

## No added benefit from nebulized amiloride in patients with cystic fibrosis

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**ABSTRACT:** In cystic fibrosis (CF) airway epithelial sodium absorption is increased 2-3 fold. Since sodium absorption is inhibited by the sodium channel blocker amiloride, our aim was to assess its therapeutic benefit in cystic fibrosis.

A randomized, double-blind, placebo-controlled, cross-over trial of nebulized amiloride was performed in 23 patients with cystic fibrosis. Amiloride or placebo was administered four times daily for two six month periods. Existing treatment was continued, and any infective exacerbations treated in the usual way. Fourteen patients completed the study.

No significant changes occurred in forced expiratory volume in one second, forced vital capacity, oxygen saturation, body weight, sputum volume, culture and rheology, serum urea, and electrolytes, white cell count and erythrocyte sedimentation rate during either treatment period. The frequency of infective exacerbations was also not different in either treatment period.

We were thus unable to confirm the benefit shown in the only other clinical trial of nebulized amiloride in cystic fibrosis and conclude that, in the presence of established treatment for cystic fibrosis lung disease, nebulized amiloride offers no additional clinical benefit.

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In cystic fibrosis (CF) the absorption of sodium across airway epithelia is increased 2-3 fold [1, 2]. As a result of the associated increase in osmotic water absorption [3, 4], the hydration of the airway surface liquid (ASL) is likely to be reduced. This may interfere with mucociliary clearance (MCC) and affect bacterial adherence, thus contributing to pulmonary infection. Application of the sodium channel blocker amiloride [5] to the mucosal surface of CF airway epithelia reduces sodium absorption both *in vitro* and *in vivo* [1, 6]. Amiloride may, therefore, have therapeutic potential in CF. Although the rationale for the use of nebulized amiloride in CF is based on inhibition of airway epithelial sodium absorption, it is possible that other effects of amiloride, such as antibacterial activity [7], inhibition of neutrophil activation [8], and anti-inflammatory effects [9], may contribute to its clinical effect.

Oral administration of amiloride does not produce sufficiently high concentrations at the airway surface to inhibit sodium absorption [10], but these can be achieved by inhalation using a nebulizer [10, 11]. The measured duration of a significant effect of nebulized amiloride on lower airway potential difference is 30 min [10]. In CF patients, inhalation of a single dose of amiloride increases mucociliary clearance, an effect which lasts up to 40 min and which may be increased with chronic administration [12].

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In a previous study of the effects of long-term (6 months) treatment in adult CF patients, nebulized amiloride was found to significantly reduce the rate of decline in forced vital capacity (FVC) and to improve sputum rheological properties [13]. However, the effects of amiloride in the latter study were investigated in the absence of any regular bronchodilator or antibiotic therapy, and were preceded by a period of intensive intravenous antibiotic treatment. The rate of decline in forced vital capacity observed, although lower during the period of amiloride treatment, was relatively high during both amiloride and placebo treatment periods in relation to pre-study baseline values. The aim of the present study was to investigate further the effects of long-term nebulized amiloride as an addition to existing medication, in order to provide a clearer indication for the role of amiloride in the context of established therapy.

### Patients and methods

#### Recruitment of patients

Local Ethics Committee approval for the main trial and for associated studies of mucociliary clearance was obtained. Patients were recruited from the adult and

paediatric cystic fibrosis clinics at the Royal Brompton National Heart and Lung Hospital. All patients had a forced vital capacity (FVC) of at least 40% of predicted values. Patients were excluded from the study if they were pregnant or awaiting heart-lung transplantation. The baseline characteristics of the 14 patients who completed the trial, together with details of their regular medication, are listed in table 1.

#### Administration of amiloride

Amiloride hydrochloride solution was prepared at a concentration of 1 mg·ml<sup>-1</sup> (3.8 mM) in 0.13% sodium chloride, and sterilized by filtration through a 2.0 µm filter. Sodium chloride, 0.13%, was used as placebo. Both were administered at a volume of 4.5 ml four times daily. The solution was nebulized to dryness using a portable Medix ultrasonic nebulizer, breathing through the mouth-piece. Pulmonary deposition of amiloride through an identical nebulizer (the Fisoneb) had been evaluated in an earlier study, and found to be faster and more efficient than through a jet nebulizer, with which it was

compared [14]. Using the Medix ultrasonic nebulizer, 10% of an administered dose of amiloride is deposited in the lung within 5 min. The ratio of central to peripheral deposition (corrected for lung volume) is 4.9, and not significantly different to that obtained using a jet nebuliser [14]. The small size of the ultrasonic nebulizer also made it more suitable for patients to use at work or school. Frequent administration was required because of the short duration of the measured effects of nebulized amiloride [11, 12].

#### Trial structure

The structure of the trial was a randomized double-blind, placebo-controlled, cross-over study. The length of each treatment arm was 6 months, and the two were separated by a 1 month wash-out period. There were 1 month run-in and run-out periods at the beginning and end of the study. The run-in period was repeated, after appropriate treatment, if a patient developed an infective exacerbation during that month. All existing medication was continued and any infective exacerbations or other acute events managed in the usual way.

Table 1. - Baseline characteristics of the 14 patients who completed the trial

Sex M/F	12/4
Age yrs	24 (2)
range	9-47
Body weight % pred	85 (4)
FEV <sub>1</sub> % pred	40 (5)
FEV <sub>1</sub> 12 months prior to trial % pred	40 (4)
FVC % pred	68 (6)
FVC 12 months prior to trial % pred	66 (5)
Oral antibiotic courses in preceding 12 months	1.1 (0.3)
Intravenous antibiotic courses in preceding 12 months	1.1 (0.4)
<b>Sputum microbiological findings</b>	<b>Patient Nos</b>
<i>Staphylococcus aureus</i>	9
<i>Pseudomonas aeruginosa</i>	8
<i>Haemophilus influenzae</i>	1
<i>Pseudomonas cepacia</i>	1
Mean daily sputum production	2.1 (0.2)
<b>Existing medication</b>	<b>Patient Nos</b>
Pancreatic supplements	13
Inhaled bronchodilators	13
Inhaled steroids	10
Inhaled antibiotics	8
Glibenclamide	1
Oral bronchodilators	5
Oral steroids	4
Oral antibiotics	10
(flucloxacillin, cefaclor or tetracycline)	
Insulin	1

Results are expressed as mean and SEM in parenthesis, unless otherwise indicated. FEV<sub>1</sub>: forced expiratory volume in one second; FVC: forced vital capacity. Sputum volume was recorded using a simple numerical scale from 0 (no sputum) to 5 (2 standard sputum pots daily).

#### Measures of outcome

Daily measurements of peak expiratory flow rate (PEFR) before and after nebulization of the trial solution were recorded by the patients throughout the trial. Patients also kept a record of compliance. Sputum volumes were recorded by the patients on a daily basis, using a simple numerical scale ranging from 0 (no sputum) to 5 (2 standard sputum pots). On a monthly basis, patients attended the hospital for clinical assessment, at which time measurements were made of oxygen saturation, forced expiratory volume in one second (FEV<sub>1</sub>), FVC and body weight. Sputum samples were collected for microbiological culture and for rheological measurements. Serum urea, creatinine and electrolytes, erythrocyte sedimentation rate (ESR) and white cell count were measured at the beginning and end of each 6 month treatment period. In five patients, mucociliary clearance was also measured at the end of each 6 month treatment period, following nebulization of the current trial medication.

#### Rheological measurements

Sputum samples were collected during physiotherapy and stored frozen at -18°C until measured. After complete thawing, any saliva in the sample was removed by blotting. Measurements were then made using the oscillation package of a Carri-med controlled stress rheometer, equipped with 2 cm flat plates at a temperature of 20°C, at a constant displacement of 500 µrad and at oscillation frequencies of 1-5 Hz [15]. Preliminary studies were carried out to ensure that the imposed stress was within the linear viscoelastic range. Recordings were made of dynamic viscosity (η'), storage modulus (G') and loss modulus (G'').

### Mucociliary clearance studies

Mucociliary clearance (MCC) was measured using a radioaerosol technique [16]. Polystyrene particles labelled with the radionuclide  $^{99}\text{Tc}$  were inhaled *via* the mouth. Initial radioaerosol lung deposition and subsequent clearance (measured every 30 min for 6 h) was monitored with two suitably collimated scintillation counters, axially opposed anteroposteriorly over the mid-sternum. A final count was made after 24 h, to estimate the proportion of the radioaerosol deposited in the non-ciliated airways (alveolar deposition). The initial topographic distribution of the aerosol within the lungs (penetration index) was ascertained by means of a gamma camera linked to a computer [17].

### Statistics

Lung volumes and sputum rheological measurements were compared using a repeated-measures analysis of variance. The Mann-Whitney U-test was used to compare means. Results are shown as mean (SEM) for convenience, unless otherwise indicated.

## Results

### Compliance and side-effects

Twenty three patients were recruited into the trial, of whom 14 completed both treatment periods. Of those who failed to complete the study, four were unable to comply with the treatment regimen, one was unable to tolerate the taste of amiloride, and three (two receiving amiloride and one receiving placebo) complained of symptoms of bronchospasm following nebulization, which was supported by an associated fall in peak expiratory flow rate (PEFR). One patient died after developing a severe infective exacerbation, but spirometry had remained stable for 5 months whilst receiving nebulized amiloride prior to this event. Five of the patients completing the trial reported a bitter taste while receiving amiloride, but the medication was otherwise well-tolerated. Mean (SEM) compliance as recorded by the patients was 85 (3)% during the active and 81 (4)% during the placebo treatment periods.

### Pulmonary function, haematological parameters and pulmonary infection

No significant change in  $\text{FEV}_1$  or FVC occurred at any time-point during either the amiloride or placebo treatment periods (fig 1). Mean (SEM)  $\text{FEV}_1$  changed from 40.1 (4.7)% predicted to 39.9 (4.8)% predicted during treatment with placebo, and from 38.8 (4.6)% predicted to 40.6 (4.8)% predicted on amiloride. Mean FVC changed from 67.8 (5.5)% predicted to 69.2 (6.3)% predicted during placebo treatment, and from 68.8 (5.6)% predicted to 66.1 (5.8)% predicted during treatment with amiloride. Comparison of the variation in lung volumes during each treatment period using a repeated-measures analysis of variance also showed no significant differences between

the two treatment periods. Retrospective analysis of recorded spirometry in the 12 months preceding entry to the trial in these 14 patients showed that no significant change had occurred in the mean  $\text{FEV}_1$  or FVC during that time (table 1). PEFR, oxygen saturation, body weight, serum urea and electrolytes, peripheral white cell count and ESR showed no significant change during either the amiloride or placebo treatment periods (table 2). There was also no significant difference in the mean number of oral or intravenous antibiotic courses required during either treatment period (table 3). When compared to the number of antibiotic courses required in the 12 months preceding the trial, the total number of intravenous antibiotic courses given during the two 6 month periods was not different. A greater number of oral antibiotic courses were prescribed during the trial period, a probable reflection of the increased frequency of clinic attendance. In patients colonized with *Staphylococcus aureus* and *Pseudomonas aeruginosa* the mean bacterial densities (number of bacteria  $\log_{10}$  per ml of sputum) were compared from the six sputum samples obtained during each of the two 6 month treatment periods. No significant

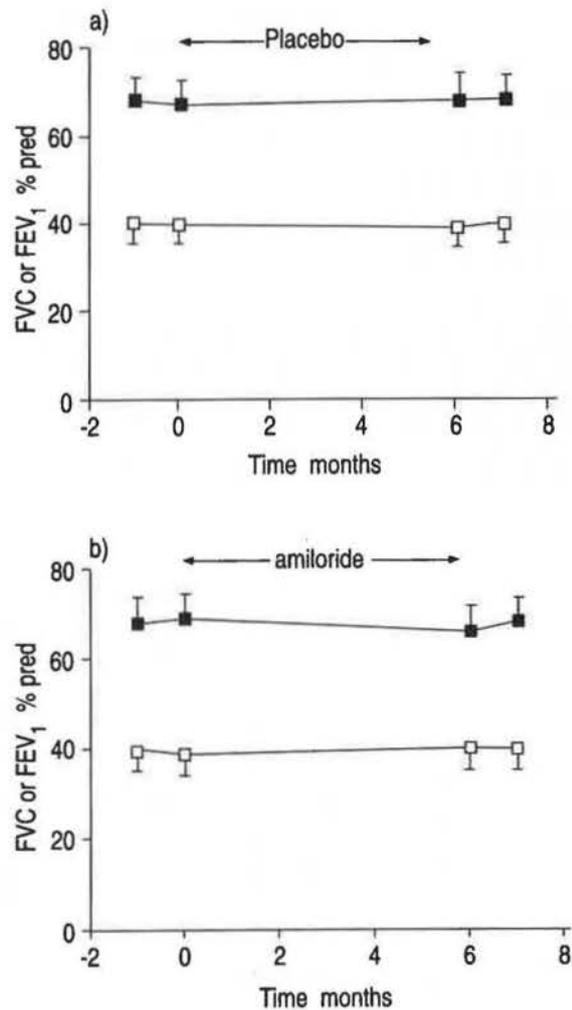


Fig. 1. - Effect of 6 months treatment with amiloride or placebo on forced expiratory volume in one second ( $\text{FEV}_1$ ) and forced vital capacity (FVC) in the 14 patients completing both treatment periods □:  $\text{FEV}_1$  % predicted; ■: FVC % predicted.

Table 2. – Effect of amiloride and placebo on peak expiratory flow rate (PEFR), oxygen saturation, body weight, serum urea, creatinine and electrolytes, peripheral white cell count and erythrocyte sedimentation rate (ESR)

	Pre-amiloride		Post-amiloride		Pre-placebo		Post-placebo	
PEFR $l \cdot \text{min}^{-1}$	326	(32)	327	(32)	308	(32)	307	(39)
O <sub>2</sub> saturation % n=12	93	(1)	93	(1)	94	(1)	93	(1)
Body weight % pred	85	(4)	86	(4)	86	(4)	86	(4)
Serum sodium $\text{mmol} \cdot \text{l}^{-1}$	139	(0.6)	139	(0.7)	139	(0.7)	139	(0.8)
Serum potassium $\text{mmol} \cdot \text{l}^{-1}$	4.3	(0.07)	4.3	(0.10)	4.3	(0.07)	4.3	(0.08)
Serum urea $\text{mmol} \cdot \text{l}^{-1}$	3.9	(0.2)	4.5	(0.2)	4.4	(0.3)	4.0	(0.4)
Serum creatinine $\text{mmol} \cdot \text{l}^{-1}$	87	(4)	73	(6)	71	(5)	84	(5)
White cell count $\times 10^9 \cdot \text{l}^{-1}$	10.7	(0.8)	11.4	(0.7)	10.9	(0.8)	12.5	(0.8)
ESR $\text{mm} \cdot \text{h}^{-1}$ n=12	13	(4)	14	(5)	13	(6)	16	(5)

Results are expressed as mean and SEM in parenthesis. n=14, unless otherwise indicated. No significant change in any parameter occurred during either 6 month treatment period.

Table 3. – Number of oral and intravenous antibiotic courses required per patient during the trial compared with those needed during the preceding 12 months

	Oral antibiotic courses	Intravenous antibiotic courses
Placebo treatment period	1.1 (0.2)	0.7 (0.3)
Amiloride treatment period	1.2 (0.3)	1.0 (0.2)
Both treatment periods	2.4 (0.4)*	1.7 (0.5)
12 month period preceding the amiloride trial	1.1 (0.3)*	1.1 (0.3)

Results are expressed as mean and SEM in parenthesis. There was no significant difference in the number of antibiotic courses required during either treatment period. The total number of intravenous antibiotic courses was not significantly different to the number required during the preceding 12 months. Significantly ( $p < 0.05$ ) more oral antibiotic courses were prescribed during the trial period than during the preceding 12 months. \*:  $p < 0.05$ .

differences were found. There were also no significant differences in the mean daily sputum volume scores (1.9 (0.2) during both the amiloride and placebo treatment periods) recorded by the patients during the two treatment periods.

#### Sputum rheology

Sputum rheological measurements were performed on a total of 112 samples from 10 of the patients completing the trial. At least four samples were analysed from each arm of the trial for each patient. The remaining four patients failed to produce sputum samples of sufficient volume for analysis. The sputum of seven of the patients was consistently purulent in appearance, and the samples from the remaining 3 patients were mucopurulent. Statistical analysis of the results using a repeated-measures analysis of variance test revealed no significant overall difference in rheological properties between the

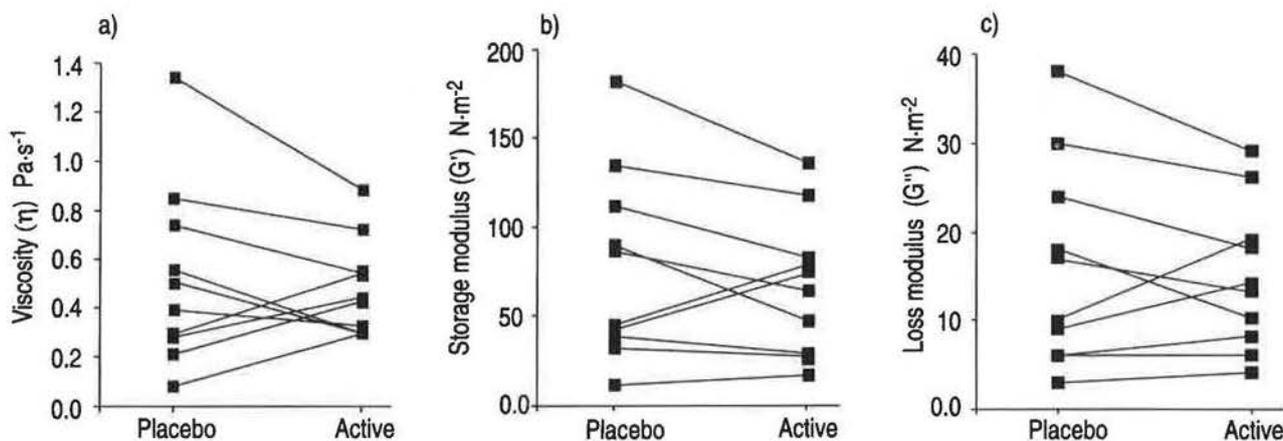


Fig. 2. – Effect of nebulized amiloride on: a) viscosity ( $\eta$ ); b) storage modulus ( $G'$ ); and c) loss modulus ( $G''$ ) at 5 Hz. Samples were measured at 20°C. Each point shown is the mean value for 4–6 sputum samples collected from that patient during each 6 month treatment period. Between 1 and 3 aliquots were measured for each sample of sputum depending on its volume.

active and placebo treatment periods. Additionally, no significant time-dependent change in rheological properties during either the amiloride or placebo treatment periods occurred. However, in the five patients from whom sputum samples showed the highest mean baseline values there was a reduction in  $\eta'$ ,  $G'$  and  $G''$  values (fig. 2). This group of patients could not be distinguished from the other five from whom sputum was obtained for rheological analysis, in terms of sputum microbiological findings, inhaled and oral medication or pulmonary function. All had macroscopically purulent sputum.

#### Mucociliary clearance

In the five patients in whom MCC studies were performed at the end of each respective trial period, alveolar deposition (amiloride=49.6 (9.0)%, placebo 46.0 (9.6)%) and penetration index (amiloride=0.75 (0.13), placebo=0.76 (0.15)) were similar for both amiloride and placebo studies. There was no significant difference between amiloride and placebo studies in the mean percentage tracheobronchial clearance at any of the 30 min time-points. Similarly, no significant difference in mean total MCC was detected between the two studies.

#### Discussion

This trial has failed to show clinical benefit from a 6 month course of nebulized amiloride in a stable group of CF patients in whom existing treatment was continued. Furthermore, no significant overall effect of amiloride on sputum rheological properties was identified. These findings contrast with those of the previous study by KNOWLES *et al.* [13] of long-term nebulized amiloride in CF patients, in that no mean decline was observed in spirometric measurements during either the active or placebo treatment periods, and no beneficial effect of amiloride was detected.

This trial was similar to that of KNOWLES *et al.* [13] in terms of the dose and frequency of administration of nebulized amiloride and the length of the treatment period. The patients taking part in this study were similar in terms of age and sputum microbiological findings to those in the previous trial, although mean FEV<sub>1</sub> and FVC were lower.

The main differences between this and the previous study were in trial design. In the trial of KNOWLES *et al.* [13], patients were given an elective course of intravenous antibiotics before each treatment period, during which all existing regular antibiotic and bronchodilator therapy was discontinued. The subsequent rate of decline in lung function observed, although lower during the amiloride treatment period, was relatively high. This decline may have resulted from withdrawal of existing regular antibiotic and bronchodilator therapy, or from the creation of unrepresentatively high baseline values as a result of the preceding intravenous antibiotic therapy. Additional differences are that no record of patient compliance was reported in the study of KNOWLES *et al.* [13]

and it did not include any children. Although a different type of nebulizer (the Fisoneb) was used for delivery of amiloride in this trial, associated studies have confirmed deposition in the lungs, and that amiloride is effective in reducing nasal potential difference using this dose and delivery system [18].

A possible explanation for the detection of an effect of amiloride in the trial of KNOWLES *et al.* [13] is that such benefit may be so small when compared to other existing therapies for CF lung disease that it is only detectable in their absence. Such benefits are unlikely to make a major contribution to the existing management of CF lung disease in the patient group studied. Alternatively, the preceding period of intravenous antibiotic therapy may have improved the access of amiloride to the airways, thus improving its therapeutic potential.

The differences in trial design discussed above, may also explain why no effect of amiloride on sputum rheological properties was observed, in spite of the much larger number of sputum samples analysed in this study. The ranges of values for dynamic viscosity and storage modulus measured in this study are similar to those previously reported for mucoid and mucopurulent CF sputum [19]. They also further illustrate the wide variation in these values both within and between patients. Thus, to detect a statistically significant beneficial effect of a therapeutic agent on sputum rheological properties, this effect would need to be large. A possible source of error in this study was the use of blotting to remove excess saliva, a procedure which may have interfered with the water content of the sol phase. However, as all samples were handled in identical fashion by a single operator, any error would be consistent between samples. Repeated freezing and thawing of human respiratory mucus has been shown to have no effect on its rheological properties [20]. This study does not, therefore, exclude the possibility of a small beneficial effect of amiloride on sputum rheological properties.

No beneficial effect of amiloride on MCC was detected, possibly as a result of the small number of patients volunteering for this part of the trial. However, major effects on MCC can be detected in as few as five patients using radioaerosol clearance techniques [21], suggesting that any possible beneficial effect of amiloride on MCC is small. In a separate study, the effects of acute administration of nebulized amiloride on MCC in seven adult CF patients have been investigated [22]. The dose and delivery system of amiloride were the same as that used in the main trial. No beneficial effect was detected. The lack of a major effect of amiloride on MCC in this patient group might explain the lack of clinical benefit of amiloride. However, no direct link between the bioelectrical effect of amiloride and its effect on MCC has been demonstrated, and in one study of the effects of inhaled amiloride on nasal potential difference (PD) and MCC, no effect on MCC was measured, in spite of a marked reduction in transepithelial PD [18]. Thus, the other effects of amiloride, such as its antibacterial activity [7], neutrophil activation [8], and anti-inflammatory effects, rather than its effects on MCC may have been responsible for the small clinical benefit previously described.

The results of this study do not exclude the potential value of amiloride or other ion transport therapies in CF. As a therapy aimed at correcting one of the basic phenotypic abnormalities in CF, it may be of less value in patients in whom bacterial colonization is established and in whom irreversible lung damage has occurred than, for example, in newly diagnosed infants. Its potential in the treatment of patients in whom airway clearance may be improved with newer therapies such as deoxyribonuclease (DNase) [23] also remain to be investigated. Further studies are required in order to explore these possibilities.

The basic ion transport abnormality in CF appears to be a defect in chloride transport. The links between this and increased sodium transport in CF have not been identified. However, for ion transport therapy to be effective in CF lung disease, it may be necessary to correct both increased sodium absorption and chloride impermeability at the same time. Compounds, such as adenosine triphosphate (ATP) and uridine triphosphate (UTP), which are effective chloride secretagogues in CF nasal epithelium [24], may be of benefit in combination with amiloride if shown to be safe for inhalational use.

This study has reconfirmed that nebulized amiloride may be safely administered to patients with CF and is generally well-tolerated with relatively few side-effects. It has also demonstrated the problems of patient compliance with a nebulized treatment administered four times daily, a factor which deterred patients from entering the trial and contributed to the high drop-out rate observed. Development of a more portable delivery system for inhalation of amiloride or identification of a longer acting compound with similar effects would assist any further long-term clinical studies. Finally, this study illustrates the difference between the effects of a trial therapy when studied alone in specialized circumstances and when added to existing medication in the routine clinical setting.

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