Modification of nasal membrane potential difference with inhaled amiloride and loperamide in the cystic fibrosis (CF) mouse

S Ghosal, C J Taylor, J McGaw

Abstract

Background **– In the airway of subjects with cystic fibrosis (CF) the combination of defective cAMP mediated chloride secretion and enhanced sodium absorption leads to dehydration of mucosal mucus and is reflected in an increased trans-epithelial potential difference (PD). The airway secretions may be less viscid and easier to expectorate if sodium (and water) reabsorption is inhibited.**

Methods **– To evaluate the response to sodium blocking agents, changes in the nasal PD in 20 transgenic CF mice were compared with 14 control mice (MF1 strain) before and after administration of nebulised amiloride and loperamide (both in a concentration of 1 mmol/l). The duration of action for both drugs was also determined after a single inhaled dose of 1 mmol/l for two minutes.**

Results **– The median basal PD was −24 mV in controls and −28 mV in CF mice ($p<0.01$). This fell in CF mice after amiloride and loperamide administration by 15 mV and 14 mV, respectively, compared with a decrease of 7 mV and 5.5 mV in controls ($p<0.01$). There was no further change in PD when loperamide was given after amiloride. This suggests that loperamide and amiloride may act on sodium absorption via similar mechanisms. Loperamide had a longer duration of action after a single administration than amiloride.**

Conclusion **– The administration of amiloride and loperamide reduces the trans-epithelial potential and inhibits sodium reabsorption in the CF mouse airway. Further studies are required to determine if the more prolonged action of loperamide could be of therapeutic use.**

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Cystic fibrosis (CF) is characterised by abnormal ion transport across epithelia.[1 2] This results in profound changes in fluid secretion and absorption in various tissues. In most patients with cystic fibrosis cystic fibrosis transmembrane conductance regulator (CFTR), a chloride channel protein, is not expressed in the apical membrane. This leads to defective cAMP mediated chloride (Cl^-) secretion. Airway epithelium also shows enhanced sodium (Na^+) absorption[2 3] which contributes to the dehydration of airway secretions. Ineffective ciliary action leads to the accumulation of viscid secretions in the lumen of airways, predisposing to endobronchial infection and limiting the effect of host defence mechanisms and antibiotics.

As there is little net movement of chloride ions in the normal human airway,[4] pharmacological approaches to activate chloride secretion via CFTR may be unsuccessful. An alternative approach is to maintain the hydration of secreted mucus by blocking the reabsorption of water and sodium ions into the airway epithelial cell. This effect has already been demonstrated both in vivo and in vitro using the sodium channel blocker amiloride,[5–7] although other investigators have found the clinical response to inhaled amiloride limited.[8] The therapeutic effect of amiloride may be limited by its relatively short duration of action (half life approximately six hours). Loperamide, which has similar action in the bowel of patients with cystic fibrosis[9] and a more prolonged half life of 11 hours, may also be beneficial in this disease.

In cystic fibrosis the nasal PD is increased mainly as a result of enhanced absorption of sodium ions.[10 11] Thus, the effects of drugs which alter the transport of sodium ions can be assessed by measuring the change in nasal PD after drug administration.[12]

Mouse models of cystic fibrosis are now available which share many of the electrophysiological properties with human airways.[13–15] We have examined the effect of the inhaled Na^+ channel inhibitors, amiloride and loperamide, on the nasal epithelium of control (MF1) and CF mice.

Methods

EXPERIMENTAL ANIMALS

Twenty CF mice were studied and 14 standard laboratory MF1 strain mice (Harlan, UK) were used as controls. Both sexes were matched for age (range 3–14 months) and weight (27–52 g). The animals were allowed food and water ad libitum. The CF mice were developed at Edinburgh[14] and were homozygous for the exon 10 insertional mutation. The breeding was subsidised by the Association Francaise de Lutte contre la Mucoviscidose, France and supplied by Charles River, UK.

A project licence approval was obtained from the Home Office to carry out the procedures.

Table 1 Changes in nasal potential difference (PD) in cystic fibrosis (CF) mice and controls after administration of amiloride and loperamide 1 mmol/l given through a nebuliser for two minutes

Mice type	*Median basal PD (mV)*	*Median PD after amiloride (mV)*	*ΔPD after amiloride (mV)*	*Median PD after loperamide (mV)*	*ΔPD after loperamide (mV)*
Control (MF1)	−24.0 (range −14 to −29) (n=14)	−16.0 (range −10 to −25) (n=14)	7.0	−15.5 (range −10 to −21) (n=8)	5.5
CF	−28.0 (range −18 to −40) (n=20) p<0.01	−14.0 (range −8 to −22) (n=16)	15.0 p<0.01	−15.0 (range −8 to −20) (n=14)	14.0 p<0.01

NASAL POTENTIAL DIFFERENCE MEASUREMENTS

The nasal potential difference was measured between a 24 G exploring catheter filled with 0.1 M KCl/2% agar and a Teflon reference cannula inserted subcutaneously and perfused at 0.5 ml/hour with 0.9% saline through a syringe pump (Perfusor Secura B Braun). The two electrodes were connected to calomel half cells (Russell) by salt-agar bridges containing 1 M KCl/2% agar. Measurements were performed using a high impedance voltmeter (Keithley Instruments Model 602) and recorded with a Bio-Rad chart recorder. Reproducibility was assessed by recording basal PD measurements from both nostrils in five control mice; all the differences were between 1 and 2 mV.

Mice were anaesthetised by intraperitoneal injection using a combination of fentanyl (0.6 mg/kg), fluanisone (20 mg/kg), and midazolam (8 mg/kg). Baseline nasal PD was then measured and the maximum reading recorded.

PROTOCOLS

Drugs were administered by inhalation over two minutes after removal of the nasal electrode. The nebulisation was achieved using a Medic-aid compressor with an Acorn system 22 nebuliser attached to a mask. The mask was occluded with parafilm and a hole made so that it fitted over the mouth and nostrils of the mice. Measurements were restarted promptly after nebulisation with the recording electrode resited in the nostril at the same depth. After each experiment the mice were allowed to recover from the anaesthetic so that the procedure could be repeated at a later date. The effect of nebulised amiloride (1 mmol/l dissolved in water, Sigma, UK) was compared with nebulised loperamide (1 mmol/l dissolved in water, a gift from Janssen Pharmaceuticals, Beerse, Belgium) given over a similar period. Changes in PD were measured after equimolar solutions (1 mmol/l) of amiloride and loperamide were administered consecutively. To assess the response to increasing drug doses, amiloride in doses of 0.1, 0.2, 0.5, 1.0, and 3.0 mmol/l and loperamide in doses of 0.1, 0.2, 0.3, 0.5, and 1.0 mmol/l were given sequentially and the PD changes recorded. In this experiment the PD changes were allowed to stabilise before the next higher concentration was given. The duration of action of the drugs was also studied after a standard single dose of both agents (1 mmol/l). The changes in PD were measured at baseline, immediately after nebulisation, and then every two hours for eight hours.

DATA ANALYSIS

The Mann–Whitney U test was used to assess significance between groups and the Wilcoxon signed rank test to assess significance within the groups.

Results

Significant differences were found between the CF mice and controls for baseline PD and the changes in PD values for both drugs (table 1, p<0.01). The median changes in PD seen in CF mice after the drugs were administered consecutively were −18 mV for amiloride (range −13 to −25) and −17 mV for loperamide (range −13 to −24) (NS, n=11). As loperamide failed to produce any further change in PD, this suggests that it may act via similar mechanisms since the Na^+ channels are already maximally inhibited by amiloride.

Figures 1 and 2 show the responses to increasing doses of drug. The maximum changes

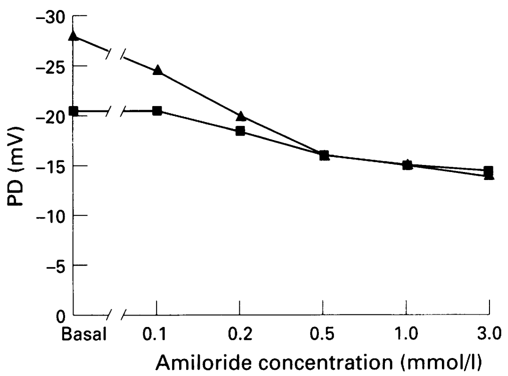

Figure 1 Median changes in potential difference (PD) with increasing amiloride concentrations in control (■) and CF (▲) mice (n=7 for both).

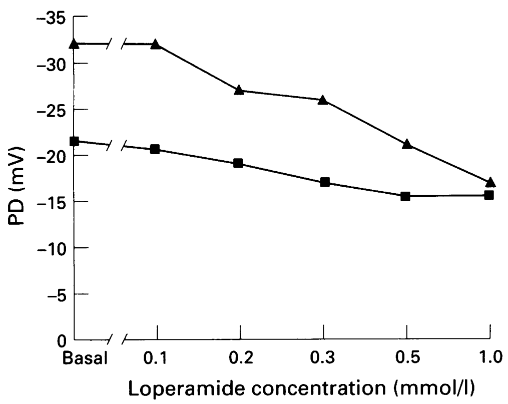

Figure 2 Median changes in potential difference (PD) with increasing loperamide concentrations in controls (■, n=8) and CF (▲) mice (n=11).

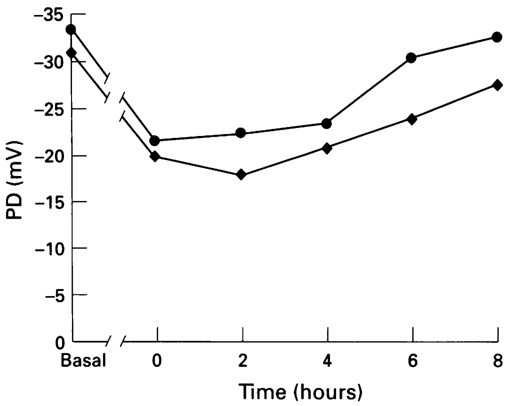

Figure 3 Duration of median changes in potential difference (PD) after a single two minute inhalation of 1 mmol/l amiloride (●) or loperamide (◆) in CF mice (n=7).

in PD with amiloride occurred at about 1 mmol/l in both CF and control mice. Although a similar pattern was observed with loperamide, the PD plateaued between 0.5 and 1.0 mmol/l in the control mice (fig 2), but this plateau was not observed in the CF mice.

The duration of action of both drugs was studied in CF mice (fig 3). Loperamide had a longer duration of action than amiloride, with the effect still present at eight hours after a single dose of 1 mmol/l while with amiloride the effect started to decay after four hours. This change in PD at eight hours after loperamide (−27.5 mV), although less than the peak effect at two hours, was still lower than the baseline value ($p<0.05$, Wilcoxon signed rank test).

Discussion

The mean basal PD observed in the CF mice was similar to published measurements on both Edinburgh and knockout mice.[14 16] The responses of the CF mice to amiloride were significantly different from the controls, showing that this CF mouse model exhibits the enhanced Na^+ absorption characteristic of the disease in humans.

A fall in nasal PD after amiloride administration has previously been seen in human and animal studies.[13 17] Similar changes have been described with loperamide using in vitro techniques in the bowel.[10] We have shown that loperamide has a relatively long duration of action compared with amiloride which suggests that less frequent administration may achieve a similar effect. We have also shown that the responses to loperamide and amiloride were concentration dependent as suggested by previous investigators.[10 18 19] However, both amiloride and loperamide were poorly soluble in water so it was not possible to achieve concentrations above 1 mmol/l for loperamide and 3 mmol/l for amiloride. Loperamide is more soluble in ethanol and since this agent has been administered previously by inhalation (L Borgstrom, personal communication), it would be interesting to test the duration of action and PD response with higher concentrations in an ethanol base.

Sodium uptake into nasal epithelia is regulated mainly by the amiloride sensitive sodium channel[20] which is a transmembrane protein consisting of three homologous subunits[21 22] present in many tissues[23] of various species. Studies using human nasal epithelium in the form of cell attached patches have shown that there is a small proportion (<6%) of non-selective cation channels involved in sodium transport[21] which cannot be blocked by amiloride. The amiloride sensitive sodium channel appears to be relatively specific and is unaffected by amiloride analogues which have less specificity for the sodium channel.[24] The effect of loperamide is probably produced by a similar action on the amiloride sensitive sodium channel since the effect of loperamide was absent after maximal inhibition of the sodium channel by pretreatment with amiloride.

In addition to blocking apical sodium absorption, amiloride has been shown to induce chloride secretion in airway tissue by hyperpolarising the apical membrane and generating a driving force for secretion of chloride ions. However, secretion of chloride ions is absent in subjects with cystic fibrosis and the inhibitory action of loperamide on secretion of chloride ions seen in canine tracheal epithelium may be of little clinical relevance.[25]

Hardcastle and colleagues found that absorption of sodium ions in the rat small bowel was quantitatively inhibited by loperamide.[26] It seemed unlikely that loperamide inhibited the sodium pump situated on the basal membrane since it did not inhibit mannose-linked absorption of sodium ions (which works via a neutral channel linked to the sodium pump) and it did not reduce the activity of the enzyme Na^+-K^+ ATPase. It was therefore suggested that loperamide acts on sodium absorbing sites situated on the apical side of the membrane. This supports our view that the changes in nasal PD observed in this study are due to inhibition of sodium ion transport alone.

In conclusion, we have shown that loperamide inhibits absorption of sodium ions in the CF mouse airway. This effect appears to be prolonged compared with amiloride. Thus, loperamide alone or in combination with other agents that stimulate secretion of chloride by calcium activated pathways such as uridine triphosphate[27] may be of use in the treatment of cystic fibrosis.

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