Solubility of fluticasone propionate and beclomethasone dipropionate in simulated lung lining fluids

Wachirun Terakosolphan[1], Mireille Hassoun[1], Abhinav Kumar[1] & Ben Forbes[1]

[1] King's College London, Institute of Pharmaceutical Science, London, SE1 9NH, UK

Summary

There is burgeoning interest in the dissolution of inhaled medicines in the lungs and speculation that a biopharmaceutics classification system (BCS) concept, analogous to that currently used for oral drug dosage forms, may be extended to pulmonary drug products. This exposes the need for reliable and relevant methods for measuring the solubility in the lungs, especially for poorly soluble drugs for which dissolution is thought to be an important determinant of their pharmacokinetics and pharmacodynamics in the lungs. Inhaled corticosteroids (ICS) are a widely used class of anti-inflammatory agents for the treatment of respiratory disease which includes drugs with low aqueous solubility, i.e. fluticasone propionate (FP) and beclomethasone dipropionate (BDP). The solubility of such drugs has been reported in a variety of simulated lung lining fluids of different compositions. The purpose of this study was to compare drug solubility in solvents typically used to represent lung lining fluid. The solvents investigated in this study were based on a physiological salt solution (Gamble's solution), a licensed lung surfactant product (Survanta®) and a synthetic simulated lung lining fluid (sLLF) based on the measured composition of human lung lining fluid. Drug solubility was compared in five solutions in total: ultrapure water, Gamble's solution, sLLF, an aqueous dilution of Survanta, and Survanta itself. The solubility of FP in these solvents was 1.92, 1.99, 2.04, 3.89 and 20.28 µg/mL, respectively. BDP solubility in the same solvents was 2.17, 1.03, 16.79, 5.78, to 37.16 µg/mL, respectively. These data illustrate that simulated lung fluids that contain surfactant have enhanced potential to dissolve inhaled steroid drug particles that have deposited in the lungs.

Introduction

Inhaled corticosteroids (ICS) are mainstay therapy for several respiratory diseases such as asthma and chronic obstructive pulmonary disease (COPD) [1], and in recent guidelines were recommended for use for all patients except those with mild, intermittent symptoms of chronic asthma (GINA 2016) [2]. Fluticasone propionate (FP) and beclomethasone dipropionate (BDP) are widely used ICS for the treatment of asthma in children and adults. In terms of pharmacokinetics, dissolution of drug particles appears to be a rate-limiting step in the disposition of these drugs after they deposit in the lungs. Particles dissolution in the lungs depends predominately on the physicochemical properties of the active pharmaceutical ingredient [3,4]. Studies in pulmonary drug product research that have focused on dissolution have often studied ICS due to their poor water-soluble profiles which potentially affect the drugs' therapeutic efficacy [5].

In order to simulate the dissolution of drugs in airways, artificial lung fluids were used as solvents in this solubility study. Lung lining fluid is a complex mixture of glycoproteins, proteins, and lipids. It is produced and secreted by alveolar epithelial type-II cells. It consists of lipid-rich lipoproteins with the lipid composition dominated by phosphatidylcholine, with a high dipalmitoyl content. Apart from phospholipids, many proteins are also present. In addition to the most abundant protein, albumin, there are four non-serum apoproteins (SP-A, SP-B, SP-C and SP-D) [6]. The most widely used simulated lung lining fluids used in pharmaceutical research, namely Survanta®, Gamble's solution, and an in-house synthetic simulated lung lining fluid (sLLF) were the solvents utilised in this study (Table 1). Survanta is a marketed artificial lung fluid, which is a bovine lung extract containing similar substances to those found in normal human lung surfactant. It is indicated for the treatment of respiratory distress syndrome in newborn premature infants with deficiency of lung surfactant and acts by replenishing and restoring the surfactant and its activity [7]. In this study, Survanta was used as an undiluted solvent and as a 21.6% v/v diluted solvent with Hank's balanced salt solution (HBSS). The latter was used to match the total lipid concentration in human lung lining fluid. Another simulated lung fluid, which is designed more to mimic the interstitial fluid in the airways, is Gamble's solution which consists of a wide range of electrolytes [8]. Finally, sLLF is an in-house developed simulated epithelial lung lining fluid, composed of a number of lipids and proteins, and designed to reflect the human lung fluid composition [9].

This study aimed to investigate the solubility of FP and BDP in the respiratory biological milieu using simulated lung lining fluids compared to ultrapure water as control.

Materials

6α,9-Difluoro-17-[[(fluoromethyl)sulfanyl]carbonyl]-11β-hydroxy-16α-methyl-3-oxoandrosta-1,4-dien-17α-yl propanoate (fluticasone propionate: FP) was purchased from Adooq Bioscience (Irwin, CA), 9-Chloro-11β-hydroxy-16β-methyl-3,20-dioxopregna-1,4-diene-17,21-diyl dipropanoate (beclomethasone dipropionate; BDP) was purchased from Medchem Express (US), and Survanta® from AbbVie Ltd. (UK). Ultrapure water with 18.0 MΩ·cm residual specific resistance was obtained using an Elgastat Maxima purifier (Elga, UK). All other reagents were obtained from standard source.

Table 1. The composition of the *in vitro* lung lining fluid simulants

Survanta®	Gamble's solution	sLLF
- Phospholipids 25 mg/mL (including 11.0 – 15.5 mg/mL disaturated phosphatidylcholine) - Triglycerides 0.5 – 1.75 mg/mL - Free fatty acids 1.4 – 3.5 mg/mL - Protein less than 1.0 mg/mL	- Magnesium chloride 0.095 mg/mL - Sodium chloride 6.019 mg/mL - Potassium chloride 0.298 mg/mL - Disodium hydrogen phosphate 0.126 mg/mL - Sodium sulfate 0.063 mg/mL - Calcium chloride dehydrate 0.368 mg/mL - Sodium acetate 0.574 mg/mL - Sodium hydrogen carbonate 2.604 mg/mL - Sodium citrate dihydrate 0.097 mg/mL	- DPPC 4.8 mg/mL - DPPG 0.5 mg/mL - Cholesterol 0.1 mg/mL - Albumin 8.8 mg/mL - IgG 2.6 mg/mL - Transferrin 1.5 mg/mL - Ascorbate 140 μM - Urate 95 μM - Glutathione 170 μM

Experimental methods

Solubility measurement

The solubility of FP and BDP was investigated separately in ultrapure water, Gamble's solution, sLLF, diluted Survanta, and undiluted Survanta by placing excess drug powder (approximately 0.5 mg) in a microcentrifuge tube with 0.5 mL of the solvent. The tubes were closed firmly and agitated to assist mixing using a vortex mixer for 5 min before being placed in a bath sonicator at 37°C for 30 min. Thereafter, they were placed into a shaking water-bath at 37°C for 48 h to allow the solutes to dissolve completely and reach their equilibrium solubility.

The microcentrifuge tubes containing the drug suspensions in different solvents were centrifuged at 13000 rpm for 10 minutes to pellet any undissolved solute. After centrifugation, 0.2 mL of the supernatant was transferred to fresh tubes and re-centrifuged as described above. After the second centrifugation, 0.1 mL of the supernatant was transferred to fresh tubes and diluted 10 times with methanol, followed by further centrifugation to sediment any precipitates from the dilution. Eventually, 0.2 mL of the supernatant was transferred to HPLC vials for analysis of each drug in specific HPLC condition (table 2). All measurements were performed in triplicate. It was assumed that the addition of methanol had dissolved the micelles from either several surfactants or phospholipids in tested solvents, so that all the solutes were detectable.

HPLC assay

The saturated solution of FP and BDP were analysed using the following conditions:

Table 2. Chromatographic condition for HPLC analysis

Chromatographic condition	Fluticasone propionate (FP)	Beclomethasone dipropionate (BDP)
Column	Luna C18, 3 μm, 150 x 4.6 mm I.D. or equivalent	
Mobile phase	Methanol: 0.6% w/v aqueous ammonium acetate solution (75:25 %v/v)	Acetronitrile:Water (65:35 %v/v)
Flow rate	1.0 mL/min	1.5 mL/min
Column temperature	40°C	65°C
Sample temperature	15°C	25°C
Detection wavelength	UV 240 nm	UV 254 nm
Injection volume	60 μL	75 μL
Retention time	FP ~ 5.5 minutes	BDP ~3.2 minutes
Run time	7 minutes	6 minutes

Results and Discussion

Different solubility profiles were observed for FP and BDP in different solvents (Figure 1). As expected, when FP and BDP were added to ultrapure water and Gamble's solution they produced the lowest concentrations, approximately 1.0 – 2.2 µg/mL, since both corticosteroid drugs are poorly water-soluble. For the solubility of FP in the rest of simulated lung fluids, it gradually increased from 2.0, 3.9, to 20.3 µg/mL in sLLF, diluted Survanta, and undiluted Survanta®, respectively. On the other hand, BDP showed higher concentrations than those from FP results. Indeed, 16.8 µg, 5.8 µg, and 37.2 µg of BDP was solubilised in 1 mL of these three solvents, respectively.

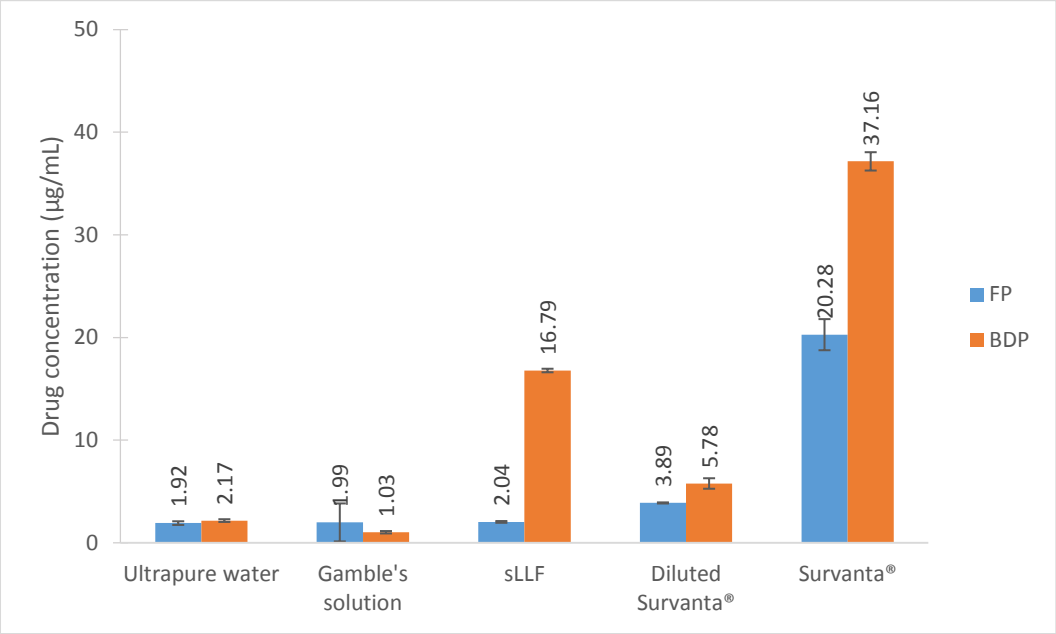

Figure 1. Solubility profile of fluticasone propionate (blue) and beclomethasone dipropionate (orange) in ultrapure water, Gamble's solution (a commonly used artificial lung lining fluid), synthetic human lung lining fluid, diluted Survanta (total lipid concentration matches the total lipid concentration in human lung lining fluid) and undiluted Survanta. Data expressed as mean ± SD (n=3).

The solubility of lipophilic drugs in respiratory tracts is proposed as a critical parameter for pulmonary drug product development under the proposed inhaled BCS concept [5]. The saturated solutions of FP and BDP, which were prepared by placing excess amount of the drugs in the promising solvents were quantified using HPLC analysis. FP and BDP have been reported to be highly water-insoluble drugs [10], with concentrations of approximately 0.1 – 0.2 µg/mL in water [11,12]. In our measurement, the concentration of FP and BDP in water are higher than those from earlier studies, 1.92 and 2.17 µg/mL, respectively. This may be attributed to sample preparation, where a longer time period was utilised (48 h) to allow for equilibrium of saturated solutions to be reached. Similarly, 1.99 µg of FP and 1.03 µg of BDP were dissolved in 1 mL of Gamble's solution, which is a simple electrolyte solution [8]. As anticipated these results were similar to the solubility profile of both drugs in water. Higher concentrations of BDP (more than 10 µg/mL) were shown in the three solvents; sLLF, diluted Survanta, and undiluted Survanta.

Several previous studies have revealed that lung surfactants can enhance the solubility of small, lipophilic drug molecules, such as gluco-corticosteroids and cationic compounds because of their composition and the ability to form liposomal structures which effectively entrap these particles within [13,14]. This explains the higher solubility in the 3 phospholipid-containing fluids. However, although the solubility of FP increased when placed into sLLF, diluted Survanta and undiluted Survanta, its concentration in those fluids was significantly lower than that of BDP. The concentrations of FP and BDP in sLLF and diluted Survanta fell between the lower value in water and the higher value in undiluted Survanta, which was attributed to the fact that the solvents had the same lipid concentration of phospholipid, 5.4 mg/mL. It was anticipated FP solubility in sLLF would be higher (closer to that of FP in Survanta rather than the observed solubility of 2.04 µg/mL, which is closer to the solubility values in water and Gamble's solution) because of the presence of the liposomal structures and specifically the presence of cholesterol, which can potentially form tight nanodomain complexes with DPPC, stabilising lamellar structures in the fluid [15]. However, the lower solubility value may be due to the presence of albumin, which studies have shown, has the ability to solubilise the cholesterol [16], and hence reduce the stability of the lamellar phase and reduce the extent to which FP particles are effectively entrapped and solubilised.

Conclusion

In this study, we investigated the solubility of drugs in simulated lung lining fluids which represent the biological milieu. Gamble's solution, which mimics the interstitial fluid deep within the lung, has a low solubilising power for FP and BDP. However, other lung surfactant simulant fluids, including sLLF, diluted Survanta, and undiluted Survanta, manifested up to 10-40x higher drug solubility, particularly for BDP. It can be summarised that poorly water-soluble drugs, including lipophilic molecules and some steroids, can be solubilised effectively in the lungs when those respirable drug particles are inhaled and deposited on a mucosal surface covered by a surfactant-containing liquid film.

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