Fluticasone particles bind to motile respiratory cilia: a mechanism for enhanced lung and systemic exposure?

Short title/running head: Fluticasone particles bind to motile respiratory cilia

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Abbreviation List

ALI = air-liquid interface;

BEBM = bronchial epithelial basal cell medium;

BEGM = bronchial epithelial cell growth medium;

CBF = ciliary beat frequency;

FP = fluticasone propionate;

HPLC = high-performance liquid chromatography;

ICS = inhaled corticosteroid;

pMDI = pressurised metered dose inhaler;

NGI = next generation impactor.

Abstract

Background. Inhaled corticosteroids are the main prophylactic treatment for asthma and diseases including COPD yet the interaction of inhaled corticosteroid particles with the ciliated epithelium remains unclear.

Research question. To investigate the earliest interaction of aerosolised fluticasone propionate particles with human ciliated respiratory epithelium.

Methods. A bespoke system was developed to allow aerosolised fluticasone propionate particles to be delivered to ciliated epithelia cultures by nebulisation and from a pMDI via a spacer with interactions observed in real time using high-speed video microscopy. Interaction with non-respiratory cilia was investigated using steroids on brain ependymal ciliary cultures. The dissolution rate of steroid particles was determined.

Results. Fluticasone propionate particles delivered by aerosol attached to the tips of rapidly beating cilia. Within 2 hours, 8.7±1.8% (nebulisation) and 12.1±2.1% (pMDI via spacer) of ciliated cells had one or more particles attached to motile cilia. These levels decreased to 5.8±1.6% ($p=0.59$; nebulisation) and 5.3±2.2% ($p=0.14$; pMDI via spacer) at 24 hours. Particle attachment did not affect ciliary beat frequency ($p>0.05$) but significantly ($p<0.001$) reduced ciliary beat amplitude. Steroid particles also attached to the tips of motile ependymal brain cilia and also reduced beat amplitude (24 hours: >2 particles bound $p<0.001$). Dissolution of fluticasone propionate particles was slow with only 22.8 ± 1.3% of nebulised and 12.8 ± 0.5% of pMDI delivered drug dissolving by 24 h.

Conclusions. Fluticasone propionate particles adhere to the tips of rapidly moving cilia with significant numbers remaining bound at 24 hours, resisting the shear stress generated by ciliary beating. *In vivo*, this mechanism may predispose to high local drug concentrations and enhance respiratory and systemic corticosteroid exposure.

Key words

Inhaled corticosteroid, respiratory cilia, particle binding, nebulisation, pMDI.

Introduction

Inhaled corticosteroids (ICS) are the mainstay prophylactic treatment for asthma and are also widely used in other respiratory diseases, including chronic pulmonary obstructive disease (COPD). Corticosteroids enter cells through the glucocorticoid NR3C1receptor , supressing inflammatory mediators via transcriptional changes in the cell nucleus [1]. Whilst suppression of inflammation is associated with clinical benefit, inhaled corticosteroid use has been linked to adrenal suppression [2]. There is also debate as to whether inhaled corticosteroids increase the risk of pneumonia [3-6] and apoptosis of cells lining the respiratory epithelium [4, 7-10].

While much is known about the delivery and action of inhaled corticosteroids, the initial interaction of inhaled corticosteroid particles with the ciliated epithelium has not been investigated.

The aim of this study was to provide insight into the earliest interactions of aerosolised steroid particles with human ciliated respiratory epithelium. To do this we developed a bespoke system that delivered aerosolised drugs to ciliated primary human epithelial cultures under direct observation using high-speed video microscopy. We demonstrated for the first time that particles of fluticasone propionate bound to the tips of rapidly moving cilia, with many still bound 24 hours later. Binding to cilia was independent of the aerosol drug delivery system used and did not rely on overlying mucus as similar binding was seen to the tips of motile brain ependymal cilia.

Methods

Primary cell cultures

Human respiratory epithelial samples were obtained from three healthy individuals and one chronic obstructive pulmonary disease (COPD) patient [11] (Supplementary table 1). Ethical approval was obtained through the Living Airway Biobank (REC reference 14/NW/0128) and UCL Research Ethics (reference 4735/001).

Epithelial brushings were grown to a ciliated epithelium at an air-liquid interface (ALI) as previously described [11, 12]. Experiments were carried out on fully differentiated ciliated cultures 35 days after exposure at ALI on the day of rinsing.

Rat brain ependymal cells were cultured as previously described [13]. After 7-14 days, a ciliary phenotype was observed by using high-speed video microscopy. Experiments were carried out in artificial cerebrospinal fluid (aCSF; 10mM HEPES, 140mM NaCl, 4mM KCl, 2mM $MgCl_2$, 2mM $CaCl_2$,, 10mM D-glucose, pH 7.35).

Aerosol delivery to ciliated cells

Two different aerosol delivery methods were used to generate aerosol particles of fluticasone propionate: a) fluticasone propionate (2mg/2ml Flixotide, GSK, UK) nebulised via a jet nebuliser, AeroEclipse XL (Trudell Medical International, UK); b) fluticasone propionate (250μg/actuation; Evohaler® GSK, UK) administered from a pressurised metered dose inhaler (pMDI) via an antistatic spacer device (InspiraChamber®; InspiRx, US). The valve of the holding chamber was removed to allow drug to settle directly on to cultures.

A system was developed that allowed nebulisation directly onto the surface of respiratory ciliated cell cultures at ALI (Supplementary Figure 1.A). This allowed the impaction of fluticasone particles on the surface of cells and their interaction with cilia to be observed in real-time and recorded using high-speed video microscopy (Motion Pro 4x camera (IDT, US);

Orca flask 4.0 camera (Hamamatsu, Japan); Nikon Ti Eclipse microscope (Nikon, Japan). Aerosol generated from the nebuliser passed via an aluminium tubing to the cultures to negate effects of static charge. A series of vents were placed in the aluminium tubing in close proximity to the ALI culture surface to reduce the pressure on the surface of the cultures from the aerosol-laden air generated by jet nebulisation. To more closely mimic physiological conditions the system was enclosed in inner and outer chambers maintained at 37°C. Fluticasone propionate was nebulised for 30 seconds and aerosol allowed to settle on the cultures for a further minute.

The second delivery method consisted of a pMDI attached to an antistatic spacer~~,~~ that had the valve removed to allow drug to settle on the cultures (Supplementary Figure 1.B). The mouthpiece of the spacer was positioned vertically, immediately above the ciliated cell cultures. The pMDI was primed by firing a shot to waste, then was shaken and immediately actuated into the spacer with the mouthpiece of the spacer sealed with parafilm to prevent the direct force of the high velocity on the culture. The parafilm over the mouthpiece was removed allowing aerosol to settle onto the culture. To encourage deposition of fluticasone propionate the pMDI was removed from the spacer immediately after actuation and replaced with a 50 ml syringe. The syringe was discharged slowly creating a flow of approximately 60 ml/min gently pushing air-containing aerosol from the spacer onto the culture below. The spacer was left *in situ* for 1 minute and the procedure repeated.

Cultures were observed using the high-speed video microscopy and videos recorded to determine the number of ciliated cells, the attachment of fluticasone particles bound to cilia, ciliary beat frequency (CBF) and ciliary beat amplitude. CBF was determined as previously described [11, 13].

Ciliary beat amplitude was determined by measuring the distance between the beginning and the end of the forward stroke. The measured amplitude was calibrated with the corresponding objective lenses and converted in μm.

Statistical analysis was performed in GraphPad Prism 7.0. One- or two-way ANOVA were conducted as appropriately, and the significance was presented as * unless specified in the figure legends. All results are presented in mean±SEM.

Aerosolised fluticasone propionate analysis

A next generation impactor (NGI, Copley Scientific, UK) [14] was used at 15 L/min (±0.5 L/min) to determine the aerodynamic particle size of fluticasone propionate from (A) the AeroEclipse XL nebuliser nebulised for 1 minute, and (B) the pMDI delivered via the spacer. The pMDI was shaken before each actuation.

To the determine the drug dose delivered to the surface of the culture from nebuliser and pMDI/spacer a 13 mm round glass coverslip coated with glycerol was placed in the cell culture well. Delivery of drug was performed as described above for the ciliated cultures and coverslips then rinsed with 1 ml of methanol.

Fluticasone propionate collected was analysed by HPLC (Agilent Technologies HPLC 1200 series, Germany). Chromatography was performed by injecting 20 μl of each sample in a 5 μm HyPURITY C18 column (250 mm length, 4.6 mm i.d., Thermo Fisher Scientific, UK) in methanol: acetonitrile (60:40, v/v), at 25ºC and a flow rate of 1ml/min. A UV wavelength of 242 nm was used for detection. Calibration solutions were ranged from 1 to 100 μg/ml. Four different calibrations were prepared and analysed.

Dissolution rate of fluticasone propionate

The respirable particle fraction of fluticasone propionate aerosols was sampled from an Evohaler 250 µg/actuation pressurised metered dose inhaler (2 actuations) and a Pari LC Sprint jet nebuliser aerosolising fluticasone propionate (Flixotide suspension 0.5 mg/2 mL, 5 min of operation). Particles were collected on a 24-well Transwell insert where the polyester membrane was replaced with a glass microfibre filter according to the method of Rohrschneider *et al.* [15]. The Transwell insert was placed in the second stage of a twin stage impinger, as described by Grainger et al.[16]. Dissolution was initiated by transferring the Transwell insert into a well containing 600 µl of dissolution medium (sodium dodecyl sulfate 0.1% w/v in phosphate buffered saline) with the same solubilising capacity towards fluticasone propionate as a biorelevant simulated lung fluid [17, 18]. The assay was conducted at 37°C on a shaking plate operated at 15 rpm. The dissolution medium was sampled over 24 hours and fluticasone propionate concentration determined by HPLC with mass balance used to calculate the percentage of fluticasone propionate dissolved at each timepoint.

The dissolution data were analysed by plotting the cumulative percentage of dissolved FP against time and fitting a Korsmeyer-Peppas model [19, 20] to determine the dissolution rate constant.

$$F = k_{KP} \cdot t^n$$

where F is the fraction of drug dissolved in time t, k_{KP} is the dissolution rate constant, n is the diffusional exponent indicating the dissolution mechanism [21].

Results

Fluticasone propionate particles bind to motile cilia

The amount of fluticasone propionate contained in particles of different aerodynamic diameters following nebulisation or delivery form the spacer is shown in Figure 1. The AeroEclipse XL produced a higher proportion of larger fluticasone propionate particles (e.g. aerodynamic diameter >3.3μm 67.5±4.5%: pMDI spacer combination 19.7±8.2%).

The total (mean±SEM) amount of fluticasone propionate depositing on the surface of the ALI culture inserts was 2.47±0.08 μg following 30-second nebulisation (2mg/2ml), and 1.77±0.42 μg following delivery from the pMDI spacer combination (3 actuations, 250 μg/actuation).

The impaction of fluticasone propionate particles onto ciliated cultures was observed in real-time using video microscopy and studied using slow motion replay. Fluticasone propionate particles impacting on the cultures were immediately propelled by ciliary activity across the culture (Supplementary video 1.A). Particles of fluticasone propionate were observed to bind to the tips of rapidly beating cilia with the earliest binding seen within seconds of aerosolization of the drug by either delivery system onto cultures.

It was possible to discern when one or two particles were attached to the beating cilia on an individual ciliated cell. However, a significant number of cells had multiple particles attached to their cilia making it difficult to count the exact number. Results are therefore expressed as the number of ciliated cells with either no particle or, one, two or multiple fluticasone propionate particles bound to the tips of motile cilia divided by the total number of ciliated cells observed.

Multiple areas of each culture were studied and each point on the graphs represents a separate field of view. Two hours following nebulisation (Figure 2.A) 8.7±1.8% of the ciliated cells

observed had either 1, 2 or multiple fluticasone propionate particles attached to their cilia. There was a significant decrease in particle binding (8.7±1.8% to 5.8±1.5%: p=0.05) 24 hours after nebulisation. Following delivery by the pMDI and spacer combination (Figure 2.B), 12.1±2.1% of the ciliated cells had either 1, 2 or multiple fluticasone propionate particles attached to their cilia after 2 hours. A non-significant reduction (5.3±2.2%: p=0.83) in the number of ciliated cells with fluticasone propionate particles bound to motile cilia was seen at 24 hours.

Particle binding reduces ciliary beat amplitude.

No reduction in ciliary beat frequency was seen following binding of fluticasone propionate particles delivered by nebulisation or by the pMDI/spacer combination (Figure 3).

However, a reduction in ciliary beat amplitude was seen following fluticasone propionate binding. A significant decrease in ciliary beat amplitude was observed, following nebulisation, when 2 fluticasone propionate particles were attached to beating cilia on a cell at 2 hours ($p<0.01$), but not at 24 hours (p=0.07) (Figure 4.A).

A significant reduction in ciliary beat amplitude was also detected when 1 or more particles of fluticasone propionate attached to cilia when observed at 2 and 24 hours after delivery via the pMDI spacer combination (Figure 4.B).

Secreted mucus is unnecessary for particle binding

To determine if the mucus layer overlying respiratory cilia is required for fluticasone propionate binding to cilia, fluticasone propionate particles were nebulised to cultures of rat brain ependymal that beat in a fluid environment in the absence of an overlying mucus layer

(Supplementary video 1.B). Twenty different fields of view from ependymal cultures were examined (Figure 5).

Two hours after nebulisation, binding of single particles of fluticasone propionate to ciliated cells was observed, but no ciliated cells had two or more particles attached (Figure 5.A). However, there was a non-significant increase in the proportion of ependymal cells with fluticasone propionate particles bound to motile cilia at 24 hours (24.5±6.0%, p=0.41) compared to 2 hours (5.5±2.6%).

The mean ciliary beat frequency in control cultures was 19.7±1.1Hz at 2 hours and 19.8±1.0Hz at 24 hours (Figure 5.B). Binding of fluticasone propionate particles to cilia did not alter ciliary beat frequency at 2 hours or after 24 hours.

The ependymal ciliary beat amplitude in control wells were 11.9±0.5μm and 12.0±0.9μm at 2 hours and 24 hours respectively (Figure 5.C). A significant reduction in ependymal ciliary beat amplitude was only observed when multiple particles of fluticasone particles were attached to cilia on an individual cell at 24 hours (>2 particles 6.6±0.8μm: $p<0.01$).

Fluticasone propionate particles dissolve slowly

We aerosolised fluticasone propionate from the pMDI and nebuliser on to Transwells and conducted a biorelevant dissolution assay. The amount of fluticasone propionate deposited on the glass fibre filter in the second stage of a twin stage impinger was established by mass balance, with 76.09 μg ± 3.01 and 30.82 ± 3.74 μg fluticasone propionate deposited by the Evohaler (250 mcg/actuation; Evohaler® GSK, UK) and fluticasone propionate suspension (0.5 mg/2ml, Flixotide, GSK, UK), respectively. After 24 h, 22.75 ± 1.31% of the mass of fluticasone propionate deposited by nebuliser and 12.77 ± 0.52% of the mass delivered by pMDI had dissolved. The profiles demonstrated that fluticasone propionate particles are slow

to dissolve with a dissolution rate constant (k_{KP}) of ~8 %/h for the nebuliser and ~2 %/h for the pMDI formulation when modelled from with a sigmoidal Korsmeyer-Peppas function (Figure 6). Further, the diffusional exponent (n) was 0.3 for nebulised drug and 0.6 for pMDI delivered drug, implying that the drug release mechanisms from the FP particles were Fickian diffusion and anomalous transport, respectively. While the drug release via the Fickian model is governed by solvent transport rate or diffusion, both diffusion and structural relaxation contribute to the drug release mechanism in non-Fickian or anomalous transport. Differences in formulation and/or aerosol generation by nebuliser and pMDI devices may affect the physicochemical characteristics of the drug particles, leading to the differences observed in the drug release profiles. These *in vitro* dissolution rates indicate that fluticasone is likely to persist in particulate form continually releasing drug for many hours after delivery to the airways. The persistence of solid particles over 24 h in the dissolution assay corresponded with what was observed in the ciliated respiratory culture experiments.

Discussion

This study provides, for the first time, insight into the earliest interactions of aerosolised steroid particles with human ciliated respiratory epithelium. Significant numbers of fluticasone propionate particles, delivered by either nebulisation or from a pMDI via a spacer device, adhered to the tips of rapidly moving cilia, with many particles remaining bound to ciliary tips after 24 hours. Although particles bound to the tips of cilia did not have a significant effect on ciliary beat frequency, they significantly affected ciliary function by reducing ciliary beat amplitude. Fluticasone propionate particles also bound to the tips of motile brain ependymal cilia that beat in cerebrospinal fluid in the absence of overlying mucus suggesting binding is not exclusive to respiratory cilia and that the mucus layer overlying respiratory cilia is not essential for binding.

Traditionally, experiments on respiratory epithelial cells have involved addition of solutions or suspensions of drugs, pollutant particles and pathogens to cultures. While valuable information has been obtained from such studies, most medicines, particles and microbes that reach the airway lumen are inhaled as aerosols. In an attempt to more closely mimic the *in vivo* situation, we developed bespoke aerosolization systems that allowed direct delivery of fluticasone propionate to the surface of human ciliated epithelial cells under direct observation using high-speed video microscopy. When fluticasone propionate was delivered by nebulisation high-speed video photography allowed real-time observation of particles impacting on ciliated cultures and initial particle movement. Although we report measurement of binding at two hours, initial binding of fluticasone particles to the tips of some rapidly moving cilia was seen within seconds. Binding of fluticasone propionate delivered by nebulisation or from a pMDI via a spacer suggests binding is not dependent on the delivery system used.

Our pilot experiments (data not shown) using nebulised fluticasone propionate showed it resulted in ciliary stasis following aerosolization and that ciliary stasis was reversible on rinsing the surface of cultures with cell culture fluid. These findings are consistent with reports of ciliary stasis when nasal sprays of budesonide and fluticasone propionate were added to ciliated cultures [22, 23]. Stasis in these studies was linked to preservatives used. Our results support this in that resuspension of fluticasone propionate particles in cell culture fluid prior to nebulisation abolished ciliary stasis. We therefore nebulised fluticasone propionate particles suspended in cell culture fluid for this study.

As mentioned, binding of fluticasone propionate particles to the tips of cilia did not affect ciliary beat frequency but significantly affected ciliary function by reducing ciliary beat amplitude. We have previously shown ciliary beat frequency to be maintained despite marked effects on ciliary beat pattern in certain phenotypes of primary ciliary dyskinesia and following viral infection [24-26] suggesting ciliary beat frequency alone is a poor measure of ciliary function. Our fluticasone propionate results strongly imply that studies where only ciliary beat frequency are used to assess ciliary function may have missed significant alterations in ciliary function and should be interpreted with caution [27].

To help determine if mucus overlying respiratory cilia was involved in particle binding, we repeated experiments using ciliated cultures of the brain ependymal layer, where cilia beat in cerebrospinal fluid in the absence of overlying mucus. The attachment of fluticasone particles to the tips of rapidly beating ependymal cilia confirmed our hypothesis that the presence of mucus is not critical to binding. As with respiratory cilia, binding of particles to the tips of ependymal cilia did not affect ciliary beat frequency but significantly reduced ciliary beat amplitude.

Fluticasone Propionate is not thought to be metabolised in the lungs with the fraction of drug inhaled contributing significantly to systemic availability [28, 29]. Indeed, absorption via the gastrointestinal tract is so low that systemic effects are thought to arise from drug absorbed from the lung [30, 31]. As fluticasone propionate is absorbed almost exclusively by the lung, the high terminal half-life values of ten hours reported after inhalation indicate relatively long residence times [32, 33]. The long half-life identified from plasma pharmacokinetic studies is supported by Esmailpour and colleagues (1997) who showed fluticasone propionate is retained in the lung tissue of patients for a long time, with drug detectable in lung tissue up to 21 hours after inhalation [34]. Although particles of fluticasone propionate trapped in mucus, following inhalation, would release drug during mucociliary transport out of the lungs it is likely that a proportion exit the lung prior to full dissolution. It is possible however, that prolonged binding of poorly soluble fluticasone propionate particles to ciliary tips may result in prolonged and increased release of drug with enhanced systemic absorption. It is likely that binding also results in a high local tissue dose. Thorsson and colleagues (1997) showed that the average plasma concentration of fluticasone propionate is approximately 1.7 times higher after multiple inhalations from a Diskhaler than after a single dose [35]. The authors speculated that extensive distribution into tissues might have been the cause of the delayed elimination seen in plasma concentrations. Our results suggest it is possible that binding of drug particles to cilia may result in a previously unrecognised ‘depot’ dose that may help to explain these findings.

Particle persistence for greater that twenty-four hours was demonstrated in the dissolution assay. Systemic absorption of fluticasone propionate from aerosol particles depositing in ciliated central lung region can be dissolution rate-limited due to non-sink conditions that arise as a result of slow drug permeation of the airway epithelium. In contrast, the aerosol fraction

depositing in the alveolar region comprises finer sized particles that will dissolve more readily, particularly under sink conditions provided by the large alveolar surface area and rapid absorption of solubilised fluticasone propionate from the alveolar fluid lining to blood. For the slowly dissolving particles deposited in the airways, binding to cilia will be important in avoiding clearance of fluticasone propionate from the lungs with mucus. The dissolution assay mimics the environment in the airways in that the membrane restricts transfer of solubilised fluticasone propionate away from the deposited aerosol particle surface resulting in an initial faster dissolution rate followed by a prolonged slower phase. A number of factors influence dissolution rate including (i) particle deposition density, which was greater in the dissolution assay than would be found at the airway mucosal surface and probably accounted for the slower dissolution rate of the pMDI-derived particles compared to the nebulised particles despite their smaller particle size (geometric diameter was not measured, but is likely to vary in the same dimension as the aerodynamic particle size), (ii) binding of multiple particles by individual cilia, which may reduce dissolution rate due to particles' proximity to each other, and (iii) rapid beating of cilia, which provides a stirring mechanism that might promote dissolution.

In summary, aerosolised particles of fluticasone propionate adhere to rapidly moving respiratory cilia, some remain bound after 24 hours, and this binding reduces ciliary beat amplitude. This binding may result in a 'depot' dose with prolonged release of fluticasone propionate affecting plasma levels and exposing local tissue to high concentrations of steroid.

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Authour contributions. COC had full access to all of the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis, including and especially any adverse effects. COC, SS and BF conceived and designed the study. DDHL, WT, AS, PR and JC designed and performed the experiments. DDHL, DC, WT, JC, CMS, SS, BF, COC contributed substantially in data analysis, interpretation and the writing of the manuscript. Finally, we would like to pay tribute to the generosity, kindness and warm nature of Acom Sornsute who passed away on 17/11/2019.

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Figures legends

Figure 1. Amount of fluticasone propionate contained in particles of different aerodynamic size. Fluticasone propionate was delivered by (A) nebulisation (2 mg/2 ml); or from (B) the pMDI spacer combination (250 μg/actuation). Aerolised FP aerodynamic particles size was determined using a Next Generation Impactor (NGI): stage 1 (≥14.10μm), stage 2 (≥8.61μm), stage 3 (≥5.39μm), stage 4 (≥3.3μm), stage 5 (≥2.08μm), stage 6 (≥1.36μm), stage 7 (≥0.98μm), and stage 8 (≥0.70μm). Bars represent mean±SEM (n=4).

Figure 2. Interaction of fluticasone propionate (FP) particles and ciliated epithelium. The graph shows the number of fluticasone propionate particles attached to ciliated cells divided by the total number of ciliated cells observed when delivered by (A) nebulisation or via the pMDI spacer combination (B)**.** Binding of particles is shown 2 and 24 hours after administration. Results from the nebuliser study represent observation of 57 fields of view of cultures from three different subjects. Results from the pMDI spacer combination represents observation of 30 fields of view of cultures from three different subjects. The lines in the graph represent the mean±SEM values.

Figure 3. Attachment of fluticasone propionate (FP) particles to cilia and effect on ciliary beat frequency (CBF). CBF of cilia on ciliated cells with different numbers of fluticasone propionate particles attached following (A) nebulisation and (B) pMDI spacer delivery. Bars in the graph represent ciliated cells with different levels of fluticasone propionate binding. For each study 3 healthy matching individuals were used. Results are expressed as mean±SEM in Hertz (Hz). N/A = not seen

Figure 4. Effect of attachment of fluticasone propionate (FP) particles on ciliary beat amplitude. Ciliary beat amplitude of cilia on ciliated cells with different numbers of FP particles attached following delivery of fluticasone propionate**: (**A) nebulisation and (B) pMDI spacer delivery. Bars in the graph represent ciliated cells with different levels of fluticasone propionate binding. Results are shown as mean±SEM in µm.

Figure 5. Attachment of fluticasone propionate to brain ependymal cilia and effect on ciliary function. Fluticasone propionate (FP) particles were nebulised onto submerged cultures of brain ependymal cilia. The number of ciliated cells with one or more FP particles attached, divided by the total number of ciliated cells, is shown in (A), 2 and 24 hours after delivery. Ciliary beat frequency (B) and ciliary beat amplitude (C) of cilia with different numbers of particles attached following delivery of FP is shown after 2 and 24 hours. Bars in the graph represent ciliated cells with different levels of fluticasone propionate binding. Results are shown as (B) mean ± SEM in Hz and (C) mean ± SEM in µm.

Figure 6. Dissolution profile for fluticasone propionate in aerosol particles emitted from the nebuliser suspension and pressurised metered dose inhaler (pMDI). The respirable fraction of the aerosols was sampled in the second stage of a twin stage Impinger and the percentage of drug released from the particles was measured using a Transwell apparatus adapted for evaluating the dissolution of orally inhaled drug products. The data points are from three dissolution profiles and were fitted with a sigmoidal Korsmeyer-Peppas function.

Supplementary material figure and video legends

Supplementary Figure 1. A schematic diagram of the aerosol drug delivery using the jet nebuliser. The system developed allow nebulisation directly onto the surface of respiratory ciliated cell cultures at ALI. The impaction of fluticasone propionate particles on the surface of cells and their interaction with cilia is monitored in real-time and recorded using high-speed video microscopy. Aerosol generated from the nebuliser passes to the cultures via an aluminium tubing that negates effects of static charge. A series of vents are placed in the aluminium tubing in close proximity to the ALI culture surface to reduce the pressure on the surface of the cultures from the aerosol-laden air generated by jet nebulisation. The system is enclosed in inner and outer chambers maintained at 37^{o}C. B. A schematic diagram of the aerosol drug delivery using the pMDI and spacer. (1) The pMDI is actuated through the spacer which the filter valve is in absent, and (2) generate an air flow by the cut opened syringe fitted into the pMDI holder to deliver the fluticasone particles land onto the ciliated culture on the bottom. The artificial air flow is followed by each actuation and then left for a minute before next actuation.

Supplementary video 1 (A) Video of the ciliated cell culture with particles of fluticasone propionate (arrowed) bound to moving cilia 30 minutes after delivery by nebulisation.

(B) Video of ciliated rat brain ependymal cultures with particles of fluticasone propionate (arrowed) bound to moving cilia 30 minutes after delivery by nebulisation. Videos were recorded at 250 frame per second and playing at 25 frames per second. The scale bars represent 10μm.

Supplementary Table 1. Patient and participant information

Patient / Participant	Disease	Airway	Gender	Age group (years)	Smoking History
Healthy 01 (GOSH25)	Healthy	Nasal	Male	0-9	NA
Healthy 02 (BS08)	Healthy	Nasal	Male	20-29	NA
Healthy 04 (67B)	Healthy	Bronchial	Female	0-9	NA
COPD 01 (JW0302)	COPD	Nasal	Female	80-89	Ex-smoker

Supplementary Table 2. Kinetic parameters from Korsmeyer-Peppas function fitting of dissolved FP as a function of time. Data represent mean ± standard deviation, n=3.

Formulation	**R^2**	**Dissolved FP at 24 h (%)**	**k_{KP} (h^{-1})**	**Exponent, *n***
Flixotide nebuliser	0.9826	21.01 ± 1.82	7.99 ± 1.65	0.31 ± 0.05
Evohaler pMDI	0.9907	12.12 ± 0.98	2.06 ± 0.13	0.56 ± 0.05

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