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A PILOT STUDY OF AEROSOLIZED AMILORIDE FOR THE TREATMENT OF LUNG DISEASE IN CYSTIC FIBROSIS

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Abstract Excessive active absorption of sodium is a unique abnormality of the airway epithelium in patients with cystic fibrosis. This defect is associated with thickened mucus and poor clearance of airway secretions and may contribute to the pulmonary disease in these patients. To study whether the inhibition of excessive absorption of sodium might affect the course of lung disease in cystic fibrosis, we performed a double-blind, crossover trial comparing aerosolized amiloride (5 mmol per liter; 3.5 ml four times daily), a sodium-channel blocker, with vehicle alone.

Fourteen of the 18 adult patients initially enrolled in the study completed the one-year trial (25 weeks for each treatment). The mean (±SEM) loss of forced vital capacity (FVC) was reduced from 3.39±1.13 ml per day during treatment with vehicle alone to 1.44±0.67 ml per day during treatment with amiloride ($P<0.04$). A measured index of sputum viscosity and elasticity was abnormal during treatment with vehicle alone and improved during treatment with amiloride. Calculated indexes of mucociliary and cough clearance also improved during amiloride treatment. No systemic, respiratory, or subjective toxic effects of amiloride were noted.

We conclude from this preliminary study that aerosolized amiloride can be safely administered to adults with cystic fibrosis. The slowing of the loss of FVC and the improvement in sputum viscosity and elasticity suggest a beneficial clinical effect. Aerosolized amiloride deserves further evaluation in the treatment of lung disease in patients with cystic fibrosis. (N Engl J Med 1990; 322: 1189-94.)

IN the airway epithelium of patients with cystic fibrosis, the combination of excessive absorption of sodium[1,2] and defective regulation of the secretory chloride channel of the apical membrane[3-6] probably leads to the dehydration of airway secretions, as reported in clinical studies.[7] These ion-transport defects probably contribute to the abnormal rheologic features and poor clearance of airway secretions,[8] obstruction of airflow, and chronic bacterial infection of the airways.[9] The sodium-channel blocker amiloride inhibits the excessive absorption of sodium (and liquid) in vitro[1,10] and in vivo[11] when applied to the luminal surface of the airway epithelium of patients with cystic fibrosis. These findings suggested that the long-term inhalation of amiloride might improve the viscosity, elasticity, and clearance of secretions, protect the airways from intraluminal obstruction, and improve airflow.[11,12]

The present study was designed as a preliminary investigation of the safety and efficacy of long-term treatment with aerosolized amiloride for airway disease in cystic fibrosis. A double-blind, crossover design was employed, and each treatment period (25 weeks) was preceded by a course of parenteral antibiotics to standardize the recent use of antibiotics among the patients and enhance the delivery of the aerosol.

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In accordance with the *Journal*'s policy, the authors have stated that Drs. Knowles and Boucher have an interest in the patent for the use of aerosolized amiloride in the treatment of lung disease in cystic fibrosis.

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METHODS

Study Subjects

Eighteen patients were recruited into the study. Guidelines for selection included the diagnosis of cystic fibrosis on the basis of clinical criteria and the results of sweat tests for chloride, partial pressure of arterial oxygen greater than 55 torr without chronic retention of carbon dioxide, normal renal function, and no long-term use of systemic steroids. Informed consent was obtained under the auspices of the Human Rights Committee of the University of North Carolina.

Study Design

A randomized, double-blind, crossover study was designed.* The nominal length of each treatment period was 25 weeks. Each period was preceded by 10 to 14 days of parenteral treatment with tobramycin and ceftazidime. Treatment with all respiratory medicines (oral and aerosolized antibiotics and bronchodilators) was withdrawn, and was withheld during the study period unless guidelines for reinstitution were met.

Two base-line measurements of pulmonary function were obtained on separate days after the completion of parenteral antibiotic treatment. Each patient also had a physical examination, blood-chemistry tests, chest radiography, quantitative microbiologic testing of sputum, complete blood count, urinalysis, and 24-hour measurement of urinary aldosterone excretion at the same time. Aerosol treatment was initiated after base-line measurements were obtained, and physical examinations, spirometry, quantitative microbiologic testing, measurements of serum electrolytes, and renal-function testing were also performed at three-to-six-week intervals from week 3 to week 25. At the end of each period (at about week 25), duplicate or single measurements were obtained as at base line, and aerosol treatment was then discontinued. Sputum was obtained from six patients for biorheologic studies of viscosity and elasticity at the end of each period.[13] A two-to-four-week washout period and treatment with parenteral antibiotics preceded the second period of aerosol therapy.

According to specific criteria, unscheduled visits and interventions with oral antibiotics (except quinolones) and bronchodilators were allowed for the treatment of mild exacerbations of disease. The therapy was discontinued after two to four weeks, but patients could receive a second (or third) course of therapy.

Criteria were also established for episodes of illness (usually major hemoptysis) that required "extraordinary intervention" with parenteral antibiotics. Safety was monitored by a blinded, nonparticipating observer who periodically reviewed coded clinical and laboratory data.

Aerosol Solution and Delivery of the Drug

Amiloride hydrochloride was dissolved in 0.3 percent saline (5 mmol per liter; pH 7.0). A nebulizer (DeVilbiss 646) and compressed-air generator (Pulmoaide, DeVilbiss) were used to nebulize and deliver 3.5 ml of drug or vehicle (0.3 percent saline) four times daily. With proper inhalational technique, this approach deposits an effective dose of amiloride (about 0.1 mmol per liter) on airway surfaces.[14,15]

Tests

Pulmonary-function tests (spirometric measurements before and after use of a bronchodilator and measurement of single-breath diffusing capacity [Gould 2400], measurement of arterial blood gases [Radiometer ABL 30], and plethysmographic measurement of lung volumes [Jaeger]) were performed according to American Thoracic Society standards[16] at least three hours after the patient had arisen and three hours after the administration of the aerosol. Quantitative bacterial cultures of sputum were performed with a technique equivalent to quantitation using mucolytic agents.[17,18] Sputum for biorheologic testing was obtained three hours after the administration of aerosol at the end of each study period in a manner designed to minimize salivary contamination.[13] Rheologic studies used the technique of the magnetic oscillating sphere,[19] and the percentage of solids was measured after microwave drying. Chest radiographs were graded according to a published scoring system.[20] The urinary aldosterone level was measured by radioimmunoassay (SmithKline BioScience). Twelve pulmonary and systemic symptoms or measures (chest tightness; shortness of breath; wheezing; cough during the first two hours of the day, during the rest of the day, or during sleep; hemoptysis; color, consistency, and volume of sputum; appetite; and general well-being) were graded weekly by the patients, using an ordinal scale and a diary designed for the study.*

Statistical Analysis

In both treatment periods 3 of the 14 patients who completed the study had episodes of acute illness that qualified for extraordinary parenteral antibiotic therapy. After the intervention, the patients resumed aerosol therapy and completed the treatment period so that toxicity could be evaluated. In these three patients, only data collected before the extraordinary intervention were used in our analyses of efficacy.

The use of complete, balanced analysis-of-variance or multiple-analyses-of-variance methods was precluded because of the irregular timing of visits. Analyses were performed by two statistical methods selected before the study. In method 1, the mean change from base line (i.e., the difference between the mean base-line value and the mean of the values during treatment) with vehicle and amiloride was computed for each subject. In method 2, the slopes from unweighted simple linear regression (including base-line data) for each subject for each period and for each variable (forced vital capacity [FVC] and forced expiratory volume in one second [FEV_1], for example) were analyzed. The difference between the slopes during treatment with vehicle and treatment with amiloride was computed for each subject. Comparisons of treatments as assessed by methods 1 and 2 were based on these differences in unweighted, two-tailed, paired t-tests and signed-rank tests, which yielded similar outcomes except as indicated. A separate analysis was performed for each response variable. To test for differences in rheologic features and bacterial counts in the sputum obtained near the end of each study period, paired t-tests were employed with log-transformed data. All hypotheses regarding efficacy and safety were specified before the analysis began. A Type I error rate of 0.05 was used to judge statistical significance. All but one investigator remained blinded to the assigned treatments during the analyses. Results are reported as means ±SEM unless otherwise indicated.

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Results

Of the 18 patients who were recruited, 3 withdrew from the study (after 3, 10, and 18 weeks) because they were unable to follow the protocol. One other patient withdrew (after 13 weeks) after hemolytic anemia associated with Epstein–Barr-virus seroconversion developed; a subsequent trial of aerosolized amiloride in the patient produced no adverse effect. Table 1 outlines the clinical features of the 14 patients who completed the study.

Seven of the 14 patients were randomly assigned to vehicle and 7 to amiloride for the first period. The mean duration of treatment with vehicle and amiloride was well matched (24.7 weeks in each period), and there was no seasonal bias in either treatment period. There was an equal degree of compliance during treatment with vehicle (79.0±6.0 percent of scheduled doses) and amiloride (80.3±4.7 percent).

Efficacy

Before treatment with vehicle and treatment with amiloride, the base-line FVC (3.66±0.28 and 3.60±0.29 liters, respectively) and FEV_1 (2.25±0.24 and 2.22±0.25 liters, respectively) were nearly identical. The mean decrease in FVC from base line (analyzed according to method 1) during treatment with vehicle (296±79 ml) was approximately twice as large as that during treatment with amiloride (160±53 ml; $P<0.04$). The data on FVC are shown in Table 2. The mean decrease in FEV_1 during treatment with vehicle (291±95 ml) was not significantly different from that during treatment with aerosolized amiloride

Table 1. Clinical Characteristics of the 14 Patients Who Completed the Study.*

CHARACTERISTIC	VALUE
Male sex (no.)	8
Age (yr) — range (median)	18–37 (25)
Hospitalizations (no.)†	0.9±0.3
FVC (% of predicted)‡	81.2±5.6
FEV_1 (% of predicted)‡	60.1±5.9
Microbiologic findings (no. of patients)§	
Pseudomonas aeruginosa	14
Staphylococcus aureus	8
Aspergillus fumigatus¶	4
Mycobacterium avium–intracellulare	2
Medications (no. of patients)	
Oral contraceptives	5
Insulin	1

*Plus–minus values are means ±SEM. FVC denotes forced vital capacity, and FEV_1 forced expiratory volume in one second.

†Number per patient in the previous year.

‡Measured after initial antibiotic treatment (base line, period 1).

§Number of patients with positive sputum cultures in previous year.

¶None had allergic bronchopulmonary aspergillosis.

Table 2. Changes in Mean Forced Vital Capacity (FVC) during the Study Periods.

Patient No.	Vehicle			Amiloride			Difference*
	Average base-line FVC	Average FVC during treatment	Change	Average base-line FVC	Average FVC during treatment	Change	
	ml						
1	3450	2925	525	2825	2760	65	460
2	4445	4190	255	4500	4490	10	245
3	6090	5067	1023	5960	5617	343	680
4	2565	2588	−23	2365	2543	−178	155
5	4475	3934	541	4250	3810	440	101
6	4245	3952	293	4430	3985	445	−152
7	3055	2547	508	2715	2392	323	185
8	4070	4108	−38	4020	3880	140	−178
9	2870	2646	224	2945	2732	213	11
10	3720	3635	85	3730	3758	−28	113
11	2970	2890	80	3055	2960	95	−15
12	3200	3125	75	3420	3387	33	42
13	4330	3805	525	4415	4048	367	158
14	1815	1741	74	1755	1787	−32	106
Mean	3664	3361	296	3599	3439	160	137
Median	3585	3380	240	3575	3572	118	109
Standard error	282	234	79	291	265	53	60
P value by t-test			0.003			0.010	0.039
P value by signed-rank test			0.002			0.014	0.038

*Change during treatment with vehicle minus change during treatment with amiloride.

(202±51 ml; $P \approx 0.20$). After treatment with an inhaled bronchodilator, a similar pattern was observed for FVC and FEV_1. There was a larger loss of FVC during treatment with vehicle (222±50 ml) than during treatment with amiloride (117±57 ml; $P = 0.03$ by signed-rank test and 0.11 by t-test), but the decrease in FEV_1 during treatment with vehicle (225±50 ml) was not significantly different from the decrease during treatment with amiloride (170±54 ml; $P = 0.32$ by signed-rank test and 0.23 by t-test). No carry-over effects between the two periods were detected. The mean duration of expiration for FVC was the same during the two periods (10.0±0.7 seconds).

The analysis of slopes (method 2) is illustrated in Figure 1. The mean rate of decrease in FVC during treatment with vehicle (3.39±1.13 ml per day) was reduced by more than half during treatment with amiloride (1.44±0.67 ml per day; $P<0.04$). The difference in the rate of decrease in FEV_1 between the two periods was not statistically significant (vehicle, 3.21±1.11 ml per day; amiloride, 2.09±0.86 ml per day; $P \approx 0.09$).

Matched pairs of sputum samples obtained from six patients who completed both periods without a need for parenteral antibiotics were analyzed for biorheologic features[21] and the results compared with normal values (Table 3). Log G*1 and log G*100, indexes of the mechanical impedance of mucus, were different for amiloride and vehicle. The loss tangents (tan δ 1 and tan δ 100), indexes of the ratio of viscous to elastic strain, were not different. Calculated indexes of mucociliary clearance[21] and cough clearance[22] differed significantly between vehicle and amiloride (Table 3). The rheologic values of sputum obtained during long-term treatment with aerosolized amiloride approximated normal values. The solid content of the sputum obtained during treatment with vehicle (7.5±3.2 percent) did not differ from that of the sputum obtained during treatment with amiloride (8.3±4.1 percent).

Several monitored indexes were not altered by amiloride treatment. Measures of nonspirometric lung function at the beginning and end of each treatment period were available only for the patients who completed both periods without parenteral antibiotics (n = 11). There was no difference between the vehicle and amiloride periods with respect to changes in arterial blood gases (alveolar–arterial gradient, 0.3±1.7 and 1.1±1.9 torr, respectively) or diffusing capacity (−0.9±0.5 and +0.3±0.8 ml per mm Hg per minute). Bacterial densities — the number of organisms ($\log_{10}$) per milliliter of sputum — were similar in the sputum from patients without antibiotic intervention and in the sputum from all patients. Total bacterial densities were 8.16±0.18 and 8.13±0.20, and densities of *Pseudomonas aeruginosa* were 7.96±0.21 and 7.79±0.35 during 12 to 25 weeks of treatment with vehicle and amiloride, respectively. No differences were noted in bacterial densities or the incidence of positive cultures for *Staphylococcus aureus* between the vehicle and amiloride periods.

Interventions

The therapeutic interventions are summarized in Table 4. The use of oral antibiotics, bronchodilators, and prednisone did not differ between the vehicle and amiloride periods when it was analyzed on the basis of individual or grouped data.

Safety

No evidence of pulmonary or systemic toxicity emerged from analyses of pulmonary function, symptoms, physical examinations, or laboratory tests (Table 5). No difference in the incidence of aspergillus or candida species in sputum cultures was observed during treatment with amiloride, nor was any new microbial or multidrug-resistant organism identified.

Discussion

Several features appeared to be critical in the design of this study of long-term inhalation of amiloride in the treatment of lung disease in patients with cystic

fibrosis. First, effective delivery of the drug to the airway surfaces was required. The concentration of amiloride in airway-surface liquid needed to produce effective blockade (more than 90 percent inhibition) of the absorption of sodium in patients with cystic fibrosis is about 0.01 mmol per liter (the median effective dose is 0.001 mmol per liter).[1,10,11] Because amiloride is rapidly cleared from human airways (half time, about 40 minutes),[14,15] a peak concentration of about 0.5 mmol per liter on airway surfaces four times daily is required to maintain an effective concentration. Optimizing the system of nebulizing the liquid, we achieved a peak concentration of about 0.08 mmol per liter of amiloride in the bronchi of patients with cystic fibrosis.[15] We accepted this dose because respirable amiloride was available only in this formulation and we expected compliance to be poor if dosage frequency exceeded four times daily. Second, because no information is available regarding outcome variables pertinent to aerosolized diuretic agents, we selected measures of outcome on the basis of the hypothesis that amiloride would inhibit the excessive absorption of sodium and would hydrate secretions and improve their biorheologic properties and clearance from the airways. The results of a spirometric test were selected as the chief criteria of efficacy because the test measures both the retention of airway secretions[23] and the functional benefit to the patient.[9] Measurements that assess the rheologic features of sputum more directly were undertaken to explore the feasibility of performing them in a therapeutic study. Third, the outcome of this initial study of the long-term use of an aerosolized diuretic agent in humans involved questions of drug safety. We monitored our patients for any adverse effect of amiloride as a potassium-sparing diuretic agent and for deleterious effects on pulmonary function. Finally, because amiloride is not approved for use in children, we selected a cohort of adults.

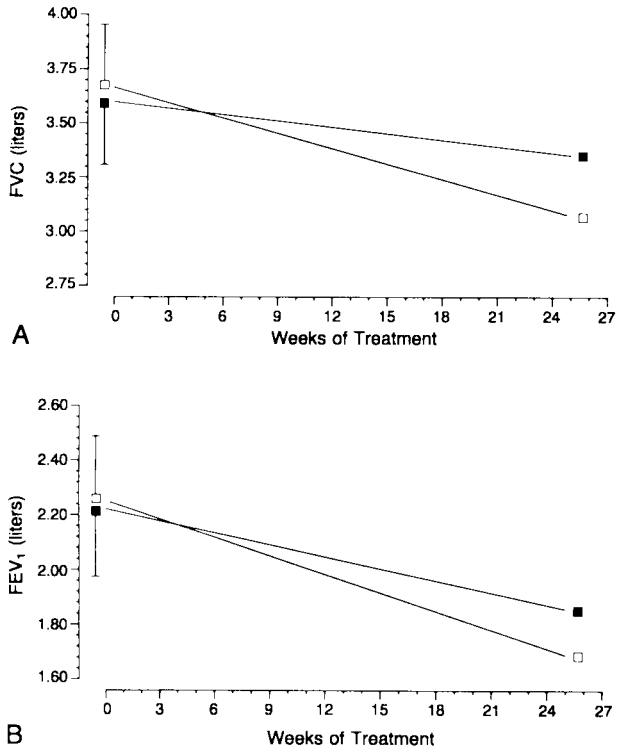

Figure 1. Changes in Mean Forced Vital Capacity (FVC) and Forced Expiratory Volume (FEV_1) during the Study Periods.

Panel A shows the mean (±SEM) base-line FVC and the mean slope of FVC as a function of time during treatment with vehicle (□) and amiloride (■). The slopes reflect 7.3 and 7.1 measurements per patient (n = 14), including base-line values, for vehicle and amiloride, respectively ($P<0.04$ for the differences in slopes). FVC decreased by 3.39±1.13 ml per day during treatment with vehicle, and by 1.44±0.67 ml per day during treatment with amiloride.

Panel B shows the mean (±SEM) base-line FEV_1 and the mean slope of FEV_1 as a function of time during treatment with vehicle (□) and amiloride (■). The slopes reflect 7.3 and 7.1 measurements per patient (n = 14), including base-line values, for vehicle and amiloride, respectively ($P = 0.09$ for the differences in slopes). FEV_1 decreased by 3.21±1.11 ml per day during treatment with vehicle, and by 2.09±0.86 ml per day during treatment with amiloride.

Table 3. Biorheologic Features of Airway Secretions after Long-Term Treatment with Vehicle and Amiloride.*

Measurement†	Vehicle (N = 6)	Amiloride (N = 6)	Normal Values[21]
Log G*1 (1 rad/sec)	2.92±0.10	2.41±0.14‡	2.17±0.08
Log G*100 (100 rad/sec)	3.32±0.09	2.67±0.13‡	2.56±0.08
Tan δ 1 (1 rad/sec)	0.41±0.02	0.32±0.04	0.29±0.16
Tan δ 100 (100 rad/sec)	0.95±0.06	1.04±0.06	0.93±0.06
Mucociliary-clearance index	0.66±0.03	0.84±0.06‡	0.91±0.02
Cough-clearance index	0.73±0.09	1.51±0.18‡	1.52±0.03

*Plus–minus values are means ±SEM.

†Log G*1 and log G*100 are indexes of the mechanical impedance of mucus. The loss tangents (tan δ 1 and tan δ 100) are indexes of the ratio of viscous to elastic strain.

‡$P<0.05$ for the comparison with values after treatment with vehicle.

The long-term inhalation of amiloride produced no pulmonary or systemic toxicity. No patient had either drug-related bronchoconstrictive symptoms or reductions in gas exchange or diffusing capacity that might indicate alveolar toxicity. No adverse effects of amiloride were seen on hematologic or liver-function tests or measurements of vascular volume, renal function, or serum electrolytes. These findings are consistent with those from animal studies[24,25] (and Boucher RC, et al.: unpublished data).

Amiloride aerosol appears to slow the decline in pulmonary function associated with cystic fibrosis. The rate of loss of FVC was reduced by approximately 50 percent during the amiloride period as compared with the vehicle period. However, a significant difference in FEV_1 was not observed. This relatively smaller change in FEV_1 parallels the pattern of spirometric changes reported in studies exploring the efficacy of antibiotics in patients with cystic fibrosis.[26] Because

Table 4. Summary of Drug Interventions in Each Study Period.*

DRUG	VEHICLE		AMILORIDE		DIFFERENCE†	
	NO. OF INTERVENTIONS	WEEKS OF USE	NO. OF INTERVENTIONS	WEEKS OF USE	NO. OF INTERVENTIONS	WEEKS OF USE
Antibiotics (oral)	22	95	24	74	−0.14 ±0.44	1.50 ±1.77
Bronchodilators (inhaled or oral)	9	23	2	7	0.50 ±0.37	1.14 ±0.79
Corticosteroids (oral)	1	3	1	2	—	—

*Medications that were administered after extraordinary intervention in three patients are not included.

†Mean (±SEM) difference per patient between values in the vehicle period and the amiloride period. No differences were statistically significant.

the effects of vehicle and amiloride were compared after parenteral antibiotic therapy, the decline in FVC during vehicle may have been more rapid than usual.

We analyzed drug interventions to determine whether the effects of amiloride on spirometric features could be attributed to differences in medical therapy. It is clear that these effects were not due to excessive intervention with bronchodilators and oral antibiotics during treatment with amiloride.

The mechanism of amiloride's beneficial effect is uncertain. The effect does not appear to reflect a direct bronchodilating activity of amiloride, because differences in FVC between the study periods persisted after the administration of beta-agonist bronchodilators, and no acute bronchodilating effect of amiloride has been observed in patients with cystic fibrosis.[14,27] The effect also does not appear to reflect a direct antimicrobial action of amiloride,[28-30] because bacterial densities did not differ between study periods. Therefore, it is more likely that amiloride exerts a beneficial effect at least in part by increasing the clearance of secretions, as has been reported in short-term studies.[27]

We hypothesized that amiloride might increase the clearance of airway secretions by improving their biorheologic properties. There are few available data that directly measure the rheologic features of sputum as an index of therapeutic efficacy.[31] To test the feasibility of performing such measurements, we collected sputum specimens at the end of each period and studied them in a blinded fashion, using techniques developed by Puchelle et al.[13] Indexes of sputum rheologic features were better after long-term treatment with amiloride than after treatment with vehicle, and they were similar to values reported for healthy subjects.[21] The biorheologic changes predict increases in the rates of mucociliary transport and cough clearance that are consistent with those reported in short-term studies of aerosolized amiloride.[27]

We did not detect the change in the percentage of solids in dried sputum that would be expected if amiloride improved the rheologic features of mucus by increasing its water content. This finding may reflect the insensitivity of the techniques employed to detect small but functionally important changes in the water content of sputum. Further studies are required to establish the mechanism of amiloride's effects on the viscosity, elasticity, and clearance of mucus.

In conclusion, this study of a small number of patients provides preliminary evidence that amiloride may be moderately effective in the treatment of adults with cystic fibrosis and established lung disease.

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Table 5. Indexes of Systemic Salt and Water Metabolism during the Study Periods.*

INDEX	VEHICLE		AMILORIDE	
	BASE LINE	DURING TREATMENT†	BASE LINE	DURING TREATMENT†
Weight (kg)	59.9±2.3	59.7±2.4	59.8±2.4	59.6±2.2
Blood pressure (mm Hg)‡				
Systolic	114.5±2.6	110.9±1.5	111.6±2.5	113.0±2.0
Diastolic	74.2±2.3	71.4±2.1	70.2±2.3	71.7±1.4
Pulse (per min)‡	91.1±4.6	100.7±3.4§	90.8±2.3	94.5±2.5
Serum sodium (mmol/liter)	139.1±0.5	138.6±0.3	138.2±0.6	139.0±0.5¶
Serum potassium (mmol/liter)	4.2±0.1	4.0±0.1	4.1±0.1	4.1±0.1
Serum urea nitrogen (mg/dl)‖	14.1±1.2	13.4±0.9	14.6±1.1	12.8±0.8§
Serum creatinine (mg/dl)**	0.8±0.1	0.9±0.1	1.0±0.1	0.9±0.1§¶
Urinary aldosterone (μg/24 hr)††	10.3±1.7	10.6±3.4	13.8±2.0	15.8±2.6

*Plus–minus values are means ±SEM.

†Values are means of all measurements recorded during the treatment period for all values except aldosterone, which reflects a single 24-hour urinary measurement at the end of the period.

‡In a standing position.

§$P<0.05$ by paired t-test for the comparison with base line.

¶$P<0.05$ by paired t-test for the comparison of the change during the amiloride period with the change during the vehicle period.

‖To convert milligrams per deciliter to millimoles per liter, multiply by 0.3570.

**To convert milligrams per deciliter to micromoles per liter, multiply by 88.4.

††Normal range, 2 to 25 μg per 24 hours. Values are for only nine patients. To convert micrograms to nanomoles, multiply by 2.774.

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