The effect of topical benzamil and amiloride on nasal potential difference in cystic fibrosis

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The lung disease in cystic fibrosis (CF) is thought to be the result of abnormal ion flux across bronchial epithelium. This consists of reduced apical chloride secretion by the defective cystic fibrosis transmembrane conductance regulator (CFTR), the product of the CF gene, and a two-fold increase in sodium absorption through apical epithelial sodium channels (ENaC) [1–3]. Attempts to correct the abnormal ion flux have consisted of gene therapy to replace the function of defective CFTR, or pharmacological therapy with chloride channel openers or sodium channel blockers. The nasal epithelium provides a useful site to test novel potential therapies as it is readily accessible and nasal potential difference (PD) can be easily and reproducibly measured [4–7].

Early attempts to correct the increased sodium reabsorption in CF used amiloride, a pyrazinoylguanidine [8], which reversibly inhibits ENaC [9]. Amiloride has been shown to reduce sodium reabsorption transport across airway epithelia and reduce nasal PD in healthy subjects and patients with CF [1, 2] but three randomized controlled trials of long term administration of aerosolized amiloride on lung function produced conflicting and disappointing results [10–12]. The most likely reason for the lack of benefit is that amiloride has a very short duration of action and is cleared rapidly from the lungs [13–16]. Longer acting sodium channel blockers might, therefore, prove more effective.

Benzamil, a benzyl substituted amiloride analogue, is a longer acting and more potent sodium channel inhibitor than amiloride in cultured human CF nasal epithelium [8, 17]. An open uncontrolled pilot study has suggested that benzamil may have a longer duration of action on nasal PD than amiloride when given topically [18]. The authors have therefore performed a randomized, double blind, placebo controlled study to compare the effects of topically applied benzamil and amiloride on nasal PD in CF.

Material and Methods

Subjects

Ten subjects, median age 22 yrs (range 18–30) were recruited from the adult clinic at Nottingham City Hospital. All had elevated sweat sodium (>60 mmolL−1) and
chloride (>70 mmol·L⁻¹) concentrations as well as clinical and radiological features consistent with the diagnosis of CF. The genotype of all but one subject were known: 3 patients were homozygous for AF508, 3 patients had one ΔF508 the other mutation being unknown and there were three patients with AF508/Q1493X, AF508/621 + 1 G>T and G551D/621 + 1 G>T respectively. Subjects were excluded from the study if there was a previous history of nasal surgery, current nasal symptoms, or if they had had a pulmonary exacerbation or upper respiratory tract infection within the preceding 2 weeks. The study was performed between September 1997 and July 1998. The study protocol was approved by the Nottingham City Hospital Ethics Committee and all subjects gave written informed consent.

Nasal potential difference measurement

Nasal PD was measured using an adaptation of the method described by Middleton et al. [19] and used previously by the current authors [7]. Briefly, the exploring electrode consisted of a paediatric urinary catheter (Foley size 8) filled with electrode cream (Signa Creme; Parker Laboratories Inc, Orange, NJ, USA), connected to a high impedance voltmeter (RS Components, Birmingham, AL, USA) by a silver/silver chloride electrode (Specialized Laboratory Equipment, South Croydon, UK). Skin drilling with a diamond tip dental burr (Kent dental, Gillingham, UK) was performed on the right forearm to superficially abrade the skin. A silver/silver chloride electrode (Specialized Laboratory Equipment) was placed over the abraded skin and electrode cream injected through a hole in the top of the electrode to allow contact. The reference electrode was connected to the same voltmeter. Before recordings were made the offset of the electrodes was measured and appropriate corrections made. The exploring electrode was passed along the floor of the nose without direct vision. The catheter was advanced until the maximal PD had been passed and then gradually withdrawn. The highest voltage stabilized for 10 s, was recorded. Three measurements were made from each nostril and a mean for each side obtained. The mean of the two sides was used to give the nasal PD reading for each subject at each time point. The exploring catheter was removed after each reading. All nasal PDs were lumen negative with respect to the reference electrode. The authors have previously shown that nasal PD measurement is repeatable in their hands [7].

Study drugs

Benzamil and amiloride were purchased from Sigma (Poole, Dorset, UK). Amiloride (1 × 10⁻³ M) and benzamil (1.7 × 10⁻³ M) solutions were prepared at their limit of solubility in 0.9% sodium chloride [17]. 0.9% sodium chloride was used as placebo.

Protocol

The study had a randomized, double blind, placebo controlled, crossover design. Randomization was performed by the Nottingham City Hospital Pharmacy using an in house random number randomization schedule. The treatment order for each subject was assigned randomly according to the randomization schedule above. The study drug was made up in the pharmacy and both subject and investigator were blinded to the nature and order of treatments. Subjects attended at the same time of day on three occasions each separated by ≥48 h. Subjects were asked to omit any inhalers or nebulized drugs on the study days. Baseline nasal PD (basal PD) was measured, 0.4 mL of the study drug (benzamil, amiloride) or placebo (0.9% sodium chloride) was applied topically to each nostril using a nasal spray (sterilized Beconase® 200; Allen and Hanburys, Uxbridge, UK) and repeat nasal PD measurements were made at 15 and 30 min, 1, 2, 4 and 8 h. The sterilization procedure allowed the nasal spray to be used as a passive carrier.

Analysis

All values are expressed as mean±SEM. Baseline nasal PD values were compared by two way analysis of variance (TWAOV) (SPSS Inc., Chicago IL, USA). The maximal change in nasal PD (PDmax), time for PDmax to return to 50% of the baseline PD (t0.5) and the area under the curve (AUC) were compared using TWAOV followed by paired Student’s t-tests where significant differences were seen. AUC was quantified using the statistical package Graphpad Prism 2.0 (Graphpad Software, Inc., San Diego, CA, USA), by calculation of the AUC which was presented as weighted means over 8 h. Increases from baseline were recorded as zero. In all analyses a p-value <0.05 was regarded as statistically significant.

Results

Basal PD values were -36.8±0.7 mV, -36.0±1.6 mV and -39.1±1.4 mV on the benzamil, amiloride and placebo study days respectively (p=0.1, NS). There was no order effect (p=0.1, NS).

Benzamil had a similar maximum effect but a more prolonged action than amiloride (fig. 1). PDmax was 20.6±0.9 mV after benzamil, 20.3±1.6 mV after amiloride and 3.6±2.3 mV after placebo. TWAOV of PDmax, AUC and t0.5 showed a significant difference between the three treatments (all p<0.0001). PDmax values for amiloride and benzamil were significantly different from placebo.

Fig. 1. – The effect of topically applied amiloride and benzamil or placebo on nasal potential difference (NPD) in 10 subjects with cystic fibrosis. Data are expressed as mean±SEM. ∆: change; □: placebo; ▲: amiloride; ▼: benzamil.
There were, however, some quantitative differences. The results, showing that it was longer acting than amiloride, with benzamil were qualitatively similar to the current 0.7 h compared to 0.6 h in the current study. Their values for benzamil, amiloride and placebo were 11.8±1.6 mV, 2.8±0.4 mV and 0.6±0.4 mV, respectively. Both benzamil and amiloride were significantly different from placebo (p<0.0001 and p<0.001 respectively). The AUC value for benzamil was significantly greater than amiloride (95% confidence interval (CI) for the difference 5.3–12.7 mV, p<0.0000). t0.5 values were 4.3±0.7 h for benzamil and 0.6±0.1 h for amiloride (95% CI for the difference 2.0–5.3 h, p<0.001). There were no side-effects reported in the study.

Discussion

In this double blind, placebo controlled crossover study it has been shown that benzamil and amiloride have a similar maximum effect on nasal PD but that the effect of benzamil is longer lasting. This suggests that benzamil has the potential to be a more useful sodium channel blocker than amiloride for the treatment of CF lung disease.

The magnitude of the nasal PDs in the CF subjects were similar to those of a previous study [7]. The maximum effect (52% increase from baseline) and short duration of action of amiloride in this study is similar to that previously reported [6, 7, 10, 18, 20]. The short duration of action of amiloride is likely to explain the lack of efficacy seen in studies of regular nebulized amiloride given either short (2 weeks) [10] or long-term (6 months) [11, 12] to the lungs in CF patients. Nebulized amiloride, given four times daily, could only be expected to block ENaC sodium channels for a fraction of the day.

The maximal effect of benzamil was similar to amiloride in this study. Benzamil produced a much more sustained increase in nasal PD than amiloride with a t0.5 seven times greater than amiloride. The effect of benzamil was still 50% of maximal at 4 h and the PD had not returned to baseline at 8 h in contrast to amiloride where the effect was only 35% of maximum at 1 h and was negligible at 2 h. These results suggest that regular administration of benzamil 3–4 times daily would be likely to produce a marked and sustained reduction in sodium reabsorption.

The only other study to compare the effect of amiloride and benzamil is an open parallel group pilot study by HOFMANN et al. [18]. In their study the maximal PD from baseline and duration of effect were measured for 6 h after different concentrations of amiloride and benzamil. Their findings, using the same concentration of amiloride (10-3 M), were similar to those of the current study with a t0.5 of 0.7 h compared to 0.6 h in the current study. Their t50 values were 4.3±0.5 h for benzamil and 0.6±0.1 h for amiloride (95% CI for the difference 2.0–5.3 h, p<0.001). There were no side-effects reported in the study.

In conclusion, it has been shown that benzamil produces a more sustained improvement in nasal potential difference than amiloride in cystic fibrosis. Benzamil may therefore hold promise as a long acting sodium channel blocker for the treatment of cystic fibrosis lung disease. Further studies are needed to address the long term benefits and safety of benzamil given regularly by nebulization to the lower respiratory tract.

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References

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