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Critical Characteristics for Corticosteroid Solution Metered Dose Inhaler Bioequivalence

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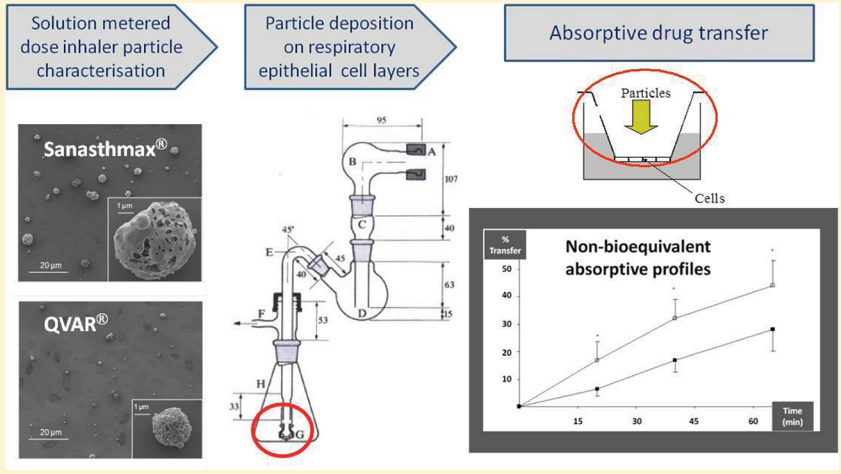

ABSTRACT: Determining bioequivalence for solution pressurized metered dose inhalers (pMDI) is difficult because the critical characteristics of such products are poorly defined. The aim of this study was to elucidate the non-aerodynamic properties of the emitted aerosol particles from two solution pMDI products that determine their biopharmaceutical differences after deposition. Novel particle capture and analysis techniques were employed to characterize the physicochemical and biopharmaceutical properties of two beclomethasone dipropionate (BDP) products: QVAR and Sanasthmax. The BDP particles emitted from the Sanasthmax inhaler were discernibly different those emitted from QVAR in terms of size (50% larger, less porous), solid state (less crystalline) and dissolution (20-fold slower). When deposited onto the surface of respiratory epithelial cell layers, QVAR delivered ~50% more BDP across the cell layer in 60 min than Sanasthmax. Biopharmaceutical performance was not attributable to individual particle properties as these were manifold with summative and/or competing effects. The cell culture dissolution−absorption model revealed the net effect of the particle formed on drug disposition and was predictive of human systemic absorption of BDP delivered by the test inhalers. This illustrates the potential of the technique to detect the effect of formulation on the performance of aerosolized particles and contribute to assessment of bioequivalence.

KEYWORDS: *aerosol formulation, inhaled corticosteroids, airway epithelial cell, pressurized metered dose inhalers, aerosol deposition*

INTRODUCTION

The redesign of a medicine that is in clinical use is a commercially attractive proposition if the new product meets a well-defined clinical or market need. The medicine regulatory agencies in most countries will consider the submission of a truncated clinical testing package to support the approval process if the redesigned product can be shown to be "bioequivalent" to the licensed medicine.[1] The use of *in vitro* data, generated using tests that demonstrate good *in vitro*−*in vivo* correlation, is an accepted means of supporting the bioequivalence of simple dosage forms, e.g. immediate release tablets and capsules. However, the application of a similar medicine redesign and approval process for more complex products is less well established.[2] In the case of inhaled preparations, the increasing availability of generic market entry products has exposed limitations in product knowledge.[3,4]

Received: August 18, 2011
Revised: December 8, 2011
Accepted: January 8, 2012
Published: January 8, 2012

Despite the availability of characterization methods such as *in vivo* imaging,[5−7] plasma pharmacokinetics[8,9] and *in vitro* measures of aerodynamic performance,[5,10−12] the issues raised in reformulating corticosteroids over the past decade[13−16] highlight the challenges associated with assigning bioequivalent status for inhaled medicines. The correlation between particle size of the inhaled medicine, treatment efficiency and side-effect profile, which has been demonstrated for β_2 agonists,[17] is weak for inhaled corticosteroids.[13,16] Without clear links between drug deposition, tissue availability and systemic exposure, the data generated to define corticosteroid performance using traditional particle size-driven paradigms is of limited use in bioequivalence assessment. The indirect nature of test methodologies and their inability to define clearly the critical product performance characteristics that drive drug bioavailability is one reason why there is uncertainty and thus a reluctance to apply the regulatory dispensation associated with the bioequivalence definition to inhaled medicines. The recent PQRI review of the issues and techniques for evaluating inhaler bioequivalence[4] did not consider integrated *in vitro* experimental modeling (reviewed recently by Sadler and co-workers[18]).

As a product, pressurized metered dose inhalers (pMDI) containing solution formulations are very different from other inhaled dosage forms, including suspension pMDI, in that they only form solid particles at the point of administration. For example, QVAR generates a liquid aerosol plume upon actuation, and then, as the propellant evaporates, respirable solid beclomethasone dipropionate (BDP) particles are formed rapidly. As a consequence the physical and chemical properties of the delivery unit, i.e. the drug particle, are not measurable in the product *in situ*. Particle properties influence not only where particles deposit but also the dynamics of postdeposition clearance, including particle dissolution and drug absorption. However, the capture and characterization of the emitted particles has not been reported. QVAR and Sanasthmax BDP pMDI formulations differ in the amount of ethanol cosolvent present (8 and 10%, respectively), the presence of glycerol (0 and 1.3% respectively) and the device design, but whether these changes affect the release of drug from particles that deposit in the lung has not been reported.

A recently developed *in vitro* method for studying the interaction of particles with the mucosal surface of an epithelial cell layer[14,19] presents an opportunity to link the fundamental properties of inhaled particles to their biopharmaceutical performance. Therefore, the aim of this study was to use a common method to collect the particles generated from pMDI inhalers, then measure their physicochemical properties and absorptive drug profiles across respiratory cell layers. Two nonbioequivalent inhalers (QVAR and Sanasthmax) were selected to generate the test particles, which were also characterized by traditional impaction particle sizing assessment. Following collection, powder X-ray diffraction, dissolution assays and dissolution−absorptive transport profiling were performed. It was anticipated that linking particle properties to biopharmaceutical performance *in vitro* and comparing the outcome to published *in vivo* data would provide a better understanding of what underpins inhaled corticosteroid solution pMDI pharmacokinetics. This approach has the potential to elucidate the critical performance characteristics that are important for inhaled product bioequivalence.

METHODS

Aerodynamic Sizing. The Andersen apparatus (Copley, Nottingham, U.K.) was assembled and used as described in the British Pharmacopoeia (2004) with air flow of 28.3 L/min. A molded rubber adapter for pressured metered dose inhalers was attached to the throat section. Inhalers were primed by actuating to waste twice before being actuated the requisite number of times to deliver 0.5−2 mg into the cascade impactor. A 50:50 v/v acetonitrile (ACN):water mixture was used to wash BDP from each of the plates and the inside of the throat using 20 and 50 mL aliquots, respectively. These solutions were assayed for BDP by HPLC. The Andersen cascade impactor stage cutoff diameter vs the inverse of the normal cumulative distribution of (% undersize/100) was plotted and used to calculate mass median aerodynamic diameter (MMAD).

Scanning Electron Microscopy. Each BDP formulation was aerosolized (3 actuations) on separate occasions into a sealed spacer (Volumatic, GSK, U.K.) and the aerosol allowed to sediment onto a coverslip on a scanning electron microscopy (SEM) stub for 2 min. The stub and coverslip were then carefully removed and immediately gold coated using a Polaron E5100 sputter coater (Quorum Technologies, Sussex, U.K.). Particles were analyzed at 15 kV [Philips SEM501B scanning electron microscope (Philips Electron Optics, Cambridge, U.K.), fitted with a Deben Pixie 3000 digital scan generator (Deben UK Ltd., Suffolk, U.K.)]. Volume mean diameter $D_{[4,3]}$ was calculated from representative SEM images (several slides from 3 separate depositions) uploaded to Image Pro Plus software (version 4.1, Media Cybernetics). Particles were spherical in shape (Figure 1) and were analyzed individually using the Best Fit Circle function following triple-point selection on the particle perimeter.

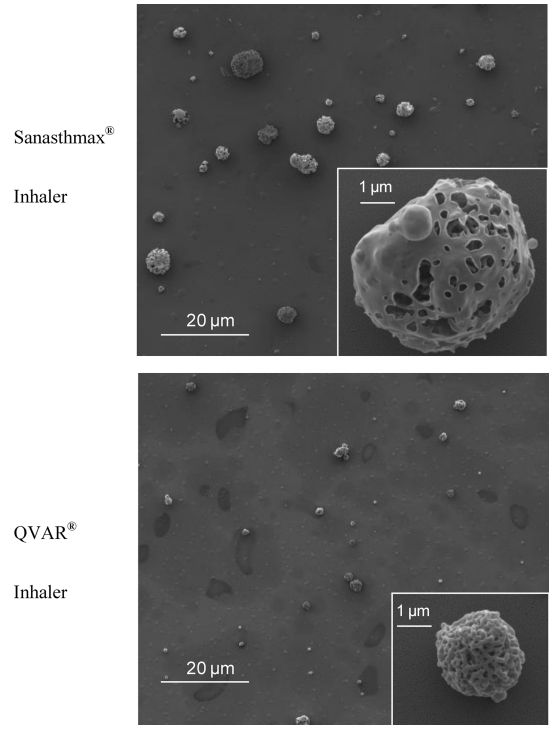

Figure 1. Scanning electron microscopy of particles from the QVAR and Sanasthmax inhalers at lower magnification (bar: 20 μm) to show the typical particle size distribution and at higher magnification (bar: 1 μm, inset) to show typical particle morphology.

Differential Scanning Calorimetry. The second stage of a Twin-Stage Impinger (TSI, Copley Instruments, U.K.) was lined with aluminum foil to allow the direct collection of particles in the solid state. QVAR or Sanasthmax was actuated 80 or 50 times, respectively, into the TSI (60 L/min) over 6 min. After disassembly the aluminum foil was removed, consolidated and analyzed by differential scanning calorimetry (DSC) using a TA 2920 (TA Instruments, U.K.). Spray trapped on aluminum foil (all the foil was used) was weighed accurately into 40 μL hermetically sealed aluminum pans with a 1 mm pinhole in the top. The samples were heated at 10 °C min^{-1} to 350 °C. Oxygen free nitrogen, used as the purge gas, was set at a flow rate of 110 mL min^{-1}. Thermogravimetric analysis (TGA) was performed at 10 °C min^{-1} up to 200 °C in open aluminum pans (TA Instruments, U.K.) using a TGA 2050 thermogravimetric analyzer (TA Instruments, U.K.).

Powder X-ray Diffraction. The dose collector on the second stage of a twin-stage impinger was lined with a Mylar polymer sheet to capture emitted particles. The inhalers were actuated into the TSI 160 times over 10−12 min, and the Mylar sheet was recovered. The sample was enclosed in a modified Mylar film powder holder to minimize moisture absorption during analysis. The powder X-ray diffraction (PXRD) data were obtained using a Bruker D8 Advance diffractometer using the following operating parameters; [λ(Cu Kα) = 1.5418 Å, voltage 40 kV, filament emission 40 mA] with 1 mm divergence slit, 1 mm receiving slit and 0.2 mm scatter slit. Samples were scanned immediately after collection under ambient conditions from 3 to 40° (2θ) using a 0.01° step width and a 1 s count time.

Dissolution. BDP particles emitted from each of the commercial pMDI products were collected in the second stage of a modified TSI (Copley Instruments, U.K.) on a 0.45 μm nitrocellulose membrane (Millipore, Bedford, MA, USA), previously soaked in the buffer, to capture the emitted drug. The BDP pMDI formulations deposited 1.2 ± 0.12 mg, and the membrane was transferred to a vertical diffusion Franz cell (diffusion area: 0.196 cm^2) with the BDP-loaded surface facing upward. The Franz cell receiver fluid, 0.1 w/v sodium dodecyl sulfate in 0.1 M phosphate buffer, pH 7.4, 37 °C, was continually agitated with a small magnetic stirrer. At fixed time intervals between 0 and 60 min, 300 μL of the dissolution medium was sampled and replaced with 300 μL of fresh medium. All samples were filtered through a 0.2 μm PTFE disposable filter (Sartorius, MI, IT) and analyzed in triplicate by HPLC.

Absorptive Profiling. The Calu-3 human bronchial epithelial cell line (passage 38−50) was grown on Transwell supports using air−interface culture as reported previously.[20] For measurement of BDP disposition it was necessary to modify the medium for use in these experiments to ensure adequate BDP solubilization while maintaining Calu-3 cell layer compatibility. HBSS supplemented with 1% v/v HEPES, 1% v/v FBS, 0.1% w/v vitamin E and 1% v/v ethanol (97% v/v) at pH 7.4 was suitable for this purpose, in which BDP solubility was 3 μg/mL. The modified medium (0.4 mL at pH 7.4) was added to each receiver well of a 24-well plate for 1 h before cell layers were used in experiments.

The deposition of BDP particles on the cell layers was performed using an adaptation of the TSI as reported previously[18,19] with an airflow of 60 L/min. Sanasthmax was actuated once (1 × 250 μg) and the QVAR actuated three times (3 × 100 μg) into the TSI. The cell layer-containing Transwell insert was removed, cleaned of any particles which had deposited on the outside of the Transwell and placed into a well containing fresh medium to measure the absorptive transfer of BDP. At each sample point, the cell layers were transferred to a new well containing fresh medium. The entire medium (300 μL) from the vacated well was transferred into a 2 mL HPLC vial, and then the well was rinsed for 5 s with 1 mL of ether to recover any BDP adhered to the plastic; this was added to the sample along with fluticasone propionate (FP) as an internal standard (50 μL, 0.1 μg/mL). At the end of the experiment, the cell layer was also collected for analysis.

Samples were processed for analysis by HPLC−mass spectrometry (MS) using a Waters Quattro triple quadrupole MS coupled with liquid chromatography (Agilent HP 1100 series). The samples were stored at −70 °C until the aqueous phase had frozen and the ether layer could be removed and evaporated to dryness. The analytes were reconstituted with 200 μL of a 70:30 v/v ACN:water mixture warmed to 50 °C. The initial mass of BDP deposited on the apical surface was calculated by mass balance at the end of the experiment following validation of recovery from the cell layer and receiver fluid (recovery = 88 ± 2.8%, n = 4). The samples were assayed by HPLC−mass spectrometry using a mobile phase consisting of 70:30 v/v ACN:water containing 0.1% formic acid using a flow rate of 0.2 mL/min. MS was operated in a positive electrospray ionization mode, with a 3 kV capillary, a 30 V cone and source block and desolvation temperatures of 100 and 450 °C, respectively. Parent ion channels of 521 and 501 m/z and daughter ion channels of 337 and 293 m/z were used for BDP and FP, respectively. Absorptive data were reported as total BDP (sum of BDP and metabolites including the active form 17-beclomethasone monoproprionate) as used in bioequivalence evaluations.[9]

Statistical Analysis. Statistical analysis of data was performed using SPSS version 16.0 with a minimal level of significance of 0.05. Nonparametric Kruskal−Wallis and Mann−Whitney tests were used to analyze the permeation data. Repeated measures of ANOVA (analysis of variance) were employed to analyze the data, and post hoc comparisons of the means of individual groups were performed using Tukey's honestly significant difference test.

RESULTS

Particle Size and Morphology. The particles emitted from the pMDIs were of a similar size to that previously reported in the literature;[8] QVAR particles gave an MMAD of 1.13 ± 1.98 μm and Sanasthmax 2.77 ± 1.89 μm (n = 4). The particles were spherical and appeared porous (Figure 1); their volume mean diameter (VMD $D_{[4,3]}$) was greater than their MMAD, 3.33 μm for QVAR and 4.99 μm for Sanasthmax (Table 1). In the case of spherical particles the MMAD−VMD

Table 1. A Summary of Key Product Characteristics for Emitted Particles and Their Expected Influence on Beclomethasone (BDP) Dissolution

	particles		
characteristic	QVAR	Sanasthmax	pred effect[a]
MMAD[b]	1.13 μm	2.77 μm	increase
VMD[c]	3.33 μm	4.99 μm	
solid state	some crystallinity	amorphous	decrease
glycerol	absent	present	unknown

[a]Predicted effect on BDP release from QVAR particles compared to Sanasthmax particles. [b]Mass median aerodynamic diameter. [c]Volume mean diameter.

dx.doi.org/10.1021/mp200415g | Mol. Pharmaceutics 2012, 9, 563−569

discrepancy is a consequence of differing particle densities, as exemplified by the design of porous particles.[21] The VMD increased by 3-fold compared to 2-fold for the particles emitted from QVAR compared to Sanasthmax, respectively, indicating that the former were comparatively less dense (direct measurement of density will be performed in future studies). The Sanasthmax formulation appeared to generate particles with a smoother surface compared to QVAR, and this may have been a consequence of the glycerol not evaporating with the propellant during particle formation (glycerol was not present in the QVAR formulation).

Solid State Properties. The DSC thermogram of BDP powder (Airflow Co, U.K.) displayed two endothermic peaks, one at approximately 100 °C which was due to volatile loss, confirmed by TGA (data not shown), and one at 212.5 ± 0.3 °C which corresponded to the melting peak (T_m) of the steroid (previously reported as 213 °C). The absence of an exothermic recrystallization peak or a glass transition suggests that this material was crystalline. The thermal profile of QVAR was similar to the BDP powder with 2 endothermic peaks in the DSC thermogram representing volatile loss (approximately 100 °C) and BDP T_m, 206.88 ± 0.87 °C (Figure 2). The significant

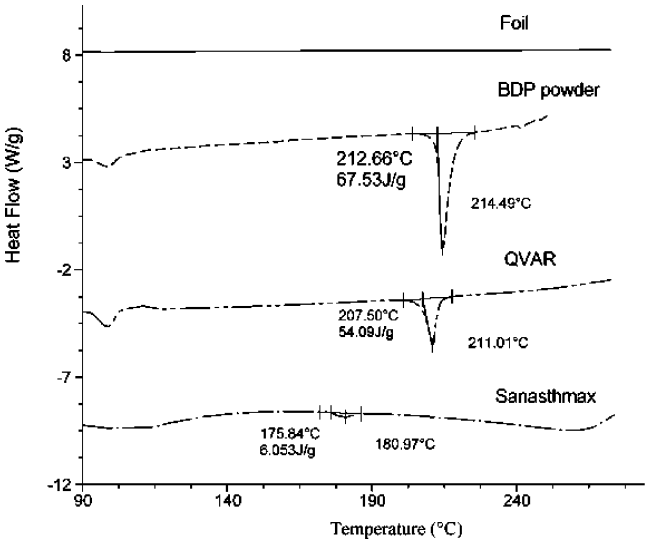

Figure 2. Differential scanning calorimetry thermograms for crystalline beclomethasone dipropionate (top), particles emitted from the QVAR inhaler (middle) and the Sanasthmax inhaler (bottom).

reduction ($p < 0.05$, ANOVA) in T_m may indicate partial crystallinity of the material. The Sanasthmax formulation did not show any evidence of crystalline BDP, and 80% of the material's weight was lost at ca.180 °C (TGA, data not shown), which suggests the small peak in the DSC trace was due to material decomposition.

The PXRD analysis of anhydrous BDP (crystalline reference) showed a typical diffraction pattern with strong peaks at ca.10, 12, 15 and 20 2θ values (Figure 3). The Mylar sheet which was used to collect the particles did not contribute peaks to the PXRD data and was therefore considered suitable for material collection. The material emitted from the QVAR inhaler appeared to show some degree of crystallinity, but the diffraction pattern contained a number of different peaks compared to the anhydrous BDP. Two of the strongest peaks in the QVAR material at ca. 2θ values of 7 and 14 were absent from the diffraction pattern recorded for the original crystalline material.

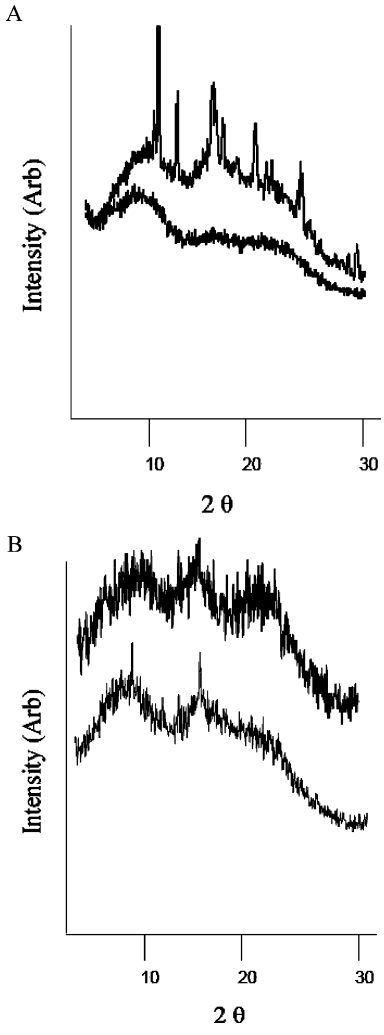

Figure 3. The diffractograms of (A) crystalline beclomethasone dipropionate (top) and the Mylar sheet alone (bottom) and (B) beclomethasone particles emitted from Sanasthmax inhaler (top) and QVAR inhaler (bottom).

The particles emitted from the Sanathsmax inhaler produced results with no peaks, suggesting that the particles were amorphous in nature.

Dissolution. After 60 min 73 ± 5% of deposited BDP had been released from the QVAR particles in comparison to 9 ± 4% of BDP from Sanathsmax (Figure 4); this corresponded to >10% of the maximum saturated solubility, and thus all tests were conducted under nonsink conditions, i.e. the drug dissolution was limited by its solubility in the medium. Linearization of the data using a first order rate model (log cumulative percent drug remaining vs time) showed that the material ejected from QVAR released the BDP much more rapidly (k = 0.23 min^{-1}) compared to Sanathsmax (k = 0.01 min^{-1}).

Absorptive Profile. Depositing the particles onto the surface of Calu-3 cell layers using the TSI produced no measurable adverse effects on transepithelial resistance of the cell layers, which remained unchanged from control: 320 ± 32 Ω cm^2, n = 3, $p > 0.05$. The amount of BDP deposited onto the cell layers from the QVAR and Sanasthmax inhalers was equivalent (range 11−145 ng; $p > 0.05$). The total amount of BDP transported across the epithelial cell layer as a function of the total dose deposited was significantly different between the two formulations (Figure 5A). The BDP from the QVAR

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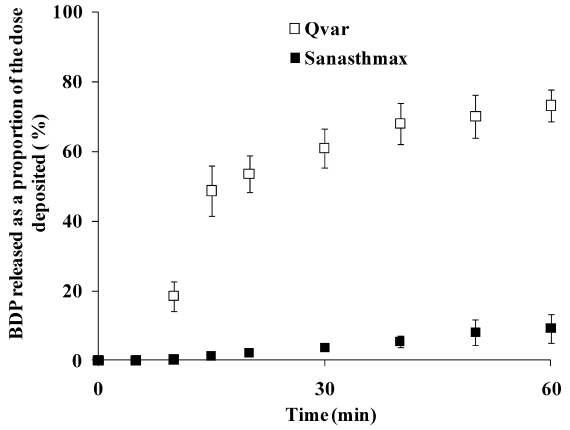

Figure 4. Dissolution of BDP particles obtained after aerosolization of solutions from QVAR and Sanasthmax pressurized metered dose inhalers. Particles were captured using the twin stage impinger (stage 2) and transferred to an adapted Franz dissolution apparatus. Data represent mean ± sd, n = 3.

inhaler transferred to the receiver chamber ~50% faster than that originating from the Sanasthmax inhaler. This profile is consistent with the pharmacokinetics of these inhaler products in human[8] and when absorptive transport was measured following administration to an *ex vivo* human lung preparation.[22] Data from these earlier studies have been adapted for presentation in a readily comparable form in Figure 5B.

DISCUSSION

Atomizing a highly evaporative drug solution into an air stream generates droplets which undergo a liquid to gas transition of the volatile components, leading to drug supersaturation and the formation of solid particles. Rather than the drug solution supplied in the product, it is the properties of the particles formed upon actuation that are relevant clinically. The aerodynamic performance of emitted particles has been well characterized,[10−12] but other properties that may affect efficacy (through local bioavailability) and safety (through systemic exposure) are largely unreported. To study the material emitted by solution

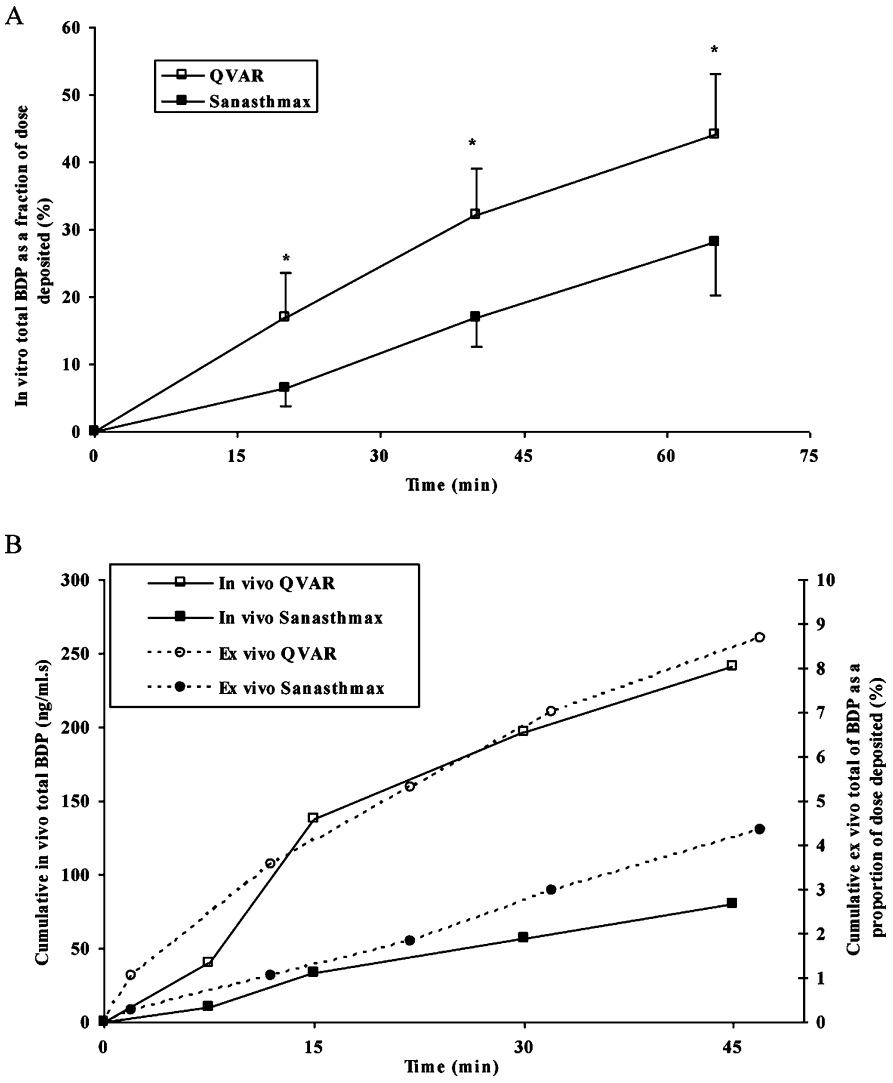

Figure 5. Delivery of BDP from QVAR and Sanasthmax pressurized metered dose inhalers. (A) Total BDP transfer across Calu-3 cell layers after deposition of particles. Data represent mean ± standard error of mean, n = 2−5 on 4−6 separate occasions. * = statistical difference between the transport of BDP from the inhalers (P < 0.05). (B) Total BDP absorption in a human trial after a 1000 μg inhaled dose (data from Woodcock et al., 2002; ref 8) and total BDP transfer from the *ex vivo* human lung after delivery of a 1000 μg aerosolized dose (data from Friewald et al., 2005; ref 22).

dx.doi.org/10.1021/mp200415g | *Mol. Pharmaceutics* 2012, 9, 563−569

pMDI actuation, it is essential that particle collection is carried out under conditions that mimic product use and that the subsequent analysis is rapid. We report the adaptation of a pharmacopeial impinger to capture particles, which were then assayed immediately.

The images of the particles confirm that both QVAR and Sanasthmax pMDIs generated solid particles when actuated into an air stream. The DSC and PXRD data indicated that crystalline material was generated from the QVAR formulation. In contrast, no crystalline material was detected from the Sanasthmax formulation by DSC (although glycerol may have interfered with this analysis) or by PXRD. Interestingly, comparison of PXRD data for QVAR and the anhydrous crystalline BDP powder reference indicated the presence of a mixture of molecular structures in the particles generated by aerosolization. BDP can form several crystal arrangements, including solvates and polymorphs, which can be detected by the appearance of additional peaks in X-ray traces.[23] More detailed investigation including single crystal structure resolution would be required to explore this further; this is not a trivial task and falls outside the scope of the current work.

For the drug delivered by these formulations to be available, the evaporation-driven liquid to solid transition that generates the particles must be reversed upon lung deposition by the process of dissolution. This process is influenced by both the interaction of the solid particles with the lung lining fluid and the rate of diffusion of the dissolved drug away from the particle surface. The dissolution data demonstrated clearly that the smaller, less dense QVAR particles dissolve more quickly than the Sanasthmax particles. This can be rationalized by considering the Noyes–Whitney determinants of the dissolution rate of solids in liquids. The more rapid dissolution of QVAR particles can be attributed to their greater surface area (based on surface area/mass ratio and porosity) for interaction with the solvent, which provides a powerful driving force. The dissolution rate modifying potential of the Sanasthmax particles is less clear: solvation may be promoted by the particles' amorphous nature, whereas the cosolvency or viscosity modifying effects of glycerol are unknown.

The absorptive transfer profiles of BDP following solid particle delivery to the surface of cultured respiratory epithelial cells mirrored the findings that QVAR delivers more BDP than Sanasthmax into the systemic circulation in healthy human subjects[8] and the perfusate of *ex vivo* human lungs.[24] This demonstrated the power of the integrated dissolution–absorption model to indicate the net effect of competing influences on drug disposition and illustrates the potential for the application of this technique to the evaluation of inhaler bioequivalence. The aggregate of evidence approach to evaluating bioequivalence utilizes *in vitro* aerodynamic assessments, pharmacokinetic studies and clinical studies,[24] but this is not always sufficient to understand the interplay of factors that influence inhaled drug therapy. For example, even carefully controlled studies have resulted in unexpected and poorly understood findings where the obtained pharmacokinetics contradicted the *in vitro* aerodynamic data and achieved clinical outcomes for drugs administered from dry powder inhalers.[25]

The cell-based dissolution–absorption model is designed to mimic the dissolution–absorption process that occurs *in vivo* and has the advantages of economy, sparing of human/animal testing, ease of manipulation, control of experimental conditions for mechanistic studies and the use of small amounts of material. In this study, absorptive drug transfer was evaluated in the absence of regional lung penetration factors, thereby allowing effects to be attributed to the form and physical chemistry of emitted particles. Other approaches have been to adapt aerosol fractionators to deposit discrete aerodynamic particle sizes (although this is not readily achieved) or to pre-engineer the particle fraction delivered according to the property to be evaluated.[18] This approach could be refined further to use different cell types to model different regions of the lungs to reflect regional deposition and possible differences in dissolution and transport, or by using primary cells to provide a more physiologically relevant model. It remains to be seen how robust and predictive such adaptations will be for discriminating differences between inhaled products.

A variety of respiratory cell culture systems are available as *in vitro* models for the study of particle–cell interaction.[26] The deposition and transfer of large hydrophilic solutes has been used to validate the use of respiratory epithelial cell layers that possess suitable barrier properties to evaluate particle deposition, dissolution and drug transfer.[18,27] Similar systems have been used to evaluate powders for inhalation,[28,29] but this is the first report to link absorptive profiles directly to delivery by inhaled drug products. Although no epithelial permeabilization was discerned in this study, the ability of the cell culture system to measure such effects may interest regulators as functional excipients are developed for inhaled formulations. The technique may also be useful for investigating the impact of different delivery forms, such as solution versus powder aerosols, on lipophilic drug disposition.[30,31]

In conclusion, solid particles were captured following actuation of the solution pMDI inhalers and significant differences in solid state properties were found despite relatively subtle differences in the product formulations. Differences between the emitted particles (i.e., size, porosity, crystallinity) were manifested functionally in different dissolution rates and absorptive profiles following particle deposition at a mucosal surface. This approach has potential for evaluating inhaler bioequivalence and formulation tolerance testing and may also provide insights that allow corticosteroid inhalers to be developed that produce an optimized therapeutic ratio (i.e., lung versus systemic drug exposure).

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ACKNOWLEDGMENTS

This work was in part funded by a grant from the Safety and Environmental Assurance Centre, Unilever Colworth, U.K. Particle sizing was performed by Steve Ingham, Institute of Pharmaceutical Science, King's College London.

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