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

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Sodium channel blockers and uridine triphosphate: effects on nasal potential difference in cystic fibrosis mice. S. Ghosal, C.J. Taylor, W.H. Colledge, R. Ratcliff, M.J. Evans. ©ERS Journals Ltd 2000.

ABSTRACT: Sodium channel inhibitors block the enhanced Na^+ reabsorption in cystic fibrosis (CF). Extracellular nucleotides facilitate Cl^- secretion *via* Ca^{2+} 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*^{tm1HGU}), ΔF508 homozygous mice (*cftr*^{tm1Cam}) 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*^{tm1HGU} CF mice (ΔPD 13 mV & 15 mV respectively) suggesting Na^+ blockade. The subsequent addition of UTP in a chloride-free vehicle increased the PD (ΔPD -8–-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 α-β-methylene-adenosine 5′ diphosphate. Suramin, a competitive antagonist of P2 purinoceptors blocked the action of UTP.

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.

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In cystic fibrosis (CF) there is a profound change in epithelial fluid transport affecting various tissues, particularly the airway, where viscid respiratory secretions accumulate [1]. In the majority of patients with CF, the cystic fibrosis transmembrane conductance regulator (CFTR), a chloride (Cl^-) channel protein, is not expressed in the apical membrane [2]. This leads to defective cyclic adenosine monophosphate (cAMP) mediated chloride (and water) secretion. However, alternative calcium (Ca^{2+}) activated pathways of Cl^- secretion are preserved [3]. CF airway epithelium also shows enhanced sodium (Na^+) absorption which also contributes to the dehydration of airway secretions [4]. Moreover, ineffective ciliary action prevents adequate clearance of mucus in the lumen of the airways [5], predisposing the patient to endobronchial infection and limiting the effect of host defence mechanisms and antibiotics.

Since the majority of CF subjects have no functional CFTR in the airway, the development of possible therapeutic agents has focused on sodium channel inhibitors and promoting non-CFTR Cl^- channel function. Sodium channel inhibitors block the enhanced Na^+ reabsorption and thereby help to retain the hydration of secreted mucus [6]. Some investigators suggest that sodium channel blockers may have no effect on CF because of hypertonic Cl^- concentration in the airway surface fluid [7]. Other investigators, however, have reported that the airway surface liquid is isotonic [8]. Extracellular nucleotides raise intracellular Ca^{2+} thus enhancing respiratory mucus hydration by facilitating Cl^- secretion *via* Ca^{2+} gated Cl^- channels [9, 10]. The authors would expect a combination of sodium blockers and Ca^{2+} activating nucleotide uridine triphosphate (UTP) acting *via* both pathways to produce less viscid secretions which are easier to expectorate [11].

Mouse models of CF are now available which share many electrophysiological properties with human airways [12, 13]. Cultured cells from respiratory epithelia of CF mice show defective cAMP mediated Cl^- conductance characteristic of the disease [14], and with increasing age, abnormalities in the lungs of these animals are being demonstrated [15]. The authors have examined the effect of inhaling two Na^+ channel inhibitors, amiloride or loperamide, combined with UTP on airway secretion by measuring changes in nasal epithelial potential difference (PD) of control (MF1) and CF mice.

Aims

To study the nasal PD changes using a combination of amiloride or loperamide and UTP in transgenic CF mice.

Methods

Experimental animals

Twenty-four insertional null mutant CF mice (*cftr*^{tm1HGU}) [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, (*cftr*^{tm1Cam}) [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^{-1} and met-etomidate 0.05 mg·30 g body weight^{-1}. Nasal PD was measured as described previously [16] between a 24G exploring catheter filled with 0.1 M KCl and 2% agar and a teflon reference cannula inserted subcutaneously and perfused at 0.5 mL·h^{-1} with 0.9% saline through a syringe pump (Perfusor secura; B Braun). The two electrodes were connected to calomel half cells (Russell, Aucutermuchty, Fife, Scotland, UK) by salt-agar bridges containing 1 M KCl and 2% agar. Measurements were performed using a high impedance voltmeter (Model 602; Keithley Instruments, Reading, Berkshire, UK) and recorded with a Bio-Rad chart recorder (Bio-Rad, Hemel Hempstead, Hertfordshire, UK).

Nasal PD changes were measured in a stepwise manner as follows: 1) basal PD was measured and the maximum reading recorded; 2) the mice were then divided into two groups for administration of sodium blockers, group a) received nebulized amiloride (Sigma, Poole, Dorset, UK) 1 mmol·L^{-1} and group b) received nebulized loperamide (a gift from Janssen Pharmaceutica NV, Beerse, Belgium) 1 mmol·L^{-1}, both for 2 min. The nasal PD was measured after the completion of nebulization; 3) a subgroup of eight mice (four control animals and four insertional CF animals) were administered nebulized sodium gluconate (Cl^- free vehicle) 0.15 mol·L^{-1} after the sodium channel blocker; 4) finally nebulized UTP (Sigma, UK) 0.1–10 mmol·L^{-1} dissolved in 0.15 M sodium gluconate was given to all mice in groups a) and b) and the PD was again measured. After each experiment the mice were given atipamezole 0.25 mg·30 g body weight^{-1} as antidote for the met-etomidate to enable them to recover more quickly from the anaesthetic.

A Mann Whitney U-test was used to assess significance between groups and Wilcoxon signed rank test to assess significance within the groups. A project licence approval was obtained from the Home Office to carry out the procedures.

Results

Nasal membrane PD in mice were lumen negative. The resting nasal epithelial PD in controls ranged -12–-27 mV (median -20 mV, n=20) and in *cftr*^{tm1HGU} mice -18–-38 mV (median -28 mV, n=24). The ΔF508 mice had a higher basal PD (range -25–-39 mV, median -34 mV, n=7).

Table 1. – Potential difference (PD) changes in cystic fibrosis (CF) mice and control animals with the amiloride uridine triphosphate (UTP) combination

Mouse type	Basal PD mV	ΔPD after amiloride 1.0 mmol·L^{-1} mV	ΔPD after UTP 1.0 mmol·L^{-1} mV
MF1 control n=6	-20 (-13–-24)	7	-5
CF insertional n=10	-28 (-20–-36)*	13*	-8$^{\#}$
CF ΔF508 n=7	-34 (-25–-39)*,**	17*,**	-15*,‡

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.

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^{2+} activated Cl^- 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 *cftr*^{tm1HGU} 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 2. – Potential difference (PD) changes in cystic fibrosis (CF) mice and control animals with the loperamide/ uridine triphosphate (UTP) combination

Mouse type	Basal PD mV	ΔPD after loperamide 1.0 mmol·L^{-1} mV	ΔPD after UTP 1.0 mmol·L^{-1} mV
MF1 control n=14	-22 (-12–-27)	6	-3
CF insertional n=14	-28.5 (-18–-38)*	15*	-12.5*
CF ΔF508 n=3	-34 (-30–-38)	16	-12

Data are presented as the median with the range in parentheses. *: p<0.01, comparison between control mice and insertional mutant mice. Statistical significance was not assessed in the ΔF508 group due to small numbers.

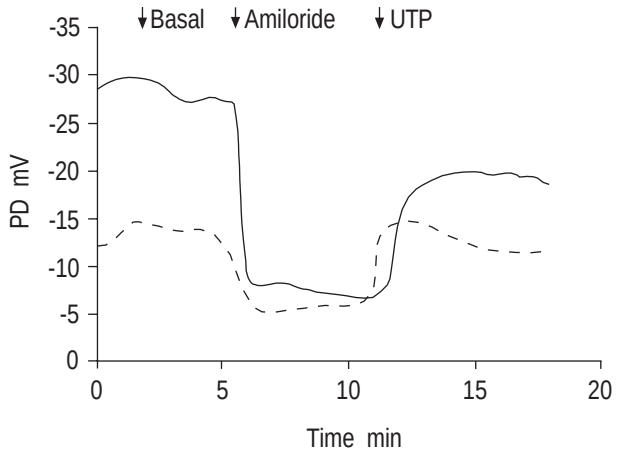

Fig. 1. – Example of nasal potential difference (PD) changes in a *cftr*^{tm1HGU} cystic fibrosis (CF) mouse (——) and a matched control (– – –) with the amiloride uridine triphosphate (UTP) combination. The PD is shown at the resting state, followed by application of amiloride causing a fall in PD which is more marked in the CF mouse. Subsequent application of UTP increases the PD and the CF mouse again shows greater changes.

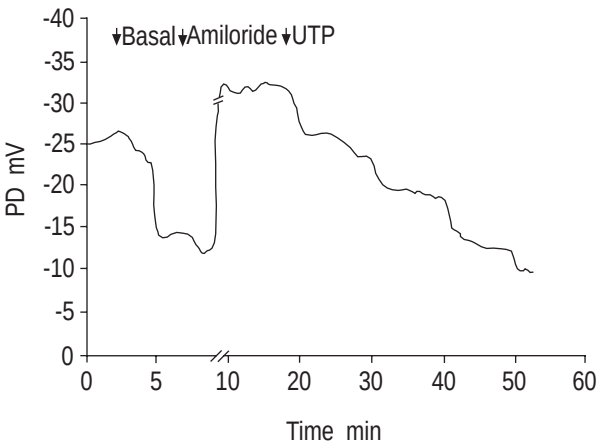

Fig. 3. – Duration of action of a single dose of cystic fibrosis (UTP) 10 mmol·L^{-1} in a *cftrtm*^{1HGU} cystic fibrosis (CF) mouse. The potential difference (PD) is shown at the resting state, followed by application of amiloride causing a fall in PD. Subsequently, the application of UTP increases the PD which declines with time reaching the value of the post-amiloride PD after 40–50 min. The action of amiloride remains for up to 4 h [14] (data not shown).

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 (*t*0.5=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

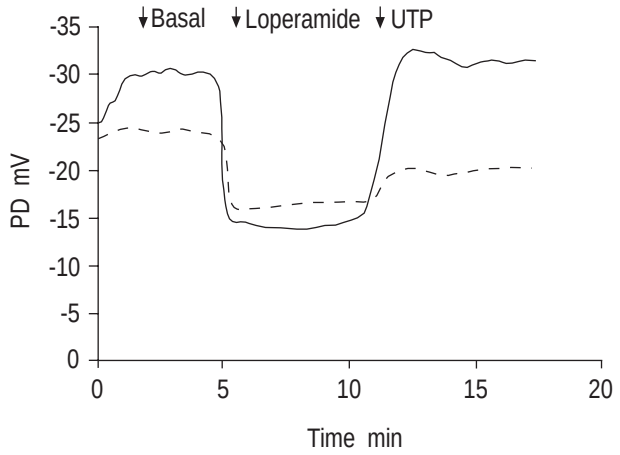

Fig. 2. – Nasal potential difference (PD) changes in a *cftrtm*^{1HGU} cystic fibrosis (CF) mouse (——) and a matched control (– – –) with the loperamide uridine triphosphate (UTP) combination. The PD is shown at the resting state, followed by application of loperamide causing a fall in PD which is more marked in the CF mouse. Subsequent application of UTP increases the PD and the CF mouse again shows greater changes.

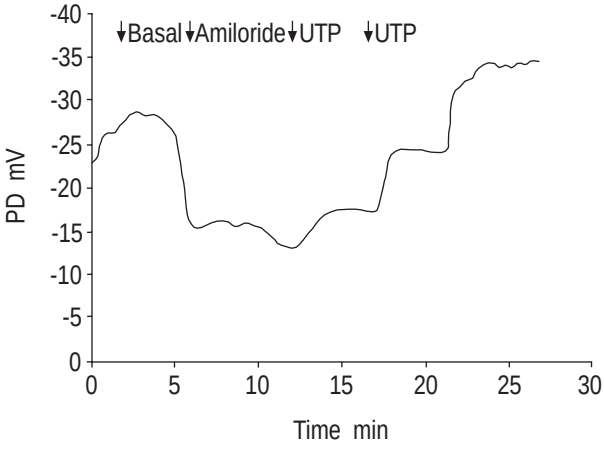

Fig. 4. – The nasal potential difference (PD) changes with logarithmic incremental doses of uridine triphosphate (UTP) from 0.1–10 mmol·L^{-1} in a *cftr*^{tm1HGU} cystic fibrosis (CF) mouse. The PD is shown at the resting state, followed by application of amiloride causing a fall in PD. Increasing doses of UTP (0.1 mmol·L^{-1}, 1.0 mmol·L^{-1}, 10.0 mmol·L^{-1}) are then applied sequentially from 0.1 to 10 mmol·L^{-1} with increasing PD changes as shown.

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 gluconate 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 gluconate) 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 $mmol \cdot L^{-1}$) 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 CF airway epithelia *via* interaction with extracellular 5-nucleotide receptors [22]. The Cl^- secretory response is mediated by chloride channels that are activated by Ca^{2+} mediated pathways. There may be additional therapeutic advantages to the use of UTP. Extracellular nucleotides induce an increase in ciliary beat frequency in human airway epithelia and goblet cel1 degranulation [23]. UTP alone, or in combination with amiloride, has been shown to increase the rate of whole lung mucociliary clearance three-fold in healthy subjects and the combination of both drugs normalized the low peripheral mucociliary clearance in CF [24].

Purine and pyrimidine nucleotides such as adenosine triphosphate (ATP) and UTP may act on extracellular P2Y receptors linked to G-proteins to produce Ca^{2+} mobilization and Cl^- secretion [25]. The CF mouse nasal tissue was pretreated with nebulized suramin (100 $\mu mol \cdot L^{-1}$) which is a competitive antagonist of P2 purinoreceptors [26, 27]. Subsequent nebulized application of UTP up to 10 $mmol \cdot L^{-1}$ 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 $mmol \cdot L^{-1}$. Attempts were made to prolong the action of UTP by the addition of 10 $mmol \cdot L^{-1}$ alpha beta methylene-adenosine 5′ diphosphate (AMP-CP), a blocker of ectonucleosidases, which regulates the breakdown of monophosphates to purine/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.

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