

## Effect of Mucolytic Therapy on Drug Delivery through Cystic Fibrosis Sputum

J. Shur<sup>1</sup>, R.Price<sup>1</sup>, J. Smith<sup>2</sup> and J. Shute<sup>2</sup>

<sup>1</sup>Pharmaceutical Surface Science Research Group, Department of Pharmacy and Pharmacology, University of Bath, Bath, BA2 7AY, UK.

<sup>2</sup>University of Portsmouth, School of Pharmacy and Biomedical Sciences, White Swan Rd, Portsmouth, PO1 2DT, UK

### Summary

Cystic fibrosis (CF) is a lethal hereditary disorder characterised by the production of viscous mucus, which poses a barrier to the effective delivery of drugs to the CF lung. We have investigated the affect of mucoactive therapy on improving the transport of FITC-dexamethasone through whole CF sputum using a barrier function assay. Confocal laser scanning microscopy (CLSM) has been used to visualise the ultrastructure of whole CF sputum and the effect of mucoactiv agents on this structure. Results suggest that UFH is able to improve transport of FITC-dexamethasone through whole CF sputum. CLSM studies of CF sputum show that UFH targets the DNA present at high concentrations in the samples. Hence, UFH possesses mucoactive properties, which may help improve drug delivery in CF.

### Introduction

Cystic fibrosis (CF) predominately affects Caucasian populations and is the most common lethal autosomal recessive hereditary disorder of the Western world (1). The disease is caused by mutations in a single gene that encodes the CF transmembrane conductance regulator (CFTR). CF is a multiorgan disease affecting the lungs, pancreas, sweat glands and gastrointestinal tract. However, 95% of morbidity and mortality is due to lung disease (2).

CF mucus is more viscous than normal airway secretions, due to the high content of DNA (3) and actin, which are products of inflammatory cell necrosis, which together with mucins form an interconnected tangled network that is held together by electrostatic, hydrogen and van der Waals forces (4). CF mucus affects the deposition pattern of aerosols on the epithelial surface of the upper respiratory tract (5) and poses a barrier to the effective diffusion of drugs in the CF lung. To improve drug delivery in CF it is vital to reduce or remove this barrier. DNA is the main component responsible for the barrier property of CF mucus. Previous work using the atomic force microscope demonstrated that mucoactive agents such as DNase, unfractionated heparin (UFH) are able to increase the pore size of synthetic DNA networks, which corresponded with its ability to improve drug transport across DNA barriers. We have investigated the ability of UFH to improve drug transport across whole CF sputum, using a novel barrier function assay. In addition, we have visualised the ultrastructure of whole CF sputum using confocal laser scanning microscopy (CLSM) and used this approach to study the effects of a range mucoactive on the structure of CF sputum.

### Materials and Method

#### *Collection of sputum samples*

Sputum samples were collected from adult patients with CF attending Southampton General Hospital and were not receiving DNase therapy. Sputum was collected by direct expectoration into a sterile container and preserved at – 70 °C until required for further use.

#### *Investigation of the ultrastructure of whole CF sputum and the effect of UFH on the structure of CF sputum.*

Sputum samples were cut into 100 mg pieces and were incubated for 2 h at 37°C with PBS and UFH in the concentration range 0.1, 1, 10 mg/ml. Treated sputum samples (100 mg) were stained simultaneously with 10 µl of YOYO-1 (Molecular Probes, Netherlands), 20 µl of rhodamine phalloidin and 20 µl of TRITC-phalloidin (Molecular Probes, Netherlands) to stain for DNA, actin and mucin respectively. Samples were incubated for 20 mins at room temperature. Following which, 8 – 10 µl of sample was teased from the sputum, placed on a glass slide and covered with a coverslip and visualised by CLSM within 2 h of preparation. A Carl Zeiss laser scanning system LSM 510 (Carl Zeiss, Jena, Germany) were used to collect images of stained sputum. Dual excitation of 488 nm and 543 nm were employed to visualise the DNA in combination with actin and mucin. Images were recorded in the planar matrix (X, Y) using the 40X oil objective.

*Investigation of the effect of mucoactive therapies on the transport of FITC-dexamethasone through whole CF sputum using a novel barrier function assay.*

The microBoyden chamber was used in the barrier function assay (Figure 1). CF sputum was incubated with phosphate buffer saline (PBS), DNase (2.9 µg/ml) and UFH (0.1 – 10 mg/ml) for 2 h at 37°C and then 20 µl were added to the upper well, while the lower wells contained 50 µl PBS buffer. The upper and lower wells were separated by an 8 µm pore sized polycarbonate filter. 10 µl of FITC-dexamethasone (10<sup>-4</sup>M, Molecular Probes, Netherlands) was layered over treated CF sputum in the upper wells and incubated for 2 h at 37°C. Following this, the chamber was dismantled and the contents of the lower wells were transferred into a 96-well plate and analysed using a fluorescence plate reader (excitation wavelength 455 nm; emission wavelength 543 nm).

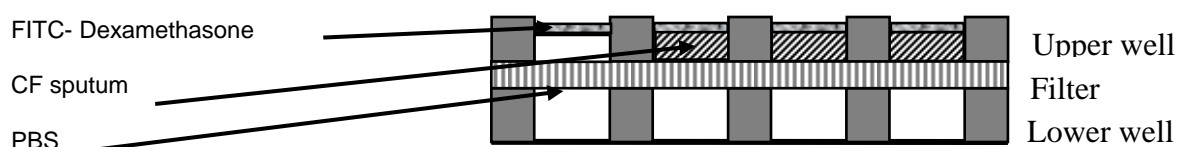


Figure 1. The barrier assay.

## Results and Discussion

### *The effect of UFH on the ultrastructure of whole CF sputum.*

The CLSM was used to visualise polymeric components of sputum from CF patients who were not on DNase therapy, as shown by Figure 2. Specimens were dual labelled using YOYO-1 for DNA and TRITC-phalloidin for mucin. The presence of a yellow colour suggested co-localisation. Figure 2A shows the presence of cell associated DNA, and mucin fibres 100s of microns in length, which form a rigid entangled network.

CF sputum samples treated with UFH 10 mg/ml have no thick DNA fibres present and have negligible DNA co-localisation with mucin. An image analysis software programme was prepared using Visual Basic to quantify the percentage area of red, green and yellow staining representing mucin, DNA and co-localisation, respectively. Five fields of each CLSM image of CF sputum treated with PBS and UFH in the concentration range of 0.1 – 10 mg/ml, which is shown by Figure 3. Samples treated with UFH (0.1 – 10 mg/ml) have significantly ( $p < 0.05$ ) reduced areas of green and yellow staining but significantly ( $p < 0.05$ ) increased areas of red staining compared to sputum samples treated with PBS. This is confirmed by Figure 2A and 2B, which show that samples treated with PBS have reduced presence of green DNA staining. Furthermore, increasing concentrations of UFH was observed to reduce the size of the mucin fibres. Hence, treatment of CF sputum with UFH has produced a physical change to the structural components of CF sputum.

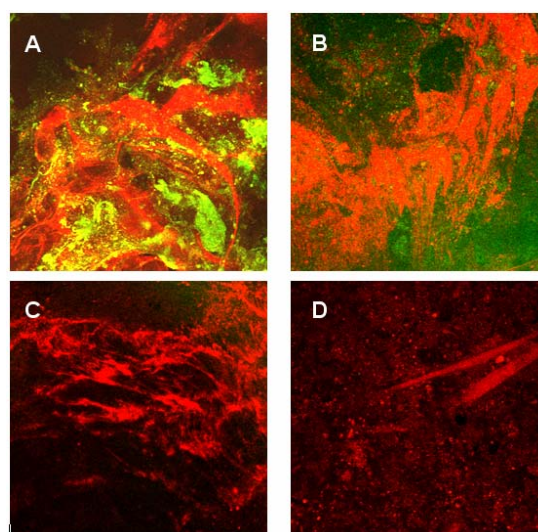


Figure 2. CLSM images of CF sputum treated with PBS (A) and UFH 10 mg/ml (B) stained with YOYO-1 and rhodamine phalloidin to stain for DNA (green) and mucin (red), respectively. The lower CLSM images are composite images of the upper two images, where the yellow colour indicates co-localisation of DNA and mucin.

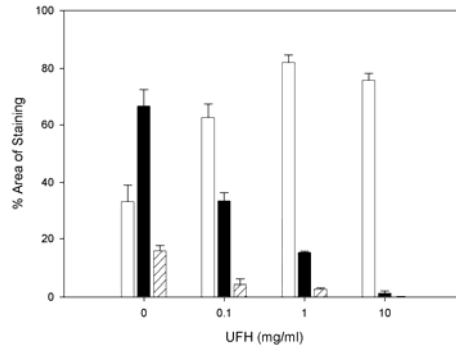


Figure 3. Percentage area of staining attributed by mucin (clear bar), DNA (black bar) and co-localisation (stripped bar) within CLSM images pre- and post-treatment with PBS and UFH (0.1 – 10 mg/ml). \* indicates statistical significance  $p < 0.05$ .

*The effect of oligosaccharides on transport of FITC-dexamethasone through DNA barriers.*

Figure 4 shows that the transport of FITC-dexamethasone through CF sputum is significantly reduced compared to 100 % diffusion through PBS control. However, after CF sputum has been treated with UFH (0.1 – 10 mg/ml) there is a significant ( $p < 0.05$ ) increase in the transport of FITC-dexamethasone through the sputum (Figure 2). All concentrations of UFH significantly ( $p < 0.05$ ) increased transport of FITC-dexamethasone through CF sputum.

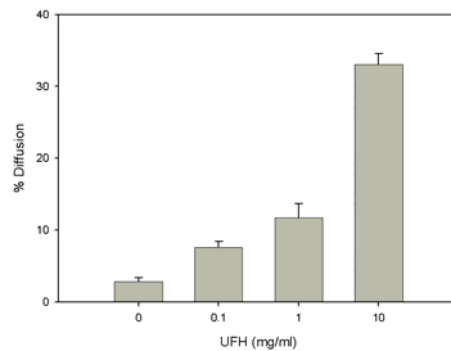


Figure 4. Effect of UFH on the diffusion of FITC-dexamethasone through whole CF mucus ( $n = 5$ ). \* indicates statistical significance  $p < 0.05$ .

**Conclusion**

Our findings using CLSM suggest that UFH changes the ultrastructure of CF sputum by acting on the DNA present in the samples. The structural changes in sputum samples treated with UFH may explain the results obtained from our barrier function assay, in which the transport of FITC-dexamethasone increased through CF sputum samples treated with UFH. These results show that UFH does possess mucoactive properties and therefore shows potential as a therapy for CF patients.

**References**

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