow with spherical morphology. Approximately 98% of TP was transported across Calu-3 epithelial cells and the concentration of interleukin-8 secreted from Calu-3 cells following stimulation with tissue necrosis factor-α (TNF-α) resulted in significantly lower level of interleukin-8 released from the cells pre-treated with TP (1.92 ± 0.77 ng·ml^{-1} TP treated vs. 8.83 ± 2.05 ng·ml^{-1} TNF-α stimulated, respectively).
Conclusions: The solution pMDI formulation of TP developed in present study was shown to be suitable for inhalation and demonstrated anti-inflammatory effects at low doses in Calu-3 cell model.

1. Introduction

Theophylline (TP), is a methylxanthine, clinically used as bronchodilator generally in form of oral sustained release formulations (Barnes, 2003). Due to its action as non-selective inhibitor of cyclic nucleotide phosphodiesterases (PDE) and competitive antagonism of adenosine receptors (Barnes, 2003, 2013; Schudt et al., 2011), causing relaxation of the airway smooth muscle cells, it is used for the treatment of asthma and chronic obstructive pulmonary disease (COPD).

TP has been associated with several side effects, narrow therapeutic index and its efficacy is dependent on the hepatic metabolism of patients, resulting in its limited use (Barnes, 2013). Furthermore TP uptake in the lung is very limited and when administered orally, less than 15% is excreted in the urine unchanged (Undem and Lichtenstein, 2001). However, TP is still being used as an add-on therapy because it is inexpensive and widely available.

Current COPD therapies have failed to slow the lung function decline in the patients (Tashkin et al., 2008; Vestbo et al., 1999; Anthonisen et al., 1994). Novel therapeutic strategies have also not been very successful, since they have focused on targeting single mediators (Ngkelo and Adcock, 2013), an approach that is not suitable for a complex disease like COPD, where multiple pathways are involved (Kaneko et al., 2013).

Corticosteroids have been used for their anti-inflammatory properties in COPD, however their use is also been contested, with some researchers questioning their benefits (Barnes, 2013; Telenga et al., 2010), although anti-inflammatory drugs are an indispensable part of the drug regimen in these patients since inflammation is prominent in COPD (Sutherland and Martin, 2003; Sethi et al., 2012; Agusti et al., 2012).

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http://dx.doi.org/10.1016/j.ejps.2015.04.016
0928-0987/

It has recently been shown that TP might be able to restore corticosteroid responsiveness and improve the anti-inflammatory response (Ford et al., 2010). *In vitro* studies have also shown that low doses of TP may enhance the anti-inflammatory effect of corticosteroids and antagonize their "resistance" (Barnes, 2003, 2006), shown by the reduction in the number of neutrophils and in the interleukin-8 concentration in induced sputum from COPD patients (Barnes, 2003).

Developing TP as an aerosol formulation for inhalation could circumvent some of the problems associated with oral TP therapy. The advantage of an aerosolized form would be an increased dose of TP delivered directly to the airways, while minimizing unwanted systemic side effects.

pMDIs are medicinal devices designed to deliver low-dose medications (~250 μg per device actuation) (Zhu et al., 2013) and possesses certain advantages over dry powder inhalers, e.g. dose consistency over product lifetime and satisfactory aerosol performance (Zhu et al., 2014).

This study aims to improve COPD treatment by formulating a low dose inhalable TP pMDI solution formulation in order to reduce the required dose via local treatment and therefore reducing side effects.

2. 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 tetrafluoroalkane (HFA 134a) was obtained from Solvay Fluor GmbH (Hannover, Germany). The water was purified through reverse osmosis (MiliQ, Millipore, France).

2.1. Determination of TP solubility in HFA 134a propellant and propellant/ethanol mixture

To determine the solubility of TP in HFA 134a and HFA 134a/ethanol mixtures, a solubility determination apparatus was used as previously described (Traini et al., 2006). In brief, the apparatus consists of upper casing, stainless filter (0.45 μm pore size) and lower casing. An excess amount of TP was placed in the lower casing of the apparatus and assembled with the filter and the upper casing. The assembly was crimped with a continuous valve and filled with approximately 7 g of HFA 134a propellant or HFA 134a/ethanol mixtures (10% and 15% ethanol (w/w), respectively). After shaking for 48 h at room temperature, the apparatus was exhausted into a dose unit sampling apparatus (DUSA) with a set airflow rate of 28.3 l/min. The apparatus and DUSA were then disassembled. The upper casing of the solubility apparatus and all components from DUSA were thoroughly rinsed with deionized water and samples collected for chemical quantification. The experiments were conducted in triplicate.

2.2. Theophylline pMDI formulation and aerosol performance

Due to the solubility of TP in HFA 134a, to obtain a stable TP solution pMDI product, 15% (w/w) of ethanol was incorporated in the formulation, to deliver 50 μg of active ingredient (through a 50 μl valve) per actuation. TP plasma concentration of 5–10 μg/ml is considered as therapeutically effective for anti-inflammatory effects and above 20 μg/ml as toxic (Barnes, 2005). Previous studies using lung microanalysis in murine and equine models have indicated lung concentrations of TP to closely follow blood (free drug) and plasma concentrations and to be of a similar order (Ingvast-Larsson et al., 1992). Thus it may be considered that 50 μg would be a reasonable dose to achieve similar concentrations in the lung to that of oral or IV dosing (assuming the normal total lung fluid volume ranges from 15 to 17 ml and the volume in the first 17 generations is 4–9 ml (Patton, 1996). Additionally, although the therapeutic dose of TP via pulmonary route is still under investigation, it is reasonable to follow the dose range of marketed solution pMDI products, such as QVAR® and Clenil Modulite® with 50 μg beclomethasone dipropionate/ dose, minimizing potential side effects.

The aerosol performance of the pMDI formulation was evaluated using an Andersen cascade impactor (ACI, Copley Scientific Ltd, UK). In brief, the airflow rate of the ACI equipped with a United States pharmacopeia throat was calibrated to 28.3 l/min with a flow meter (Westech Scientific Instruments, Bedfordshire, UK). Five doses of the formulation were fired into the impactor assembly using an 0.33 mm spray orifice actuator (636E00G, Bespak Europe Ltd, Norfolk, UK). After cascade impaction, the ACI was disassembled and each part was thoroughly rinsed with deionized water into volumetric flasks followed by sample collection for chemical quantification.

2.3. High performance liquid chromatography

The TP concentration in the samples was quantified using a Shimadzu HPLC system consisting of a LC20AT pump, a SIL20AHT autosampler and an SPD-20A UV–VIS detector (Shimadzu, Sydney, Australia). The samples were injected onto a reverse phase C18 column (4.6 × 150 mm and 5 μm, XBridge™ Shield, Waters, USA) with a 55%, v/v methanol/water mobile phase at a flow rate of 1 ml/min. The detection wavelength was 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 $\mu g \cdot ml^{-1}$ was confirmed with a R^2 value >0.999.

2.4. Morphology study

To investigate particle morphology post-deposition, particles on Stage 5 of the ACI, representing particles with an aerodynamic diameter of 1.1–2.1 μm, were collected on adhesive carbon tape and sputter-coated with 15 nm gold (Sputter coater S150B, Edwards High Vacuum, Sussex, UK), according to a previously reported method (Zhu et al., 2013, 2014). A field-emission Scanning Electron Microscope (SEM) (Zeiss Ultra Plus, Carl Zeiss NTS GmbH, Germany) was used to assess particle morphology and electron micrographs were taken at magnification of 5,000 and 15,000, respectively.

2.5. Calu-3 cell viability assay

Calu-3 human airway Calu-3 cells are an immortalized cell line used as a respiratory model of human respiratory function, structure and inflammatory response (Haghi et al., 2014). 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 72-h incubation, according to a previously published method (Scalia et al., 2013). 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 following which increasing concentrations of TP were added to each well. To assess the viability, 72 h after dosing the cells with TP, 20 μl of the CellTiter 96® Aqueous assay (MTS reagent) (Promega, Madison, USA) was added to each well. The plates were incubated for 3 h 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