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Research paper

Towards the bioequivalence of pressurised metered dose inhalers 2. Aerodynamically equivalent particles (with and without glycerol) exhibit different biopharmaceutical profiles *in vitro*

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ABSTRACT

Two solution-based pressurised metered dose inhaler (pMDI) formulations were prepared such that they delivered aerosols with identical mass median aerodynamic diameters, but contained either beclomethasone dipropionate (BDP) alone (glycerol-free formulation) or BDP and glycerol in a 1:1 mass ratio (glycerol-containing formulation). The two formulations were deposited onto Calu-3 respiratory epithelial cell layers cultured at an air interface. Equivalent drug mass (~1000 ng or ~2000 ng of the formulation) or equivalent particle number (1000 ng of BDP in the glycerol-containing versus 2000 ng of BDP in the glycerol-free formulation) were deposited as aerosolised particles on the air interfaced surface of the cell layers. The transfer rate of BDP across the cell layer after deposition of the glycerol-free particles was proportional to the mass deposited. In comparison, the transfer of BDP from the glycerol-containing formulation was independent of the mass deposited, suggesting that the release of BDP is modified in the presence of glycerol. The rate of BDP transfer (and the extent of metabolism) over 2 h was faster when delivered in glycerol-free particles, 465.01 ng ± 95.12 ng of the total drug (20.99 ± 4.29%; BDP plus active metabolite) transported across the cell layer, compared to 116.17 ng ± 3.07 ng (6.07 ± 0.16%) when the equivalent mass of BDP was deposited in glycerol-containing particles. These observations suggest that the presence of glycerol in the maturated aerosol particles may influence the disposition of BDP in the lungs.

1. Introduction

The efficacy of inhaled corticosteroids for the treatment of asthma is well documented, with national and international treatment guidelines recommending their use for control of mild persistent, moderate and severe asthma [1,2]. Beclomethasone dipropionate (BDP) is one of the most commonly used corticosteroids in the treatment of asthma and, after deposition in the lung, undergoes rapid metabolism via esterase enzymes to the active metabolite, beclomethasone-17-monopropionate (BMP) [3]. Several devices have been developed to deliver BDP to the pulmonary system including nebulisers, dry powder inhalers and pressurised metered dose inhalers (pMDI). pMDIs are the most common devices for delivery of BDP with marketed products including solution-based inhalers, e.g. QVAR® by 3M Ltd. and Clenil® by Chesi Ltd. (also licenced as Sanasthmax® and Becloforte®) and suspension inhalers, e.g. Becotide® by GSK Ltd.

Historically, BDP pMDIs were formulated in chlorofluorocarbon (CFC) propellant; however, with the regulatory phase out of CFCs, reformulation in more environmental friendly hydrofluoroalkanes (i.e. HFA 134a and 227) was undertaken. During the CFC–HFA transition, different reformulation approaches were explored resulting a plethora of patents within the field [4]. For example, BDP was formulated as an ultrafine inhalation aerosol by solubilising the drug in an ethanol-HFA 134a-based system [5]. Similarly, Modulite™ technology utilised an ethanol-HFA-based solution, but additionally incorporated non-volatile components, such as glycerol, to generate a larger mass median aerodynamic diameter (MMAD), equivalent to that of the original CFC-based systems [6]. While the former approach required extensive clinical trials due to different regional lung deposition [7], the Modulite technology was

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tuned to have a similar deposition profiles to that of the former CFC formulations.

Several clinical studies have evaluated the ultrafine and Modulite products and compared these to suspension CFC formulations in terms of pharmacokinetics (systemic exposure) and clinical efficacy [3]. The ultrafine BDP formulations with a mass median aerodynamic diameter (MMAD) ca. 1.1 μm resulted in greater systemic uptake and area under the curve (AUC) blood plasma concentrations compared to the larger Modulite (MMAD ca. 2.6 μm) or CFC formulations (MMAD ca. 4 μm) [3]. The area under the plasma concentration curve for the BMP in the Modulite formulations was reported to be markedly different than QVAR, but not dissimilar to the CFC formulations even though the MMAD's were different [8]. In addition, the Modulite formulations were shown to have similar clinical outcomes to CFC formulations in terms of Forced Expiratory Volume and morning serum cortisol concentrations [9]. A recent review by Derom and Pauwels has summarised clinical studies utilising BDP in different HFA formulations [3]; however, while these studies provide an insight into the pharmacokinetics and dynamics of HFA BDP systems, direct comparison between two formulations are difficult due to the nature of the study designs.

Interestingly, *ex vivo* and *in vitro* studies comparing ultrafine and Modulite-based pMDIs have shown significant differences in drug disposition [10,11]. A study by Freiwald et al., employing an extracorporally ventilated and re-perfused human lung lobe, showed that Modulite pMDI formulations had a significantly slower tissue penetration rate that could be attributed to the particle topology and dissolution rate [11]. A subsequent study by Grainger et al. produced similar findings using an *in vitro* Calu-3 epithelial cell line-based respiratory drug absorption model and a Franz diffusion cell method to measure dissolution [10]. Particles generated from QVAR® (containing HFA, BDP and ethanol) and Sanasthmax® (containing HFA, BDP, ethanol and glycerol as a particle performance modifier) products were compared in terms of their dissolution and transport across an air interface Calu-3 cell line after aerosol deposition. The particles generated by the QVAR formulation dissolved more rapidly and resulted in more rapid transport across the epithelial cell layer; however, this difference could not be attributed to a single particle property, since the particle size (MMAD QVAR = 1.1 μm; Sanasthmax = 2.8 μm) crystallinity and non-volatile excipients (i.e. only Sanasthmax contained glycerol) varied [10].

Current approved approaches to assess bioequivalency of oral inhaled products include the following: unit dose sampling, cascade impaction, spray pattern and plume geometry. There are, however, general issues regarding the *in vitro* tests [12]. Cascade impaction techniques are useful for general understanding of lung deposition; however, the results are not well correlated with regional lung deposition and the fate of particles deposited on the airway epithelium, the fist barrier encountered by microparticles after inhalation.

In order to study how individual inhaler formulation variables affect the biopharmaceutical performance of the particles generated, it is important that each variable is studied in isolation. The presence of glycerol as a non-volatile additive in the formulation is interesting since glycerol is a common excipient used in many areas of drug delivery. For example, glycerol is routinely used as emollient, humectant, gel vehicle, plasticiser, taste masker, transdermal patch additive and osmotic agent [1,2,13]. Glycerol has a relatively low diffusivity when compared to an aqueous solution [3,14]. Thus, the influence of glycerol on drug transport across the respiratory epithelium would be interesting and provide important information regarding the potential effect on bioavailability.

Two solution-based pMDIs with identical aerodynamic properties and aerosol dosimetry, but whose particles contained either drug alone or drug and glycerol at a 1:1 ratio have been developed [15]. Semi-empirically derived equations were used to relate HFA, ethanol, non-volatile components and valve components variables to the aerosol particle size distribution and formulate solution pMDIs with equivalent fine particle doses ($\leqslant$5 μm) and MMAD. These inhalers generated particles with very different physicochemical properties: while the particles from each inhaler formulation were amorphous, the morphology and thermal properties of the glycerol-containing particles were significantly different than those of the glycerol-free particles.

Following the successful formulation and characterisation of two formulations that had identical aerosol profiles, this article investigates the role of glycerol on the dissolution and absorptive transport of BDP across the respiratory epithelium using an air interface Calu-3 cell-based drug absorption model.

2. Materials and methods

2.1. Materials

BDP was supplied by Teva Pharmaceuticals (Harlow, UK). Actuators and pMDI valves were provided by Bespak Ltd. (Norfolk, UK). pMDI cans were provided by Presspart Ltd. (Lancashire, UK). Ethanol ($\geqslant$99.5%) was supplied by Sigma-Aldrich (Gillingham, UK) and glycerol ($\geqslant$99%) provided by Sigma-Aldrich (Gillingham, UK). HFA134a (1,1,1,2-tetrafluoroethane) and HFA 227 (1,1,1,2,3,3,3-heptafluoropropane) were supplied by (Mexichem Fluor, Runcorn, UK).

Dulbecco's Modified Eagle's Medium (DMEM, without phenol red and L-glutamine, including sodium bicarbonate and 15 mM HEPES), non-essential amino acids solution (x100), CelLytic™ M Cell Lysis (50 mM Tris–HCl, pH 8, 150 mM NaCl, 1% NP-40, 0.5% sodium deoxycholate, 0.1% SDS), mammalian protease inhibitor cocktail, HEPES (4-(2-Hydroxyethyl) piperazine-1-ethanesulfonic acid sodium salt) and (±)-α-Tocopherol (Vitamin E) were purchased from Sigma-Aldrich (Sydney, Australia). Phosphate buffered saline (PBS), Foetal bovine serum (FBS), L-glutamine solution (200 mM), Trypsin-EDTA solution (2.5 g/L trypsin, 0.5 g/L EDTA) and Hank's balanced salt solution (HBSS) were obtained from Invitrogen (Sydney, Australia). The Calu-3 cell line (HTB-55) was obtained from American Type Cell Culture Collection (ATCC, Rockville, USA) and was used between passages 35 and 40. Transwell cell culture inserts (0.33 cm^2 polyester, 0.4 μm pore size) were purchased from Corning Costar (Lowell, MA, USA). Sterile culture plastic-wares were obtained from Sarstedt (Adelaide, Australia). All solvents and chemicals were analytical grade and were supplied by Sigma (Sydney, Australia). Water used throughout the experiment was purified by reverse osmosis (MilliQ, Sydney, Australia).

2.2. Preparation of solution pMDIs with equivalent aerodynamic size distributions

Formulations exhibiting equivalent MMAD, geometric standard deviations (GSDs) and stage deposition profiles were prepared using methods described previously [15]. The formulations were designed such that evaporation of the volatile components would result in aerosol particles containing either drug alone or drug with 50% w/w glycerol, thus allowing the effect of glycerol on dissolution and transepithelial transport to be studied (Table 1).

These two formulations are referred to 'glycerol-free' and 'glycerol-containing' throughout the remainder of the text.