Sodium channel blockers and uridine triphosphate: effects on nasal potential difference in cystic fibrosis mice


ABSTRACT: Sodium channel inhibitors block the enhanced Na⁺ reabsorption in cystic fibrosis (CF). Extracellular nucleotides facilitate Cl⁻ secretion via Ca²⁺ gated Cl⁻ channels. A combination of these effects may produce less viscid secretions in CF which are easier to expectorate.

This study examined the effects of combining sodium channel blockers with uridine triphosphate (UTP) on nasal membrane potential difference (PD) in CF insertional null mutant mice (cftr<sup>nm</sup>), ΔF508 homozygous mice (cftr<sup>rm</sup>Cam) and matched control animals.

Median basal PD in the insertional CF mice and ΔF508 CF mice were -28 and -34 mV respectively. These values were significantly different to the control animals (-20 mV). Amiloride and loperamide reduced the PD in cftr<sup>nm</sup>CF mice (ΔPD 13 mV & 15 mV respectively) suggesting Na⁺ reabsorption. The subsequent addition of UTP in a chloride-free vehicle increased the PD (ΔPD -8.7-12.5 mV). ΔF508 mice showed significantly greater responses compared with CF insertional null mutant mice (p<0.05). The action of UTP was brief and not prolonged by the addition of Ca²⁺. The authors have examined the effect of UTP on nasal membrane potential difference in CF insertional null mutant mice.

In conclusion, this study demonstrated dose dependant nasal membrane potential changes in differences mice with uridine triphosphate in the presence of sodium channel blockers suggestive of chloride secretion. More stable analogues of uridine triphosphate in combination with long acting sodium channel blockers such as loperamide may have therapeutic potential in cystic fibrosis.

Keywords: Cystic fibrosis mice nasal potential difference sodium channel blockers uridine triphosphate

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Methods

Experimental animals

Twenty-four insertional null mutant CF mice (cfr<sup>tm1HGU</sup>) [10] were studied in the experiments. Their breeding was subsidized by the Association Francaise de Lutte contre la Mucoviscidose, France and supplied by Charles River, Margate, Kent, UK. Twenty standard laboratory MF1 strain mice (Harlan, Bicester, Oxfordshire, UK) were used as controls. Both sexes were matched for age (range 3–14 months) and weighed 20–42 g. The animals were allowed food and water ad libitum. A further group of seven CF mice homozygous for the ΔF508 mutation, (cfr<sup>tm1Cam</sup>) [11] were also studied using the same protocols.

Nasal potential difference measurements

Mice were anaesthetized by intraperitoneal injection using a combination of ketamine 5 mg 30 g body weight<sup>-1</sup> and met-etomidate 0.05 mg 30 g body weight<sup>-1</sup>. Nasal PD was measured as described previously [16] between a 24G met-etomidate to enable them to recover more quickly procedures.

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Results

Nasal membrane PD in mice were lumen negative. The resting nasal epithelial PD in controls ranged -12 to -27 mV (median -20 mV, n=20) and in cfr<sup>tm1HGU</sup> mice -18 to -38 mV (median -28 mV, n=24). The ΔF508 mice had a higher basal PD (range -25 to -39 mV; median -34 mV, n=7).

The changes in nasal PD after amiloride or loperamide administration were significant in both CF and control groups (p<0.01) compatible with sodium channel blockade. No significant PD changes were observed when sodium gluconate (low chloride vehicle) was administered after the sodium channel blockers. Subsequently UTP dissolved in 0.15 M sodium gluconate increased PD in both groups, indicating that Ca<sup>2+</sup> activated Cl<sup>-</sup> secretion was occurring. UTP given in 0.9% saline solution after sodium channel blockers were administered had no effect on PD. The PD changes after amiloride/loperamide and UTp administration were significant in the cfr<sup>tm1HGU</sup> mice compared to controls (p<0.01; tables 1 and 2). Similar but larger PD responses were obtained in the ΔF508 mice with sodium channel blocking agents and UTP (tables 1 and 2). An example of PD changes observed with the above combinations of drugs are shown in figures 1 and 2.

The duration of action of UTP (with the nasal mucosa in sodium blocked state after amiloride) is shown in figure 3. The PD changes after UTP were short lasting with PD reverting to the value of the post amiloride PD within 30–40 min following a single administration of 10.0

<table>
<thead>
<tr>
<th>Mouse type</th>
<th>Basal PD mV</th>
<th>APD after amiloride 1.0 mmol L&lt;sup&gt;-1&lt;/sup&gt; mV</th>
<th>APD after UTP 1.0 mmol L&lt;sup&gt;-1&lt;/sup&gt; mV</th>
</tr>
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<tbody>
<tr>
<td>MF1 control</td>
<td>-22 (-12 to -27)</td>
<td>6</td>
<td>-3</td>
</tr>
<tr>
<td>CF insertion</td>
<td>-28 (-18 to -38)*</td>
<td>15*</td>
<td>-12.5*</td>
</tr>
<tr>
<td>CF ΔF508</td>
<td>-34 (-30 to -38)</td>
<td>16</td>
<td>-12</td>
</tr>
</tbody>
</table>

Data are presented as the median with the range in parentheses. *: p<0.01; *: p<0.05, comparison between control and CF groups. **: p<0.05; *: p<0.01, comparison between ΔF508 and insertional CF groups.

<table>
<thead>
<tr>
<th>Mouse type</th>
<th>Basal PD mV</th>
<th>APD after loperamide 1.0 mmol L&lt;sup&gt;-1&lt;/sup&gt; mV</th>
<th>APD after UTP 1.0 mmol L&lt;sup&gt;-1&lt;/sup&gt; mV</th>
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<td>-5</td>
</tr>
<tr>
<td>CF insertion</td>
<td>-28 (-20 to -36)*</td>
<td>13*</td>
<td>-8*</td>
</tr>
<tr>
<td>CF ΔF508</td>
<td>-34 (-25 to -39)**</td>
<td>17**</td>
<td>-15**</td>
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mmol-L\(^{-1}\) UTP. The action of UTP was also found to be dose dependent from a range of 0.1–10.0 mmol-L\(^{-1}\) (n = 3, fig. 4). Higher concentrations did not produce further increase in PD.

**Discussion**

The basal PD measurements in the two groups of CF mice were significantly greater than the controls suggesting an ion transport abnormality similar to that seen in humans. The ΔF508 mice had higher nasal PD at rest compared to insertional CF mice indicating a more severe phenotype. Previous studies in both humans and mice have demonstrated a fall in nasal epithelial PD after amiloride administration [10, 17]. The expected fall in PD was observed in all groups after administration of the sodium channel blocking agent amiloride. Similar changes have been shown with loperamide in *in vitro* studies in the bowel [18]. It has previously been shown that loperamide is effective on the CF mouse respiratory mucosa as a sodium channel blocker (change in ΔPD -14 mV versus amiloride -15 mV) and had a longer duration of action (8 h versus amiloride 4 h) [14]. It has a relatively longer duration of action when administered systemically in humans (time (t)0.5 hours =11 h) when compared to amiloride (t0.5=6 h) [19] suggesting that less frequent administration would achieve sufficient sodium channel blockade. A fall in PD was seen with loperamide consistent with Na\(^+\) blockade. The subsequent addition of UTP (0.1–10 mmol-L\(^{-1}\)) dissolved in a chloride-free vehicle increased the PD in all groups suggesting that Ca\(^{2+}\) activated Cl\(^-\) secretion was occurring.

The resting chloride conductance of the nasal epithelium is reflected by a small increase in negative nasal PD...
following perfusion with a low Cl\(^{-}\) solution in the sodium blocked state [20]. This seems to be a necessary step because the PD changes of Cl\(^{-}\) secretion are not observed without a low Cl\(^{-}\) environment which provides a driving force for apical chloride conductance. However, in the current study, the authors failed to observe any nasal PD change after low Cl\(^{-}\) solution was applied to the sodium blocked epithelium by nebulization. It is believed that this is due to differences in the technique of PD measurement: a) the current setup was designed to emulate drug delivery as it would happen in human clinical practice (i.e. by nebulization of droplets containing drug/vehicle which were deposited on the epithelium). Intermittent withdrawal and reinsertion of the nasal recording bridge was necessary to administer the drugs. Other investigators [10, 18, 19] used a double lumen catheter placed continuously in the nostril throughout the experiment. Various drugs/vehicles were then perfused through one lumen and PD recorded through the other; b) the change in PD following low Cl\(^{-}\) solution may be brief. Due to the current technique requiring intermittent withdrawal of the nasal bridge, the authors may be placing the catheter after the PD change has already occurred. This was demonstrated when O. Pirzada and C.J. Taylor (Division of Child Health, University of Sheffield, Sheffield, UK) compared the two methods (personal communication): no change was obtained with nebulization of sodium glucconate after sodium blockade as has been described earlier. However when sodium gluconate was perfused into the nostril preblocked with loperamide, PD changes were observed, which were present as long as the perfusion was continued, but disappeared 1–2 min after the perfusion was stopped; c) difference in depth of catheter placement: Double lumen catheters typically cannot be advanced >5 mm into the nostrils [21], but the current nasal catheter usually was sited at a depth of 8–12 mm into the nostril. The authors feel that because of these technical differences they did not observe the changes with low Cl\(^{-}\) solution (sodium glucconate) given alone whereas the responses to the drugs were clear and reproducible.

The authors have now demonstrated that the addition of inhaled UTP (0.1–10.0 mmol L\(^{-1}\)) produces a dose dependent change in nasal epithelial PD reflecting Cl\(^{-}\) secretion. Higher doses did not produce any further effect. In the presence of amiloride, UTP stimulates Cl\(^{-}\) (and water) secretion across normal and cystic fibrosis epithelia [26, 27]. Subsequent nebulized application of UTP up to 10 mmol L\(^{-1}\) failed to produce any nasal PD change indicating that UTP probably acts on these receptors.

The action of UTP was brief, lasting for 30–40 min after a dose of 10 mmol L\(^{-1}\). Attempts were made to prolong the action of UTP by the addition of 10 mmol L\(^{-1}\) alpha beta methylene-adenosine 5’ diphosphate (AMP-CP), a blocker of ectonucleosidases, which regulates the breakdown of monophosphates to uridine/pyrimidine + phosphate [28]. However, this was ineffective, suggesting that the breakdown of uridine monophosphate (UMP) to uridine and phosphate is not the rate limiting step in the action of UTP.

Phenotypic and electrophysiological differences exist between CF affected human subjects and mouse models. This partly reflects the proportional species differences between the two main Cl\(^{-}\) channels in the airway epithelium, (the CFTR Cl\(^{-}\) channels and the Ca\(^{2+}\) activated Cl\(^{-}\) channels) [29], and also the expression of a proportion of wild type CFTR [30] in the mutant mouse lung. The inclusion of functional CFTR, introduced to maintain viability, may explain why the Edinburgh CF mice in particular have lower than expected nasal epithelial PDs. In the murine bowel however, CFTR mediated Cl\(^{-}\) transport plays a major role and alternative Ca\(^{2+}\) mediated Cl\(^{-}\) secretion is absent [27]. Fatal bowel obstruction therefore occurs in a large proportion of knockout CF mice because they have no residual CFTR. However, intestinal obstruction and poor growth was rare in the Edinburgh mice because they express a proportion of wild type CFTR. Thus the proportion of functional CFTR expressed in the Edinburgh mouse may limit the usefulness of this model.

In contrast to the Edinburgh model the ΔF508 model of CF mouse has increased basal PD changes and shows a larger response to sodium channel blockers and UTP. Since this model carries the mutation affecting up to 80% of human CF patients it may be more relevant model for the study of human disease.

Long acting sodium channel blockers such as loperamide in combination with UTP may have the potential to alleviate CF lung disease by augmenting respiratory mucus hydration, reducing viscosity and increasing mucociliary clearance. However, for practical therapy and convenience, a UTP analogue with similar properties and a longer action is probably required.

In conclusion, the combination of inhaled amiloride loperamide and uridine triphosphate produce nasal potential difference changes suggestive of reduced Na\(^{+}\) absorption and increased Cl\(^{-}\) secretion in the nasal mucosa of cystic fibrosis mice.

References
3. Rugolo M, Mastrocola T, Whorle C, et al. ATP and A1 adenosine receptor agonists mobilize intracellular calcium and activate K\(^{+}\) and Cl\(^{-}\) currents in normal and cystic


