**Are respiratory cell lines proving useful in pharmaceutical development?**

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Summary

The respiratory tract provides a gateway to the body for both the intentional and inadvertent delivery of drugs and other substances. Intentional drug delivery includes the delivery of drugs intended for their local effect in, for instance, the treatment of asthma and COPD. Unintentional delivery can include the inhalation of consumer products such as perfumes, household and pharmaceutical sprays.

For drug delivery to the lungs, once deposition has occurred, the efficacy of the drug will depend on a number of factors. In the upper airway the deposited particles must avoid removal by mucociliary clearance, solid particles must (usually) dissolve in the fluid lining the epithelium and drug molecules must (usually) be able to cross the barriers provided by mucus and the epithelium. When considering the respiratory region of the lung (alveoli), particles must survive clearance by alveolar macrophages, be able to cross the barrier provided by the alveolar surfactant and also cross the epithelial cells. Cell-based models are valuable tools in studying how these barriers affect drug efficacy and the recent developments in such models are reviewed with a focus on the use of three dimensional (3D) models to study the effect of mucus on drug absorption.

We have further developed and validated a 3D model of SPOC1 rat tracheal cells as a potential secretagogue-free model to predict the effect of mucus hyper-secretion on drug absorption in the lung.

Introduction

The respiratory tract provides a gateway to the body for both the intentional and inadvertent delivery of drugs and other substances. Intentional drug delivery includes the delivery of drugs intended for their local effect in, for instance, the treatment of asthma and COPD. Unintentional delivery can include the inhalation of consumer products such as perfumes, household and pharmaceutical sprays.

For drug delivery to the lungs, many studies focus on the efficient generation of an aerosol and its deposition in the lung. However, once deposition has occurred, the efficacy of the drug will depend on a number of factors. In the upper airway the deposited particles must avoid removal by mucociliary clearance, solid particles must (usually) dissolve in the fluid lining the epithelium and drug molecules must (usually) be able to cross the barriers provided by mucus and the epithelium. When considering the respiratory region of the lung (alveoli), particles must survive clearance by alveolar macrophages, be able to cross the barrier provided by the alveolar surfactant and also cross the epithelial cells. *In vitro* models are valuable tools in studying how these barriers affect drug efficacy.

In addition to drug delivery, cell-based models are an important alternative to animal experiments in toxicity testing. In particular, since animal testing for cosmetics and chemicals has been prohibited under the 7th Amendment to the Cosmetics Directive (Council Directive 76/768/EEC) and REACH (registration, evaluation, authorization and restriction of chemicals) in the European Union (EU) from March 2013[1].

There are different types of cell-based models. The simplest involves a monolayer of cells cultured on rigid plastic supports and are termed two-dimensional (2D). In their simplest form, these are based on a single cell type which can be (i) primary cells (taken from an animal or human e.g. normal human bronchial epithelial cells (NHBE) and alveolar macrophages), sustainable for a limited amount of time, or (ii) an immortalised cell line (derived from an animal or human e.g. 16HBE14o-, Calu-3, A549, RAW 264.7 cells. These are widely used in screening for new molecular entities (NMEs) but, while useful for rapid screening, have certain limitations; they often lack the required phenotype of the cells they are representing and are unable to replicate the complexity of tissues and organs. The cross-talk between different cell-types in tissues is being recognised as increasingly important in drug action.

This has led to the development of what have been more recently termed, three-dimensional (3D) cell-based models. This refers to cells cultured in the presence of an extracellular matrix, usually on permeable supports. With respect to the lung, examples include NHBE, Calu-3 and 16HBE14o- cells cultured on permeable supports at air-liquid interface (ALI), to enhance differentiation of the cells to better reflect the characteristics of the native epithelium. For drug permeability studies, the primary requirement is the formation of tight junctions. Further to this, physiologically-controlled mucin secretion by cells of the upper airways might be considered desirable.

The 3D model can be extended to include different cell types (co-cultures). Such an approach is usually employed in the lung to better understand the interaction between, for instance, immune and epithelial cells, either alone or in

contact with particles. An example of a 3D co-culture system comprised of 4 cell types is that of Klein et al (2013)[2]; in which alveolar epithelial cells (A549) were cultured on the apical surface of a Transwell membrane and endothelial cells were cultured in the basolateral chamber. After 3 days, macrophages and mast cells were seeded on top of the A549 cells and the cells cultured at ALI. Further development of such models involves the introduction of 'lung on a chip' models to represent the dynamic features of the lung such as the microcirculation[3] and breathing[4]. One of the limitations of these models is that few are validated against in vivo responses.

We are aiming to develop the SPOC1 rat tracheal epithelial cell line as a 3D *in vitro* cell culture system capable of accurately modelling the effect of mucus on drug permeability. Such a model could be used to predict the effect of mucus hyper-secretion on drug absorption in the lung and also monitor the effect of drug administration on the secretion of mucus. Previously, the SPOC1 cell line has been shown to secrete the airway mucins MUC5AC and MUC5B in response to the physiological secretagogue, ATP. This is in contrast to the Calu-3 cell line which has been shown, both in our laboratory and by Kreda et al[5], not to respond to ATP. Since it is important that a cell-based system reflects the native epithelium as closely as possible in regard to the function being studied, the SPOC1 cell line was chosen as one potential model to characterise and develop [6, 7]. We have already shown the potential of SPOC1 for its use in permeability experiments in the presence of mucus [8]. This work explores the model further, most notably without the use of a secretagogue to enhance mucin secretion.

Experimental Methods

For permeability experiments, SPOC1 cells were cultured at air-liquid interface (ALI) on Geltrex™-coated inserts (Transwell®). Trans-epithelial electrical resistance (TER) was measured before and after each experiment. Prior to adding the drug of interest (testosterone (2 μm)) formulated with FITC-dextran (FD4) (250 μM) to the apical chamber, the cells were either washed at periodic intervals with basal medium to remove accumulated mucus, or left with the mucus layer intact. Samples were removed from the basolateral chamber at 15 minute intervals for up to 75 minutes and analysed using fluorescence spectrophotometry for FD4 and LC-MS for testosterone content. The apparent permeability coefficient ($P_{app}$) of each compound was calculated. At the beginning and end of the permeability experiment the medium was collected from the apical chamber and analysed for its mucin content using an Enzyme-linked Lectin Assay (ELLA).

Results and Discussion

TER measurements indicated that the experimental conditions did not compromise the integrity of the cell layer (pre-experimental TER was an average 204 $\Omega.cm^2$ (± 15.77, n=8), falling to a post-experimental average of 190 $\Omega.cm^2$ (± 13.1, n=8), a fall of 6 %). In addition, the results of the ELLA established that mucin was present in unwashed cultures at approximately twenty times the level of washed cultures (previously the use of ATP (100 μM) as a secretagogue enhanced mucin secretion to three times basal levels [3]) (Figure 1). The presence of more mucus did not affect the $P_{app}$ of the marker FD4. However, higher mucus levels significantly decreased the $P_{app}$ of testosterone from 16.96 x $10^{-6}$ $cm^{-1}$ $s^{-1}$ to 9.85 x $10^{-6}$ $cm^{-1}$ $s^{-1}$ (unpaired t-test, $P < 0.05$) (Figure 2). While testosterone is not a drug delivered via the lungs, mucus is well-recognised to present a barrier to the diffusion of testosterone (as reviewed by Khanvilkar et al. [9]). Therefore, this result validates the use of the model to study the effect of mucus on the absorption of drugs that are delivered via the airways.

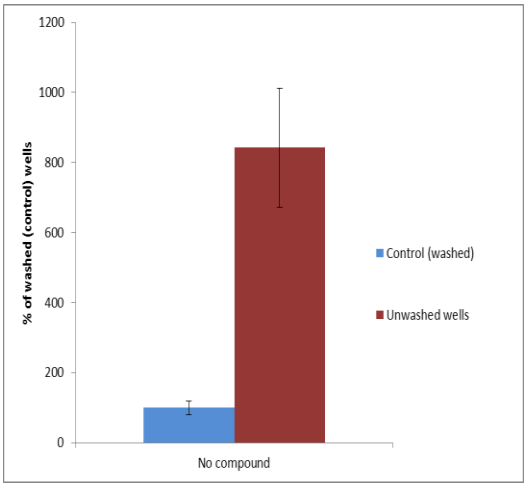


Figure 1 Mucin content of unwashed wells expressed as a percentage of washed wells. Mean ±SD; n=4

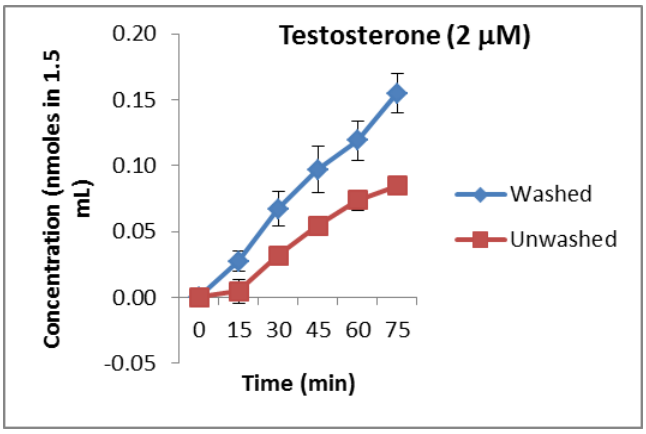


Figure 2 Effect of a higher mucin content on drug transport across SPOC1 cells. Mean ±SD; n=4

Conclusions

We have further developed SPOC1 rat tracheal cells grown at ALI as a potential secretagogue-free model to predict the effect of mucus hyper-secretion on drug absorption in the lung. Furthermore, this model may be used to assess the effect of inhaled drugs on mucus secretion and thus provide a vital tool in the drug development process.

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