Effect of oral digoxin, topical ouabain and salbutamol on transepithelial nasal potential difference in patients with cystic fibrosis

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1. Airway epithelium in cystic fibrosis is characterized by a defect in chloride secretion across the apical membrane and an increase in sodium absorption. The increased rate of sodium absorption can be inhibited in vitro by ouabain, a Na⁺-K⁺-ATPase inhibitor, and in cystic fibrosis patients the number and activity of nasal epithelial Na⁺-K⁺-ATPase pumps is increased.

2. We have performed a series of studies to determine whether drugs which modify airway epithelial Na⁺-K⁺-ATPase activity in vitro can modify nasal potential in cystic fibrosis patients in vivo. As transepithelial nasal potential difference measurements were used to study the effect of drug modulation of airway epithelial ion transport in vivo, the repeatability of the technique was first evaluated. In order to assess the effectiveness of the technique used for measuring nasal potential difference, a pilot study was carried out using topical amiloride, a drug which has previously been shown to inhibit airway epithelial sodium transport in vivo. We then studied the effects of ouabain and digoxin, two inhibitors of Na⁺-K⁺-ATPase, and salbutamol, a drug which activates Na⁺-K⁺-ATPase, on nasal potential difference.

3. In study 1, nasal potential difference measurements were repeated on non-consecutive days in 20 patients with cystic fibrosis and 20 healthy individuals. Healthy subjects had a mean (SEM) potential difference value of −19.5 (0.9) mV, with a 95% range for a single estimate of 75–133%. In patients with cystic fibrosis, the mean (SEM) potential difference was −40.4 (2.1) mV, with a 95% range for a single estimate of 74–136%.

4. In an initial pilot study, the effect of topical amiloride on nasal potential difference was investigated on two consecutive days in four cystic fibrosis patients and four healthy control subjects, in a double-blind, placebo-controlled, randomized cross-over study. Nasal transepithelial potential was measured before and at 5, 15, 30, 45 and 60 min after the intranasal administration of 0.4 ml of a fine spray of 1 mmol/l amiloride or 0.9% saline placebo to both nostrils. Amiloride was associated with a maximal reduction in nasal potential difference at 15 min of 49% and 41% in cystic fibrosis patients and control subjects, respectively. Compared with saline, the amiloride response was significant in both groups (P<0.025).

5. In study 2, the effect of topical ouabain and salbutamol on nasal potential difference was investigated in ten cystic fibrosis patients and ten healthy control subjects, in a double-blind, placebo-controlled, randomized cross-over study. Nasal transepithelial potential was measured before and at 5, 15, 30, 45 and 60 min after the intranasal administration of either 0.4 ml of a fine spray of 5 mg/ml salbutamol, 0.25 mg/ml ouabain or 0.9% saline placebo to both nostrils. There was no significant change in nasal potential difference with either ouabain, salbutamol or placebo in either healthy control subjects or patients with cystic fibrosis.

6. In study 3, we performed a randomized, double-blind, placebo-controlled cross-over study of oral digoxin on nasal potential difference, spirometry and sweat electrolytes for 2 weeks in 11 patients with cystic fibrosis. During the treatment period, patients had a mean (range) serum digoxin level after the first and second week of therapy of 0.9 (0.3–1.4) µg/l and 1.1 (0.4–2.2) µg/l, respectively. There was no significant difference in the change in nasal potential difference measurements, forced expiratory volume in 1 s and sweat Na/Cl concentrations between the digoxin and placebo trial periods.

7. In conclusion, neither topical ouabain nor systemic digoxin had any effect on nasal potential difference in cystic fibrosis. Inhibitors of Na⁺-K⁺-ATPase are therefore unlikely to find a role in the treatment of cystic fibrosis. The lack of a detrimental effect of salbutamol on nasal potential difference is reassuring, as β-agonists are widely used in patients with cystic fibrosis.

INTRODUCTION
Cystic fibrosis (CF) airway and nasal epithelium

Key words: cystic fibrosis, digoxin, Na⁺-K⁺-ATPase, ouabain, salbutamol
Abbreviations: CF, cystic fibrosis; FEV₁, forced expiratory volume in 1 s; FVC, forced vital capacity; PD, potential difference.
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is characterized by reduced chloride secretion [1–3] and a 2–3-fold increase in sodium absorption [1, 4, 5]. These changes in ion flux play a significant role in the pathophysiology of CF by affecting both the secretion and absorption of fluid across sweat ducts and epithelial linings [6]. The increased sodium absorption results in a raised transepithelial nasal potential difference (PD) in CF [7, 8].

Attempts to correct the ion transport defect therapeutically have aimed to either increase chloride flux using the extracellular nucleotides (ATP or UTP) [9, 10] or to inhibit sodium absorption using amiloride, an apical sodium-channel blocker [11]. Although amiloride inhibits nasal PD [12] and improves airway mucociliary clearance [13], it is rapidly cleared from the airways [14]. This may partly explain why the use of nebulized amiloride as a treatment for CF has proved relatively disappointing, with studies showing little or no effect on reducing the rate of decline in lung function [15, 16].

The increase in sodium reabsorption across CF nasal epithelium is due to increased activity of sodium entry channels on the apical surface of airway epithelium [17] and a concomitant increase in basolateral Na+-K+-ATPase [18]. Inhibition of the basolateral Na+-K+-ATPase pumps could therefore provide an alternative mechanism whereby the increase in sodium reabsorption could be corrected. Na+-K+-ATPase is inhibited both in vitro and in vivo by the cardiac glycosides ouabain and digoxin [4, 19, 20], and the binding kinetics of these agents suggest that they would have a longer duration of action than amiloride [21, 22]. Previous studies have demonstrated that ouabain causes near cessation of sodium absorption across nasal epithelium in vitro [4] and, when given intravenously, ouabain reduces the transepithelial airway PD in dogs [23]. Although there are no drugs which specifically activate Na+-K+-ATPase, β-adrenoceptor agonists such as salbutamol stimulate Na+-K+-ATPase in vitro [24].

We performed a repeatability study of nasal PD measurements and a pilot validation study with amiloride, followed by two studies looking at the effect of two Na+-K+-ATPase inhibitors (oral digoxin and topical ouabain) and salbutamol, a drug which activates Na+-K+-ATPase [24], on transepithelial nasal potential measurements in patients with CF. The aim of these studies was to determine whether drugs which inhibited Na+-K+-ATPase could play a role in the treatment of CF.

SUBJECTS

All patients with CF had a sweat sodium level of >70 mmol/l, as well as characteristic radiological and clinical features of the disease and stable lung function during the study period. Subjects were excluded if they had current nasal symptoms, had undergone nasal surgery or had received intravenous or oral antibiotics within the 3 weeks before the trials began. All healthy subjects were non-smokers, were free from any respiratory or nasal symptoms and were not taking any medication. Written consent was obtained from all subjects and all studies were approved by the Nottingham City Hospital Ethics Committee.

METHODS

Nasal PD measurements

Measurements of nasal potential were carried out using a slight modification of the method described by Alton et al. [7]. A 60 ml catheter syringe containing undiluted electrode cream (Signa, Orange, NJ, U.S.A.) was inserted into a short arm of a T-junction plastic connector and taped securely with adhesive tape (Sleek). The exploring electrode was a rubber urinary catheter (Foley size 8) which contained a silver/silver chloride electrode. One of the side holes at the tip of the catheter was sealed with adhesive in order to leave one contact area. The distal end of the lead ran through the plastic connection to emerge from the bare short arm and was connected to the positive pole of a high impedance voltmeter (RS Components, Birmingham, Alabama, U.S.A.). The bare short arm was sealed with resin to reduce airlocks while the catheter was firmly attached to the long vertical arm using adhesive tape (Sleek). The reference silver/silver chloride electrode and the tip of the catheter were placed in a common pool of electrode cream for calibration. Stable baseline values <±5 mV were judged acceptable. The reference electrode was taped over a small abraded area on the forearm which had been made using a diamond-tipped dental burr (SLE Ltd, London, U.K.). Electrode cream (Signa) was then injected through a hole in the electrode to allow contact. To ensure correct functioning of the equipment before insertion of the catheter into the nose, the PD of the tip of the index finger was noted, values ranging from −30 mV upwards. The tip of the catheter was inserted into the nostril downwards and medially along the floor of the nose without direct vision. The maximum stable PD was then recorded to the nearest mV between two to four times in both nostrils. All PD measurements were lumen-negative with respect to the reference electrode.

Lung function

Forced expiratory volume in 1 s (FEV1) and forced vital capacity (FVC) were measured as the highest of three blows on a Vitalograph spirometer (Vitalograph, Buckingham, U.K.).

Digoxin

Serum digoxin levels were measured 12 h after the last dose by a homogenous enzyme immunoassay.
The intra- and inter-batch coefficients of variation of the assay were 3.4% and 5.5%, respectively.

Sweat test

Sweat production was stimulated by the process of pilocarpine iontophoresis during which 0.2% pilocarpine nitrate was applied to the skin. The intra- and inter-batch coefficients of variation were 2.6% and 1.76%, respectively, for the measurement of sweat sodium, and 0.99% and 1.7%, respectively, for the measurement of chloride.

PROTOCOLS

Study I: short-term repeatability of nasal PD measurements

Twenty adult CF patients, 11 male [mean age 21 (range 16–28) years, mean baseline FEV1 1.71/s (48% predicted), FVC 2.61 (65% predicted)], and 20 healthy control subjects, nine male [mean age 24 (range 19–38) years], were studied. Nasal PD was measured on two non-consecutive days within the same week, and at the same time of day on each occasion. To validate this method with respect to the detection of drug-induced changes, we performed a pilot study measuring the effect of amiloride on nasal PD.

Pilot study: effect of topical amiloride on nasal PD measurements

Four adult CF patients, four male [mean age 24 (range 21–29) years, mean baseline FEV1 1.61/s (38% predicted), FVC 2.51 (52% predicted)], and four healthy subjects, two male [mean age 26 (range 20–37) years], were investigated. The study had a double-blind, placebo-controlled, randomized cross-over design. Subjects were studied on two occasions with at least 5 days between each visit. Each visit was at the same time of day. One subject who was on regular inhaled β-agonist therapy refrained from using these drugs for at least 6 h before each visit. Patients who were taking nebulized antibiotics omitted the morning dose on the day of each visit. Blood pressure and pulse rate were recorded at the start and end of each visit for safety purposes. On each occasion, nasal transepithelial potential was measured before and at 5, 15, 30, 45 and 60 min after the intranasal administration of 0.4 ml of a fine spray of drug or placebo to both nostrils. Solutions of 5 mg/ml salbutamol (Allen and Hanburys, Greenford, Middlesex, U.K.), 0.25 mg/ml ouabain (Laboratoire Nativelle, France) and placebo (0.9% saline) were used. Salbutamol and ouabain were in aqueous form.

Study 3: effect of oral digoxin on nasal PD

Eleven adult CF patients, six male [mean age 23.5 (range 18–31) years, mean baseline FEV1 1.71/s (50% predicted), FVC 2.71 (62% predicted)], were studied in a randomized, double-blind, placebo-controlled cross-over study of 2-week treatments, and a 2-week washout between treatments was performed. Visits were carried out on days 1, 7 and 14 of each treatment period at the same time of day. At the first visit, each patient had an electrocardiograph for safety purposes. Blood pressure, radial pulse, nasal PD, spirometry and blood electrolytes were measured at each visit. Measurements on day 1 were made before starting drug or placebo treatment. On the last visit of each treatment period, a sweat test was performed. In the active treatment phase, all patients received a loading dose of 1.5 g of digoxin orally (Wellcome, Crewe, Cheshire, U.K.) over the first 24 h, followed by a maintenance dose of 250 mg once daily to 250 mg twice daily according to body weight. A second investigator monitored digoxin levels on day 7, adjusting the dose in order to keep levels within the therapeutic range. A further digoxin level was measured on day 14. Patients on the placebo arm of the trial also had dose changes made by the second investigator to ensure that the trial remained double-blind. Placebo sucrose and digoxin tablets were inserted into empty digoxin capsules to prevent either the subjects or investigator from identifying prescribed medication.

ANALYSES

Each nasal PD value was taken as the mean of the highest two values from each nostril for each visit, and an overall mean was calculated for each subject. In patients with CF, the mean PD was compared with body mass index, FEV1 and FVC
using regression analysis (Microsoft Corporation, Redmond, Washington, U.S.A.). Repeatability was measured as the 95% range for a single estimate and was calculated using the equation: 95% range = \pm t_{0.05} SD of the difference/\sqrt{2} [25]. The magnitude of the difference between PD measurements increased with increasing means (r = 0.612, \( P < 0.001 \)). Data for all three studies was therefore log-transformed (\( \log_{10} \)) before analysis [26, 27]. The antilog of the 95% range for a single estimate was converted to percentages to give a meaningful expression of repeatability. Further interpretation of repeatability was made by calculating the intra-class correlation coefficient by dividing the between-subject variance by the total variance within both the healthy control subject group and CF patient group (SPSS, Chicago, IL, U.S.A.) [25, 27]. Repeatability was also expressed as the coefficient of variation to enable comparison with previous studies.

The change in nasal PD values after treatment with ouabain, salbutamol and placebo were compared using repeated measures two-way analysis of variance (SPSS, Chicago, IL, U.S.A.). Comparisons of the nasal PD between the saline and amiloride treatments were made using the Mann–Whitney U-test (Microsoft Corporation), as numbers of subjects in both groups were small. In the digoxin study, the changes in nasal PD, FVC and FEV\(_1\) measurements from baseline to day 7 and 14 were compared for digoxin and placebo using Student's paired t-tests (Microsoft Corporation). Sweat tests at the end of each treatment period were also compared using the paired t-test. In all analyses, a \( P \) value < 0.05 was regarded as statistically significant.

RESULTS

Study I: Repeatability of nasal PD measurements

Comparison of healthy subjects and CF patients. The mean (SEM) PD value was -19.5 (0.9) mV in control subjects and -40.4 (2.1) mV in patients with CF. In three CF patients, the PD value was below -30 mV on one or more occasions and overlapped with the control group. This was further reduced to an overlap of two subjects when the overall mean of both visits was compared. Amiloride significantly reduced PD in both healthy subjects and CF patients (\( P < 0.05 \)) (Fig. 1).

Repeatability. There was no significant order effect between the first and second tests in either healthy subjects or CF patients (\( P = 0.7 \)). The within-subject SDs, the 95% ranges for a single estimate, intra-class correlation coefficients and the coefficients of variation for both healthy subjects and CF patients are shown in Table 1.

Relationship between PD and other clinical variables in CF patients. There was a negative correlation between nasal PD measurements and both FVC (\( r = -0.63, \ P = 0.003 \)) and FEV\(_1\) (\( r = -0.52, \ P = 0.019 \)). There was no correlation between nasal PD and body mass index (\( r = -0.33, \ P = 0.16 \)).

Power calculations. Repeatability measurements from this study were used to make power calculations for the subsequent studies [28]. There was a 95% chance of detecting a 26% and 25% change in nasal PD values in CF patients and healthy individuals, respectively, when using ten subjects, changes which are considerably smaller than previously seen with amiloride therapy, which causes a greater than 50% fall in nasal PD [12].

Pilot study: effect of amiloride on nasal PD measurements

In CF patients, the mean (SEM) baseline PD measurements before application of topical amilor-

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**Table 1. Results following two consecutive nasal PD measurements in 20 healthy control subjects and 20 patients with CF**

<table>
<thead>
<tr>
<th></th>
<th>Control subjects</th>
<th>CF patients</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean (SEM) PD</td>
<td>-19.5 (0.9)</td>
<td>-40.4 (2.1)</td>
</tr>
<tr>
<td>Mean difference</td>
<td>( \log_{10} ) 0.024</td>
<td>( \log_{10} ) 0.0097</td>
</tr>
<tr>
<td>Between-subject SD</td>
<td>( \log_{10} ) 0.084</td>
<td>( \log_{10} ) 0.089</td>
</tr>
<tr>
<td>Within-subject SD</td>
<td>( \log_{10} ) 0.0695</td>
<td>( \log_{10} ) 0.063</td>
</tr>
<tr>
<td>95% range for a single estimate</td>
<td>75–133%</td>
<td>74–136%</td>
</tr>
<tr>
<td>Coefficient of variation</td>
<td>17</td>
<td>21</td>
</tr>
<tr>
<td>Intra-class correlation coefficient</td>
<td>0.69</td>
<td>0.79</td>
</tr>
</tbody>
</table>

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**Fig. 1. Mean (SEM) nasal PD measurements after administration of saline or amiloride in four patients with CF and four healthy control subjects**
Na+-K+-ATPase inhibitors in cystic fibrosis

![Graph](image)

Fig. 2. Mean (SEM) nasal PD measurements over time after administration of topical ouabain, salbutamol and saline in ten patients with CF and ten healthy control subjects

![Graph](image)

Fig. 3. Mean (SEM) nasal PD measurements from 11 patients with CF after 2 weeks of treatment with oral digoxin and placebo.

Nasal PD measurements, lung function and sweat test. There was no significant difference in baseline values of FEV₁, FVC, nasal PD and sweat test (Fig. 3, Table 2) at the start of the digoxin and placebo periods. There was no evidence of a treatment order effect for any of the variables measured. There was no significant difference in the change in nasal PD measurements, FEV₁ and sweat Na/Cl concentrations between the digoxin and placebo trial periods (Table 2). There was a small but significant fall in FVC after 14 days of digoxin therapy compared with placebo (Table 2). No side effects were seen on either digoxin or placebo limbs. Blood electrolytes did not differ between the digoxin and placebo limbs.

**DISCUSSION**

The aim of this study was to determine whether drugs which modify Na⁺-K⁺-ATPase in vitro could modify airway epithelial ion transport in CF in vivo. While the action of cardiac glycosides on myocardial function has been recognized for centuries [19], no previous studies have looked at their effect on airway PD in either healthy subjects or CF patients. There was good theoretical evidence to suggest that cardiac glycosides would reduce airway epithelial PD. Ouabain, a Na⁺-K⁺-ATPase inhibitor, causes potent inhibition of airway epithelial sodium transport in vitro [4], and Na⁺-K⁺-ATPase activity is increased in nasal epithelium in CF [18].

We used a slight modification of the technique described by Alton et al. [7] to study the effect of Na⁺-K⁺-ATPase modulation on airway epithelial transport in vivo. Transepithelial PD within the

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nasal cavity depends on the characteristics and integrity of the epithelium, with a maximal PD reading generated by the ciliated epithelial lining covering the inferior surface of the turbinate [29]. Several measurements were taken from each nostril to ensure that the highest readings were taken from the correct site. The measurement of nasal PDs appears to discriminate between patients with CF and healthy control subjects, although in the repeatability study there was some overlap between the groups. We found a similar relationship between nasal PD and indices of disease severity, such as lung function and body mass index, to that previously described [7].

Whereas several studies have documented the use of nasal potential measurements, data have not been obtained on the repeatability of this technique in large numbers of CF patients, although Alton et al. [7] described a repeatability similar to ours in a small sample of healthy subjects. The 95% range for a single estimate and intra-class correlation coefficients are the preferred methods for assessing repeatability [25-27]. The 95% range gives an estimate of the range in which the true value of any single estimate is likely to lie, whereas the intra-class correlation coefficient estimates the proportion of the variation of a measurement that is due to the difference between subjects. The latter has the advantage that it can be used to compare measurements on different scales. The repeatability of nasal PD measured as intra-class correlation coefficient was comparable to that seen in studies of bronchial challenge tests in asthma [30].

Having established the repeatability of the technique, we validated it by using amiloride as a positive control before investigating the effect of two \( \text{Na}^+\text{-K}^+\text{-ATPase} \) inhibitors, ouabain and digoxin, on nasal PD. Amiloride caused a comparable change in nasal PD to that seen in other studies [12]. Digoxin was chosen as the most suitable cardiac glycoside for oral administration because it is well absorbed and drug levels can be monitored with ease [22]. Oral digoxin has a peak cardiac effect at 5h, with a half-life of 36h [22]. After short-term administration (over days to weeks), digoxin produces widespread inhibition of cardiac \( \text{Na}^+\text{-K}^+\text{-ATPase} \), although after several months this effect may be lost due to pump upregulation [31]. The effect of digoxin on nasal PD was investigated over 2 weeks to avoid this phenomenon. We decided to use digoxin, rather than a topical lipophilic cardiac glycoside, as its pharmacokinetics have been well characterized when given orally. Ouabain was chosen for topical application because of its water solubility and its rapid onset of action [22]. A cardiac response to intravenous ouabain occurs within 15 min and ouabain has a half-life of 19–24h [21, 32]. The topical route was chosen to maximize local drug concentrations while minimizing systemic effects.

Neither ouabain nor digoxin had any demonstrable effect on transepithelial nasal PD. As ouabain was administered at high concentrations, has a high affinity for the cardiac glycoside receptor and is rapidly acting, it seems unlikely that inadequate dosage or rapidity of action can explain the negative results. It is more likely that poor absorption resulted in inadequate penetration of the drug to the basolateral membrane. Another possible explanation is that ouabain was rapidly cleared by ciliary clearance mechanisms, but if this were the case amiloride would likewise have been ineffective. Inadequate

Table 2. Lung function, nasal PD and sweat electrolytes in 11 patients with CF following 2 weeks of treatment with oral digoxin and placebo. All data were log10-transformed before analysis. \( P \) values are given for comparisons between baseline and measurements after the first and second week of therapy.

<table>
<thead>
<tr>
<th></th>
<th>Day 1</th>
<th>Day 7</th>
<th>Day 14</th>
<th>Day 1/7</th>
<th>Day 1/14</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Mean PD (SEM)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(mV) digoxin</td>
<td>-37.9</td>
<td>-35.4</td>
<td>-38.2</td>
<td>0.06</td>
<td>0.52</td>
</tr>
<tr>
<td>(mV) placebo</td>
<td>-39.9</td>
<td>-40.8</td>
<td>-38.9</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Mean FVC (SEM)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(l) digoxin</td>
<td>2.9</td>
<td>2.8</td>
<td>2.6</td>
<td></td>
<td></td>
</tr>
<tr>
<td>(l) placebo</td>
<td>2.5</td>
<td>2.5</td>
<td>2.6</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Mean FEV1 (SEM)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(l) digoxin</td>
<td>1.8</td>
<td>1.8</td>
<td>1.7</td>
<td>0.64</td>
<td>0.20</td>
</tr>
<tr>
<td>(l) placebo</td>
<td>1.7</td>
<td>1.7</td>
<td>1.7</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Mean sweat sodium (SEM)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(mmol/l) digoxin</td>
<td>117.2</td>
<td>113.2</td>
<td>113.4</td>
<td></td>
<td></td>
</tr>
<tr>
<td>(mmol/l) placebo</td>
<td>113.4</td>
<td>113.3</td>
<td>113.4</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Mean sweat chloride (SEM)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(mmol/l) digoxin</td>
<td>117.3</td>
<td>113.3</td>
<td>113.4</td>
<td></td>
<td></td>
</tr>
<tr>
<td>(mmol/l) placebo</td>
<td>117.5</td>
<td>114.7</td>
<td>114.7</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

\( P \) values are given for comparisons between baseline and measurements after the first and second week of therapy.
drug penetration is unlikely to explain the lack of effect of oral digoxin, however, as a similar plasma concentration to those obtained in this study has previously been shown to cause widespread inhibition of tissue $\text{Na}^+\text{-K}^+\text{-ATPase}$ in healthy subjects [31] after a similar length of treatment. Although a small rise in nasal PD was seen after the first week of digoxin, the increase was not statistically significant and was absent by the second week. While small changes in nasal PD may have been missed, the present study had sufficient power to detect a clinically meaningful change. Because the cardiac effect of digoxin requires 40% of myocardial $\text{Na}^+\text{-K}^+\text{-ATPase}$ activity to be inhibited to produce a therapeutic effect [33], it is possible that a 40% inhibition of airway epithelial receptors was insufficient to alter nasal potentials. We measured nasal PD at a time when tissue levels of digoxin were high and were associated with a reduction in $\text{Na}^+\text{-K}^+\text{-ATPase}$ activity. It is unlikely that we would have seen a different effect had we chosen a different time point for PD measurement. There was no difference in response in patients taking corticosteroids.

An alternative explanation for the lack of effect of digoxin is that, unlike the heart and kidneys, airway epithelium does not selectively concentrate digoxin [22]. Thus, the concentration of digoxin in airway epithelium would have been insufficient to cause significant $\text{Na}^+\text{-K}^+\text{-ATPase}$ inhibition. It is also possible that a small inhibitory effect of digoxin may have been negated after upregulation of uncoupled $\text{Na}^+\text{-K}^+\text{-ATPase}$ pumps [33]. Consistent with the lack of effect on nasal PD, sweat sodium concentration was unaltered by oral digoxin. As ouabain-inhibitable basolateral $\text{Na}^+\text{-K}^+\text{-ATPase}$ pumps are present in sweat ductal cells, similar explanations may be put forward to explain the lack of effect at this site [34].

A small fall in FVC was seen after digoxin treatment on day 14. This was probably a spurious result resulting from a type-2 error due to the number of comparisons performed. The reduction in FVC seen (300ml) was small and is unlikely to be clinically significant.

The lack of effect of oral digoxin and topical ouabain on nasal PD contrasts with a study in dogs where intravenous ouabain reduced tracheal PD [23]. The most likely explanation for this difference is that the relative doses used were far greater in the study in dogs [21]. Similar doses would result in toxicity in man [21, 23]. We considered a further study using intravenous ouabain or digoxin, but although higher initial peak drug concentrations would have been obtained, intravenous therapy would not be practical clinically [21]. Giving a more lipophilic cardiac glycoside by the inhaled route might be one way of circumventing this problem, but suitable preparations for inhalation are not currently available.

In addition to looking at the effect of the cardiac glycosides, we investigated the effect of topical salbutamol on nasal PD measurements. $\beta_2$-Adrenoceptors stimulate sodium absorption across CF airway epithelium in vitro [5, 35, 36], and $\beta_2$-adrenoceptor agonists stimulate $\text{Na}^+\text{-K}^+\text{-ATPase}$ in several tissues [24, 37]. We were concerned that their use in the clinical management of CF could exacerbate secretion dehydration. However, in the present study, it was reassuring that topical salbutamol did not alter nasal PD.

In conclusion, although previous studies have documented an increase in basolateral epithelial $\text{Na}^+\text{-K}^+\text{-ATPase}$ activity in CF [18], and cardiac glycosides inhibit sodium reabsorption across airway epithelium in vitro, we were unable to demonstrate any effect of oral digoxin or inhaled ouabain on nasal PD in vivo. $\text{Na}^+\text{-K}^+\text{-ATPase}$ inhibitors are therefore unlikely to find a role in the treatment of CF.