Comparison of Albuterol Sulphate and Base Dry Powder Particulate Deposition Using the Calu-3 Lung Epithelial Model

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INTRODUCTION

To effectively predict the fate of formulated inhalation compounds delivered to the lung, a model of the airway epithelium should reflect drug permeability and transport characteristics *in vivo*. Most cell-based system established for this purpose, study drug transport using wet models and thus do not necessarily represent *in vivo* conditions. Recently, air-interface models have been established that increase the relevance of *in vitro* transport studies to the *in vivo* state (1, 2). The aim of our study was to elucidate the dissolution and diffusion process of deposited dry drug particulates (albuterol) after aerosolization onto the epithelial surface and compare these to conventional *in vitro* 'glass' models. Two forms of albuterol were investigated (albuterol base and albuterol sulphate), to evaluate the effects of lipophilicity and aqueous solubility on the mechanism of transport

METHODS

An immortalized sub-bronchial epithelial cell line (Calu-3) known to form tight intercellular barriers and have similar protein expression profile to *in vivo* cells (3) was seeded at $5x10^5$ cells/cm^2 in 0.33 cm^2 polyester Transwell inserts and cultured at the air-liquid interface to allow for differentiation and polarization of the cells (4). On day 12 the cells were used for drug deposition studies.

Albuterol sulphate and albuterol base particles were both micronized using an air jet mill (Trost Air Impact Pulveriser, Trost Equipment Corporation, USA) and their particle size measured by laser diffraction (Malvern Mastersizer 2000, Malvern Instruments Ltd., UK). The x-ray powder diffraction pattern for each micronized powder was analysed using a D5000 XRD (Siemens, Munich, Germany). Particle morphology was studied using a field-emission scanning

electron microscope (SEM) at 5 keV (Zeiss Ultra plus, Carl Zeiss Pty Ltd, Sydney, Australia) and dissolution studies were conducted using a modified Franz cell method, described previously (5). For the cell deposition studies, drug powder was loaded into size 3 hydroxy propyl methyl cellulose (HPMC) capsules, which were placed into an Aerolizer® connected to the mouthpiece of a Twin Stage Impinger (TSI). The set up for drug deposition in the TSI followed methods described by Grainger *et al.* (2), where the lower jet assembly of TSI was replaced by a Transwell insert allowing deposition of particles <6.4 μm directly onto the epithilia. The TSI was connected to a vacuum pump and solenoid, which was adjusted to an inlet airflow of 60 L/min. After particle deposition, the insert was removed from the TSI and placed into 600μL of Hanks buffered salt solution (HBSS) and the receiver chamber was sampled over 4 hours. At each time point, the basal receiver chamber media was replaced. At the end of the experiment, drug remaining on the epithelial surface and inside the cells was recovered by washing and cell lysis, respectively. Transepithelial electrical resistance (TEER) values of the cell monolayer measured before and after the experiment validated the integrity of the epithelium. Analysis of albuterol base or sulphate concentration from both the Franz cell and epithelia cell studies was conducted using a validated high performance liquid chromatography assay.

RESULTS AND DISCUSSION

X-ray powder diffraction suggested both forms of albuterol to be crystalline in nature. This was further confirmed by SEM images, where the particles were columnar/angular in nature.

Dissolution/diffusion studies conducted using the modified Franz cell indicated that the albuterol base and sulphate had different profiles when studying equivalent masses (ca. 2 mg) on the diffusion membrane. For example, albuterol sulphate particles were rapidly wetted and 100% of drug dissolved and was transferred into the reservoir within 20 minutes. In comparison, albuterol base took 240 minutes for 100% to be transferred to the reservoir compartment of the Franz cell (Figure 1). Such observations may be expected since the sulphate form has a greater aqueous solubility when compared to the base (~250 mg/ml compared to 15 mg/ml).

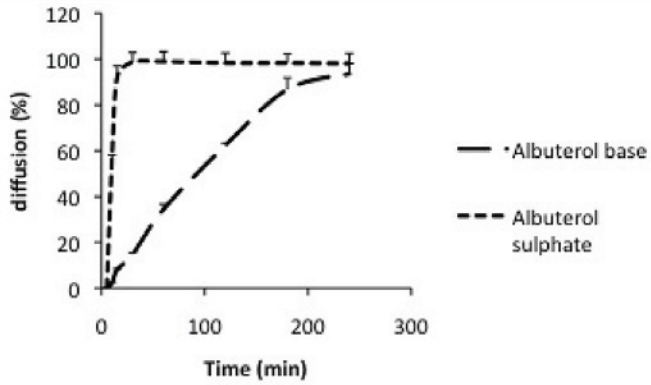

Figure 1. Albuterol base and sulphate release data from the diffusion membrane of the Franz cell over 4 hours (n=3).

Drug deposition studies on the epithelial cell lines suggested a very different behavior to that observed in the Franz cells studies. Various masses of drug (between 30 and 330 μg of each drug) were deposited on the cell surfaces and, in general, the percent of drug transferred to the basal compartment (i.e., trans or paracellular diffusion and/or transport) was calculated over a 240 min period. For albuterol base, 100 % of drug had transferred to the basal compartment after 240 mins while for the albuterol sulphate this value was around 60% (Figure 2).

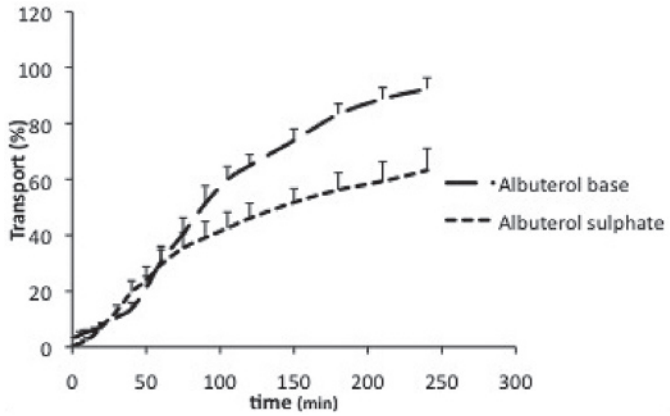

Figure 2. Albuterol base and sulphate transport data through calu-3 cell monolayer over 4 hours (n=9).

In comparison to the Franz cell measurements, albuterol base diffusion/transport was quicker than the sulphate. Recent studies have suggested that albuterol is actively transported via organic cation transporters (6). Since a localized saturated drug concentration is likely to be encountered at the point of dry drug particle deposition, the rate of transfer will be limited to the maximum pumping capacity of the influx transporter at the cell surface. Furthermore, under such a hypothesis, the difference between flux of base and sulphate drug across the cell line is likely to be due to the relative lipophilicity of each molecule; where the free base is likely to also have a passive diffusion component.

CONCLUSION

The use of simple dissolution/diffusion *in vitro* and cell-based methods to determine the fate of drugs after deposition in the lung has been evaluated using albuterol sulphate and base. While conventional 'glass methodologies,' such as the Franz cell, may be applied to study inhalation systems, this study has suggested cell models may be more representative of the *in vivo* condition.

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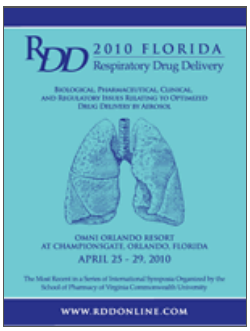

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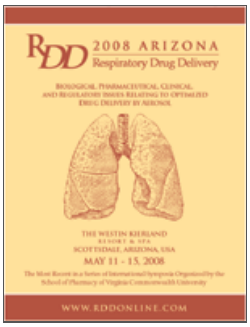

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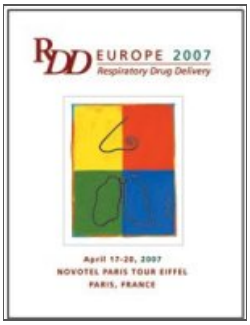

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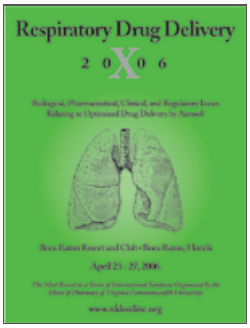

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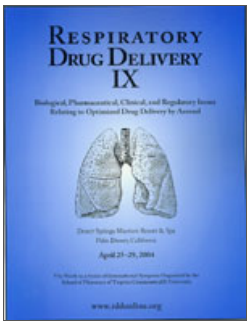

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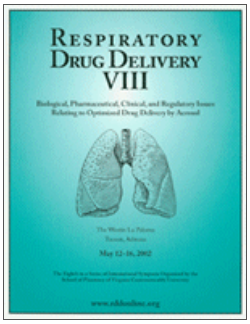

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