Selective Aldosterone Blockade with Eplerenone Reduces Albuminuria in Patients with Type 2 Diabetes

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Previous studies have shown that the selective aldosterone blocker eplerenone, in doses of up to 200 mg/d, reduces albuminuria in patients with type 2 diabetes. This study was conducted to ascertain whether lower doses of eplerenone (50 or 100 mg/d) co-administered with the angiotensin-converting enzyme (ACE) inhibitor enalapril would produce a similar antialbuminuric effect while obviating the hyperkalemia observed previously. After open-label run-in with enalapril 20 mg/d, patients with diabetes and a urinary albumin:creatinine ratio (UACR) ≥50 mg/g were randomly assigned to receive enalapril plus one of three double-blind daily treatments for 12 wk: placebo, eplerenone 50 mg (EPL50), or eplerenone 100 mg (EPL100). After week 4, amlodipine 2.5 to 10 mg/d was allowed for BP control (systolic/diastolic BP ≤ 130/80 mmHg). The primary study end points were the percentage change from baseline at week 12 in UACR and the incidence of hyperkalemia. Secondary end points included percentage changes from baseline in UACR at weeks 4 and 8 and changes from baseline in systolic and diastolic BP. Treatment with EPL50 or EPL100 but not placebo significantly reduced albuminuria from baseline. By week 12, UACR was reduced by 7.4% in the placebo group, by 41.0% in the EPL50 group, and by 48.4% in the EPL100 group (both eplerenone groups, $P < 0.001$ *versus* placebo). The incidences of sustained and severe hyperkalemia were not significantly different in any of the three treatment arms and did not differ on the basis of quartile of estimated GFR (all NS). For the secondary end points, both eplerenone treatment groups significantly reduced albuminuria from baseline as early as week 4 ($P < 0.001$), whereas placebo treatment (including enalapril) did not result in any significant decreases in UACR. Systolic BP decreased significantly in all treatment groups at all time points, but, generally, all treatment groups experienced similar decreases in BP. Co-administration of EPL50 or EPL100 with an ACE inhibitor as compared with an ACE inhibitor alone significantly reduces albuminuria in patients with diabetes without producing significant increases in hyperkalemia.

Clin J Am Soc Nephrol 1: 940–951, 2006. doi: 10.2215/CJN.00240106

Extensive investigation has established a pivotal role for the renin-angiotensin-aldosterone system (RAAS) in mediating cardiac and renal injury. In hypertensive patients, increased activity of the RAAS correlates with increased end-organ damage (1). Interventions that block angiotensin II (AngII) action or reduce AngII production reduce end-organ damage in patients with hypertension, congestive heart failure, or nephropathy (2–5). Several recent studies have demonstrated clearly that pharmacologic interruption of the RAAS retards progression of nephropathy in patients with type 2 diabetes (5,6–8). Whereas most attention has focused on the role of AngII in causing end-organ damage, several lines of evidence recently suggested that aldosterone *per se*, beyond the influence of renin and AngII, is an important mediator of both cardiovascular and renal injury (9–13).

Received January 18, 2006. Accepted May 25, 2006.

Published online ahead of print. Publication date available at www.cjasn.org.

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Although many studies have demonstrated a beneficial effect of interruption of the RAAS in retarding progressive renal disease (5,6–8), these interventions do not differentiate between the relative contributions of renin or AngII *versus* aldosterone alone. Studies in several animal models have succeeded in dissociating the relative contributions of aldosterone from the rest of the RAAS (14–17). In addition, clinical studies have supported the postulate that mineralocorticoid receptor (MR) blockade may confer an antialbuminuric effect in patients with and without diabetes (18–22). Although suggestive, these studies had one or more of the following deficiencies: (*1*) They were composed of small numbers of patients; (*2*) patients were given a fixed high dose of the MR antagonist; (*3*) the studies were of short duration; or (*4*) they did not use an angiotensin-converting enzyme (ACE) inhibitor as a comparator, the preferred agent to treat this condition. Consequently, a systematic study in patients with albuminuria was needed to determine (*1*) whether MR blockade would be additive to the effects of ACE inhibitor monotherapy, (*2*) which dosages would be efficacious, and (*3*) whether this could be accomplished without provoking hyperkalemia.

Eplerenone is a selective aldosterone blocker that has been

ISSN: 1555-9041/105-0940

shown to be an effective antihypertensive agent (23–26), to prevent cardiovascular and renal end-organ damage in patients with essential hypertension (22,27), and to reduce morbidity and mortality in the short- and long-term in patients with heart failure and left ventricular systolic dysfunction after myocardial infarction (28,29). This clinical study was conducted to compare the effect of coadministration of two different doses of the selective aldosterone blocker eplerenone or placebo combined with an ACE inhibitor, enalapril, on albumin excretion in patients with type 2 diabetes mellitus and albuminuria, and to evaluate the effect of each treatment on the incidence of hyperkalemia.

Materials and Methods

Study Design and Treatments

This multicenter, randomized, double-blind, placebo-controlled, parallel-group trial compared the effects of concomitant eplerenone plus enalapril *versus* enalapril plus placebo on albuminuria and serum potassium levels in patients with type 2 diabetes. The study included a 1-wk screening period followed by a 2- to 4-wk open-label run-in period with enalapril 20 mg/d and a 12-wk, double-blind treatment phase. Eligible patients were randomly assigned to one of three treatment arms during the double-blind treatment phase: placebo/enalapril 20 mg once daily, eplerenone 50 mg/enalapril 20 mg once daily (EPL50), or eplerenone 100 mg/enalapril 20 mg once daily (EPL100). Add-on hydrochlorothiazide was not allowed. Study medications were taken at the same time each day (morning), with or without food. Patients were instructed to take study medication 23 to 25 h before a clinic visit but not on the morning of the visit; instead, the dose was administered at the clinic after all study visit procedures were completed. Because the goal of the study was to discern the relative effects of eplerenone on urinary albumin:creatinine ratio (UACR) independent of BP lowering, the intent of the study design was to achieve equivalent reduction of BP in all three treatment arms. To achieve this, add-on therapy with amlodipine was permissible. At week 4 and thereafter, when BP was uncontrolled (systolic BP [SBP]/diastolic BP [DBP] >130/80 mmHg), open-label amlodipine was added. The initial amlodipine dose was 2.5 mg/d and could be doubled every 2 wk up to 10 mg as needed for BP control. If at week 10 the patient's DBP was ≥95 mmHg and the patient had been uptitrated to amlodipine 10 mg, then the patient was withdrawn from the study. If symptomatic hypotension occurred at any time during the study, if DBP was ≥110 mmHg, if SBP was ≥180 mmHg at two consecutive visits 1 to 3 d apart, or if serum potassium was elevated >5.5 mmol/L on two consecutive occasions 1 to 3 d apart, then the patient was withdrawn.

Patient Population

Patients who were eligible for the study included male and nonpregnant female adult patients with type 2 diabetes and albuminuria (UACR ≥ 50 mg/g) measured by first-morning-void urine samples. Patients were required to have glycosylated hemoglobin (HbA_{1c}) ≤ 8.5%. Patients may or may not have had a history of mild to moderate hypertension (SBP/DBP ≥ 140/90 mmHg), but only patients with a DBP <110 mmHg and an SBP <180 mmHg were eligible for inclusion. Before randomization, patients must have had a serum potassium level ≥3.5 and ≤5.0 mmol/L.

Key exclusion criteria were as follows: orthostatic hypotension; secondary hypertension; a history of severe or malignant hypertension; a history of New York Heart Association class II, III, or IV heart failure, myocardial infarction, coronary angioplasty, or bypass surgery within the past 6 mo or unstable angina pectoris or arrhythmia that required treatment; a history of stroke or transient ischemic attack within the past 6 mo or known presence of hemodynamically relevant stenosis of the arteries perfusing the brain; administration of spironolactone, guanethidine, or reserpine within 30 d of double-blind treatment; active participation in a weight-reduction program during the course of the study; acute or chronic hepatic disease; a calculated creatinine clearance <70 ml/min per the Cockcroft-Gault formula; or any clinically significant, abnormal laboratory values that could preclude the patient from safely participating in the study.

All patients provided written informed consent to participate in the study. The study design and consent form were approved by the Institutional Review Board of each institution. The study was conducted in accordance with ethical principles that were based on the Helsinki Declaration.

Study End Points

There were two primary end points: (*1*) the percentage change from baseline at week 12 in UACR and (*2*) the incidence of hyperkalemia. Baseline UACR reflected the clinical state at the end of the run-in period, just before randomization. Secondary end points of the study were (*1*) percentage changes from baseline in UACR at weeks 4 and 8; (*2*) change from baseline in SBP at weeks 4, 8, and 12; and (*3*) change from baseline in DBP at weeks 4, 8, and 12. In addition, the safety and the tolerability of each treatment were evaluated.

Measurements

Urinary albumin excretion was measured at the central laboratory by first-morning-void urine samples at weeks 0, 4, 8, and 12. Heart rate, trough cuff BP, electrocardiograms, and adverse events were evaluated in the clinic at weeks 0, 2, 4, 6, 8, 10, and 12. At each visit, two seated measurements of BP were taken, and the average of these was computed; all descriptive statistics and inferential analyses of BP are based on this average value. Fasting clinical laboratory safety assessments, including serum potassium, were performed during screening and at weeks 0, 4, 8, and 12 or at early termination (serum potassium was additionally measured at weeks 2, 6, and 10), all at the central laboratory. Because investigators often underestimate the frequency of adverse events, we used laboratory-based measurements of potassium as a more rigorous index of the incidence of hyperkalemia. Therefore, hyperkalemia is reported separately from other adverse events. Physical examinations were performed during the screening period and at week 12 or early termination.

Estimated glomerular filtration rate (eGFR) was calculated using the full Modification of Diet in Renal Disease (MDRD) Study prediction equation (30). At the time the protocol was initially written (2001), the Cockcroft-Gault formula was the best estimation of renal function available, so it was used as the basis for entry criteria. However, for these analyses, we had the benefit of the more recently derived MDRD equation; consequently, all analyses use the MDRD to predict eGFR.

Statistical Analyses

Sample sizes were computed in the natural-log scale of change from baseline to week 12. For log-UACR, to detect a treatment difference of 0.56, with an SD of 1.0, at an overall type I error rate (α) ≤0.05, a sample of size 68 per treatment arm was required to achieve a power of ≥90%. Assuming a 15% dropout rate, a total of 240 patients (80 per treatment group) were to be enrolled in the study. Power calculations were performed using nQuery Advisor version 4.0 (Statistical Solutions, Saugus, MA). All analyses were computed using SAS version 8.12 (SAS Institute, Cary, NC).

Efficacy Analyses. The primary efficacy analysis was based on UACR percentage change from baseline to week 12. Additional efficacy analyses were performed on BP changes from baseline to week 12. All analyses were completed for all patients who had a baseline UACR and an end point UACR within 7 d of the last dose of double-blind study medication (UACR intention-to-treat cohort). UACR distribution of baseline and postbaseline (weeks 4, 8, and 12) changes and percentage changes (from baseline to weeks 4, 8, and 12) were explored. Because of a high degree of skewness, extreme outliers, and multimodality in the data, even subsequent to a natural-log transformation, nonparametric statistics were used for analysis (31,32). Specifically, Wilcoxon's rank-sum test was used to compare medians between treatment groups, and Wilcoxon's signed rank statistic was used to test for location shift from zero (absolute or percentage changes from baseline) within treatment groups. Similar to the methods of Schjoedt *et al.* (32), pivotal analyses were based on percentage change from baseline, although absolute changes also were reported when appropriate. When confidence intervals were reported, they were constructed at a 99% confidence level. However, population dispersion was depicted by quartiles. To account for multiplicity and to maintain an overall type I error rate of 0.05, all pair-wise *P* values were compared with 0.01 for significance. Treatment-by-subgroup factor interaction was evaluated by ANOVA on the log-transformed UACR data. Because of concerns about nonnormality of the data, this approach was used to evaluate interactions only. Spearman rank correlations were computed to establish association between parameters at weeks 4, 8, and 12.

Safety Analyses. Safety analyses (potassium elevations and adverse events) were performed for all randomly assigned patients who took at least one dose of double-blind study medication. Any laboratory assessment of serum potassium that was drawn >1 d after the last dose of study medication was excluded from the evaluation of hyperkalemia incidence. Sustained hyperkalemia was defined as a serum potassium >5.5 mmol/L on two consecutive occasions 1 to 3 d apart, and severe hyperkalemia was defined as a serum potassium ≥6.0 mmol/L on any occasion. *P* value testing was performed using the Cochran-Mantel-Haenszel test. Logistic regression with incidence of hyperkalemia as the binary response and treatment as an ordinal explanatory variable was fit to evaluate a possible trend in the incidence of hyperkalemia across treatment groups.

All adverse events were summarized by treatment group. Adverse events with onset >7 d after the last dose of study medication were excluded from the adverse event analysis, with the exception of serious adverse events, which were included when they occurred up to 28 d after the last dose of study medication.

Results

Patient Disposition, Demographics, and Baseline Characteristics

A total of 268 patients from 43 sites were randomly assigned and treated in the study. One patient who was randomly assigned to the placebo group did not receive any study medication and was not included in any analysis. Of the 268 treated patients, 91 in the placebo group, 91 in the EPL50 group, and 86 in the EPL100 group received at least one dose of study medication. Most patients (>87%) in each treatment group completed the planned 12 wk of treatment (Figure 1). Five (5.5%) patients in the placebo group discontinued early from the study because of an adverse event. Early discontinuations in the eplerenone-treated groups were due primarily to elevated potassium levels; four (4.4%) patients in the EPL50 group and seven (8.1%) patients in the EPL100 group withdrew. No patient in any treatment group discontinued as a result of inadequate BP control.

Baseline demographics and characteristics are presented in Tables 1 (categorical factors) and 2 (continuous factors). The placebo group contained more women than either eplerenone treatment group (Table 1). This merits attention, given that men and women have a different natural history in renal disease and that eplerenone has important potential for hormonal action. Consequently, we conducted a statistical analysis that demonstrated that there was no difference in the decline in UACR in the male cohort as compared with the female cohort (ANOVA interaction $P = 0.34$). Although UACR seemed to have an uncharacteristic aberration in its values in the raw scale, the general distributions across the treatment groups were similar.

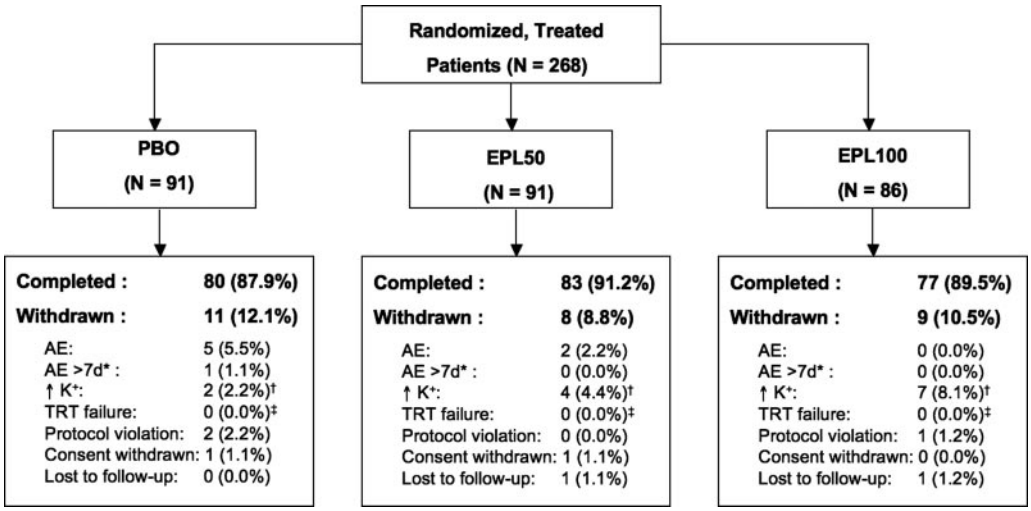

Figure 1. Patient disposition. PBO, placebo + enalapril 20 mg/d; EPL50, eplerenone 50 mg + enalapril 20 mg/d; EPL100, eplerenone 100 mg + enalapril 20 mg/d; AE, adverse event; TRT, treatment. *AE occurred >7 d after last dose. †Serum potassium >5.5 mmol/L on two consecutive occasions. ‡Diastolic BP (DBP) ≥110 mmHg or systolic BP (SBP) ≥180 mmHg; a DBP ≥95 mmHg at week 10 after treatment with add-on amlodipine for at least 2 wk.

Table 1. Baseline characteristics: Categorical variables[a]

Demographic or Baseline Characteristic	PBO ($n = 91$)	EPL50 ($n = 91$)	EPL100 ($n = 86$)
Gender (n [%])			
female	41 (45)	31 (34)	30 (35)
male	50 (55)	60 (66)	56 (65)
Ethnicity (n [%])			
white	87 (96)	87 (96)	79 (92)
black	3 (3)	1 (1)	7 (8)
Asian	0 (0)	2 (2)	0 (0)
not listed	1 (1)	1 (1)	0 (0)
Selected concurrent medications			
dihydropyridine CCB	53 (58)	44 (48)	37 (43)
HMG-CoA reductase inhibitors	23 (25)	17 (19)	15 (17)

[a]CCB, calcium channel blocker; PBO, placebo/enalapril 20 mg once daily; EPL50, eplerenone 50 mg/enalapril 20 mg once daily; EPL100, eplerenone 100 mg/enalapril 20 mg once daily; HMG-CoA, β-hydroxy-β-methylglutaryl-CoA.

Table 2. Baseline characteristics: Continuous variables[a]

Demographic or Baseline Characteristic	PBO ($n = 91$)	EPL50 ($n = 91$)	EPL100 ($n = 86$)
Age (yr)	60 (53, 66)	58 (52, 66)	58 (53, 66)
Body mass index (kg/m^2)			
female	33.3 (30.57, 36.79)	30.9 (27.76, 34.76)	31.0 (28.36, 36.31)
male	30.8 (27.43, 33.03)	29.7 (26.64, 32.23)	29.2 (26.92, 33.38)
BP (mmHg)[b]			
systolic	146 (134,158)	140 (130, 152)	140 (132, 154)
diastolic	88 (78, 95)	83 (78, 90)	85 (80, 90)
Heart rate (beats/min)	72 (68, 80)	74 (68, 80)	75 (68, 80)
Serum creatinine (μmol/L)[c]	($n = 88$)	($n = 89$)	($n = 82$)
	80 (61.9, 88.4)	80 (70.7, 88.4)	80 (61.9, 88.4)
eGFR (ml/min per 1.73 m^2)[c]	($n = 88$); 74 (60.5, 82.2)	($n = 89$); 73 (62.1, 83.6)	($n = 82$); 75 (62.8, 85.9)
UACR (mg/g)[c]	($n = 87$); 280 (105.1, 762.2)	($n = 89$); 422 (153.8, 856.0)	($n = 83$); 240 (90.7, 577.8)
Hemoglobin A_{1c} (%; mean [SD])	7.9 (0.02)	8.1 (0.02)	8.2 (0.02)

[a]Unless otherwise noted, data are median (25th quartile, 75th quartile). eGFR, estimated GFR; UACR, urinary albumin:creatinine ratio.
[b]Hypertension was not a requirement for randomization.
[c]For the intention-to-treat population.

The distributions of all other parameters were similar across treatment groups.

Efficacy Results

Effect of Treatment on Albumin Excretion. Eplerenone treatment (both EPL50 and EPL100 treatment groups) significantly reduced albuminuria from baseline as early as week 4 and continued throughout weeks 8 and 12 ($P < 0.001$ for all comparisons; Figure 2). Placebo treatment (including enalapril) did not result in any significant decrease from baseline in UACR. There were no significant differences in UACR reduction between the eplerenone treatment groups ($P = 0.48$, 0.61, and 0.55 at 4, 8, and 12 wk, respectively).

Figure 3 displays the median percentage change in UACR from baseline to week 12 by quartile of baseline eGFR and treatment group. Albuminuria seemed to be reduced more in the highest eGFR quartile (eGFR ≥85 ml/min per 1.73 m^2). The treatment effect was not significantly different across eGFR quartiles (ANOVA interaction $P = 0.59$). There was a significantly greater reduction in UACR in the EPL100 group *versus* the placebo group in quartiles 1, 3, and 4 ($P = 0.01$, 0.03, and 0.01, respectively) and in the EPL50 group *versus* the placebo group in quartiles 1 and 4 ($P < 0.001$). There were no significant differences between eplerenone treatment groups.

Effect of Treatment on BP. The time course of BP changes by treatment group is summarized in Figure 4. Both SBP (Figure 4A) and DBP (Figure 4B) decreased in all three treatment groups at weeks 4, 8, and 12. SBP was significantly reduced from baseline in all three treatment groups at weeks 4, 8, and 12 (all $P < 0.001$, except for placebo treatment at week 4, which was $P = 0.02$). Although BP reduction was slightly less in the placebo group, there were no significant differences in BP reduction between treatment groups, with the exception of a significant difference in SBP at week 4 between the EPL50 group and the placebo group ($P = 0.01$). It is important to note that there were no significant differences in BP between treatment groups at week 12. Equivalence of BP at week 12 facilitates delineation of relative effects of eplerenone on urinary protein *per se* independent of BP lowering. Because of the anticipated confounding effect of add-on amlodipine administration beginning

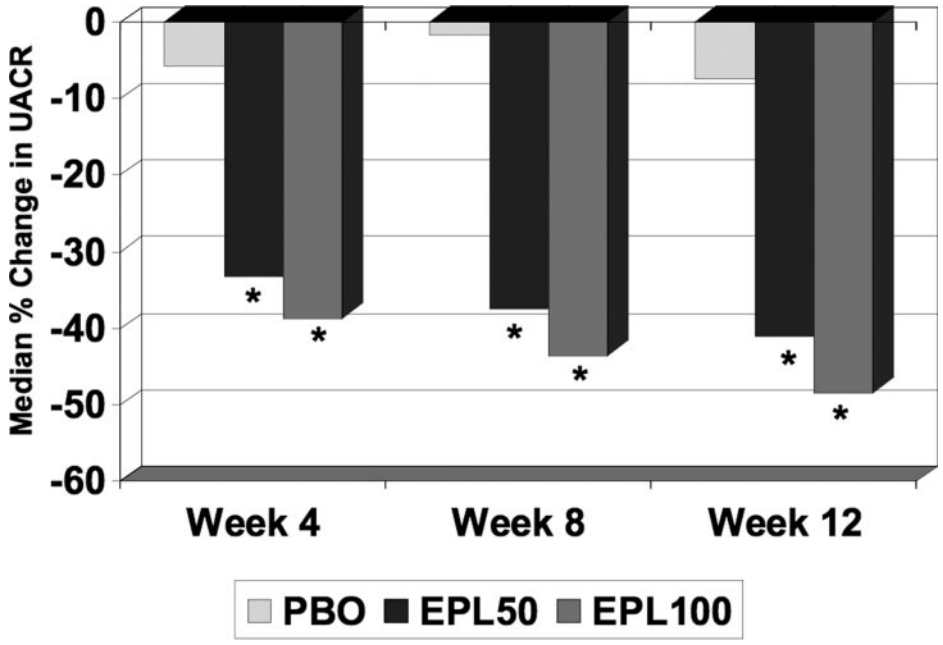

Figure 2. Overall percentage change from baseline urinary albumin:creatinine ratio (UACR) over time by treatment group. *$P <$ 0.001 *versus* PBO.

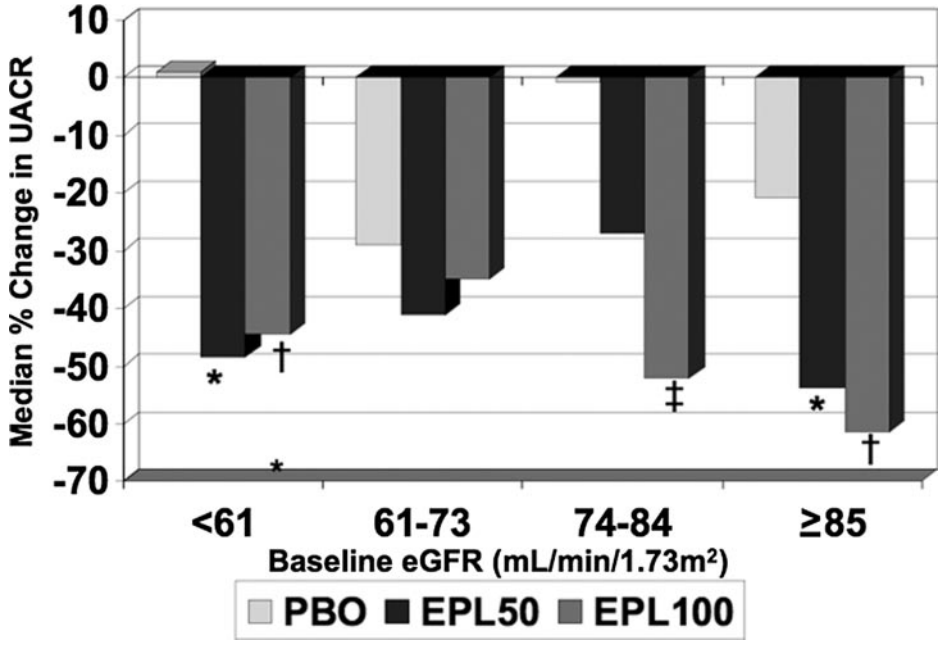

Figure 3. Percentage change in median UACR from baseline to week 12, by quartile of baseline estimated glomerular filtration rate (eGFR) and treatment group. *$P < 0.001$ *versus* PBO; †$P = 0.01$ *versus* PBO; ‡$P = 0.03$ *versus* PBO.

at week 4, a comparison of antihypertensive efficacy of eplerenone from week 4 to the end of study is inappropriate.

The numbers of patients in the efficacy analysis who required add-on antihypertensive treatment with amlodipine to achieve target BP goals (SBP/DBP $<$ 130/80 mmHg) at week 10 (the last week that amlodipine could be added) was 64 (74%) of 87 patients in the placebo group, 62 (70%) of 89 patients in the EPL50 group, and 48 (58%) of 83 patients in the EPL100 arm. Statistical comparisons among the treatment groups demonstrated no significant differences in the number of patients who required add-on treatment for BP.

SBP and UACR were somewhat correlated, particularly by week 12. Spearman correlation coefficients between percentage changes from baseline in UACR and SBP at weeks 4, 8, and 12 were 0.16, 0.19, and 0.34, respectively. The correlations were significantly different from zero.

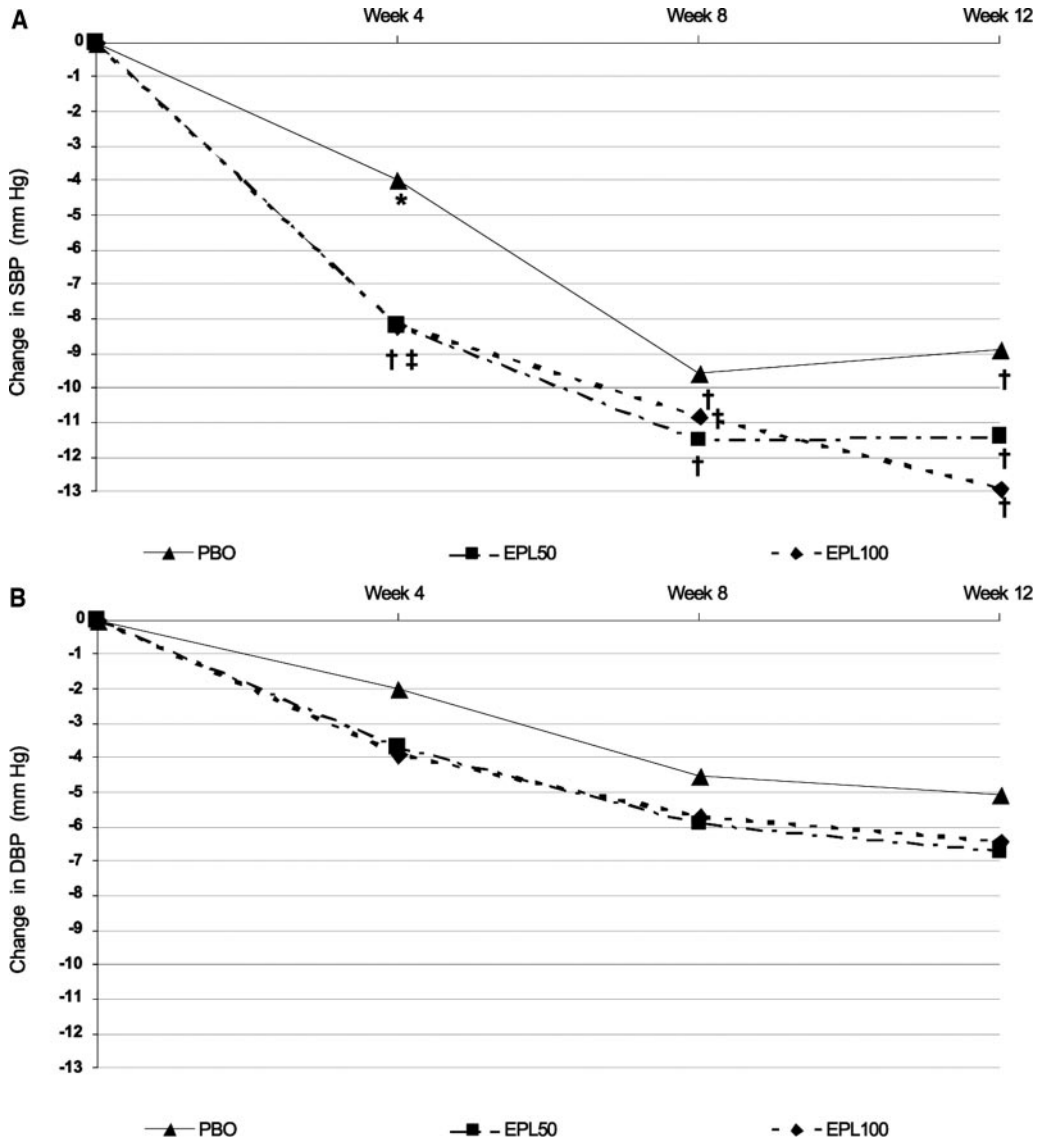

Figure 4. Time course of BP changes, by treatment group, for SBP (A) and DBP (B). *$P = 0.02$ *versus* baseline; †$P < 0.001$ *versus* baseline; ‡$P = 0.01$ for EPL50 *versus* PBO.

Table 3. Median eGFR over time

Treatment Group	Median (Q_1, Q_3; ml/min per 1.73 m^2)			
	Baseline	Week 4	Week 8	Week 12
PBO	74 (60.5, 82.2)	68 (56.9, 81.1)	74 (63.0, 83.4)	72 (63.2, 83.5)
EPL50	73 (62.1, 83.6)	69 (57.8, 79.0)	71 (58.2, 82.3)	67 (59.5, 81.6)
EPL100	75 (62.8, 85.9)	65 (57.2, 82.3)	70 (61.3, 83.5)	66 (57.0, 85.1)

Q_1, 25th percentile; Q_3, 75th percentile.

Effect of Treatment on eGFR. Table 3 presents median eGFR values over time, from week 4 to week 12. Figure 5 displays the absolute change from baseline eGFR at weeks 4, 8, and 12.

eGFR and UACR were weakly correlated. Spearman correlation coefficients between percentage changes from baseline in eGFR and UACR all were <0.17. No correlations were found between percentage changes in eGFR and SBP.

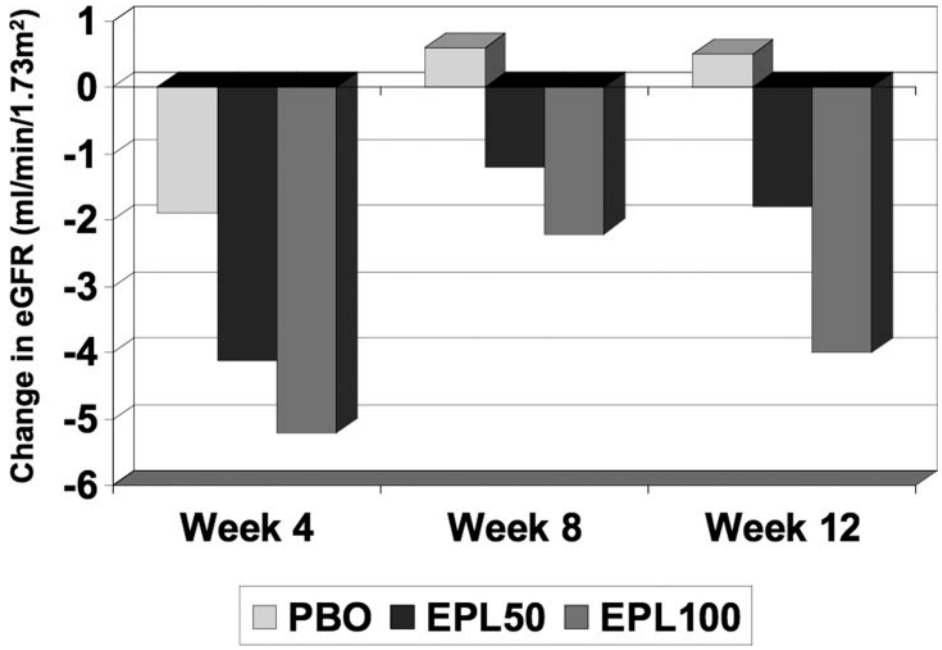

Figure 5. Overall absolute change from baseline eGFR over time by treatment group.

Safety Results

Serum Potassium Elevations. The incidences of sustained (>5.5 mmol/L on two consecutive occasions) and severe (≥6.0 mmol/L at any time) hyperkalemia by treatment group are presented in Table 4. There was no difference in the incidence of either sustained or severe hyperkalemia among the three treatment groups (all NS; Table 4). A trend analysis indicated that there was no trend in the incidence of hyperkalemia with respect to whether a patient was on placebo, EPL50, or EPL100 ($P = 0.29$ for sustained and 0.38 for severe hyperkalemia). The rates of hyperkalemia generally were low among all three treatment groups. There was no difference in the incidence of either sustained or severe hyperkalemia on the basis of quartile of eGFR ($P = 0.27$ and 0.12, respectively). An evaluation of the time to onset of either sustained or severe hyperkalemia failed to suggest conclusively a particular time period when increased monitoring for hyperkalemia would be particularly beneficial.

Adverse Events. Adverse events that occurred in ≥3% of patients are given in Table 5. All treatments generally were well tolerated. The frequency of patients who discontinued treatment as a result of adverse events was five (5.5%) of 91 in the

Table 4. Incidence of hyperkalemia by treatment and eGFR quartile

eGFR Quartile (ml/min per 1.73 m²)	PBO (*n* [%])	EPL50 (*n* [%])	EPL100 (*n* [%])	*P*[a]	
				Within Quartile[b]	Treatment × Quartile Association[c]
Sustained hyperkalemia ($k^+ > 5.5$ mmol/L on two consecutive occasions)					
<61	1/25 (4.0)	1/20 (5.0)	1/19 (5.3)	0.84	0.27
61 to 73	0/19 (0.0)	1/26 (3.8)	1/20 (5.0)	0.37	
74 to 84	0/25 (0.0)	0/22 (0.0)	0/19 (0.0)	—	
≥85	0/19 (0.0)	0/21 (0.0)	1/24 (4.2)	0.26	
Severe hyperkalemia ($k^+ \geq 6.0$ mmol/L)					
<61	2/25 (8.0)	1/20 (5.0)	0/19 (0.0)	0.22	0.12
61 to 73	0/19 (0.0)	1/26 (3.8)	1/20 (5.0)	0.37	
74 to 84	0/25 (0.0)	0/22 (0.0)	1/19 (5.3)	0.18	
≥85	1/19 (5.3)	0/21 (0.0)	3/24 (12.5)	0.29	
Total	88	89	82	259	

[a]Based on the Cochran-Mantel-Haenszel test.
[b]*P* value testing for the association between the incidence of hyperkalemia and treatment within each quartile of eGFR.
[c]*P* value testing for association in the incidence of hyperkalemia between treatment and quartile of eGFR.

Table 5. Incidence of adverse events in ≥3% of patients

Adverse Event	PBO ($n = 91$; n [%])	EPL50 ($n = 91$; n [%])	EPL100 ($n = 86$; n [%])
Any adverse event	43 (47.3)	31 (34.1)	38 (44.2)
Coughing	4 (4.4)	6 (6.6)	3 (3.5)
Peripheral edema	5 (5.5)	1 (1.1)	5 (5.8)
Asthenia	1 (1.1)	1 (1.1)	3 (3.5)
Influenza symptoms	1 (1.1)	2 (2.2)	3 (3.5)
URI[a]	2 (2.2)	1 (1.1)	3 (3.5)
Menstrual disorder[b]	0/41 (0.0)	1/31 (3.2)	0/30 (0.0)
Headache	4 (4.4)	2 (2.2)	2 (2.3)
Injury, accidental	3 (3.3)	0 (0.0)	1 (1.2)
Dizziness	5 (5.5)	1 (1.1)	0 (0.0)

[a]Upper respiratory infection.
[b]Female patients only.

placebo group, two (2.2%) of 91 in the EPL50 group, and 0 patients in the EPL100 group. Serious adverse events occurred in five (5.5%) of 91 patients in the placebo arm, two (2.2%) of 91 patients in the EPL50 arm, and two (2.3%) of 86 patients in the EPL100 group. None of these serious adverse events was judged by the investigator to be related to study medication.

There were no incidences of gynecomastia or female breast pain in the study. In the placebo group, impotence was reported in one patient. In the EPL50 group, one patient experienced impotence and one patient experienced a menstrual abnormality (vaginal bleeding). No patients in the EPL100 group reported any sex-hormone–related adverse events. None of these sexual adverse events was judged by the investigator to be related to study medication.

There was no impact of treatment on HbA_{1c} (Wilcoxon's P values: placebo *versus* EPL50, 0.55; placebo *versus* EPL100, 0.85; EPL50 *versus* EPL100, 0.84). Furthermore, the mean change from baseline in HbA_{1c} was the same for all groups (Kruskal-Wallis $P = 0.87$).

Discussion

Although aldosterone was classically described to act on renal epithelial cells, recent evidence suggests that the most significant contribution of aldosterone to both cardiovascular and renal disease results from nonepithelial, fibrotic, and proinflammatory effects at a number of target organs, including the heart and the kidneys. Epstein (9,10,33) reviewed the rapidly emerging preclinical evidence for aldosterone as a mediator of progressive renal disease. Consistent with previous findings using spironolactone (34), eplerenone has been demonstrated to confer renal protection independent of its effects on BP (15,17). In concert, these preclinical studies in diverse rat models demonstrated that the renal injury and fibrosis that are induced by aldosterone infusion are characterized by a florid inflammatory component that involves macrophage infiltration and cytokine upregulation. In addition, treatment with eplerenone reduced osteopontin and expression of proinflammatory molecules, with consequent attenuation of renal damage and inflammation (15), suggesting that the protective effects of eplerenone are mediated by selective aldosterone blockade and are independent of BP.

Several recent clinical investigations have confirmed the antialbuminuric effects of aldosterone blockade in both patients with and without diabetes. Sato *et al.* (35) demonstrated that in patients with diabetic nephropathy, the antiproteinuric effect of ACE inhibitors reverted to baseline in patients who manifested aldosterone rebound and that nonselective aldosterone blockade in conjunction with ACE inhibitor therapy resulted in an additional and significant decrease in urinary albumin excretion. Bianchi *et al.* (20) demonstrated that nonselective aldosterone blockade with spironolactone effectively reduced proteinuria in patients with chronic kidney disease (CKD). This was a prospective, uncontrolled study of 42 patients with idiopathic chronic glomerulonephritis who were already taking standard therapies, including ACE inhibitors, angiotensin receptor blockers (ARB), and diuretics. Schjoedt *et al.* (36) recently demonstrated that aldosterone escape occurs in 40% of patients with type 1 diabetes and diabetic nephropathy and is associated with an accelerated rate of decline of GFR. More recently, Rossing *et al.* (21) demonstrated that spironolactone added to a maximally recommended dosage of an ACE inhibitor or an ARB resulted in a significant ($P < 0.001$) 33% reduction in albuminuria in patients with type 2 diabetes and nephropathy. Spironolactone treatment induced an insignificant and reversible reduction of GFR in these patients.

Our study extends and amplifies these earlier studies by using two different dosages of a selective aldosterone blocker in addition to an ACE inhibitor in a substantial number of patients with diabetes and albuminuria. Eplerenone in doses of 50 and 100 mg, when added to baseline ACE inhibitor therapy, significantly reduced UACR in comparison with treatment with placebo and background ACE inhibitors. Both of the eplerenone treatment groups demonstrated significant reductions in albuminuria as early as week 4 (Figure 2), before the allowance of add-on amlodipine for BP control. In the placebo group, UACR was not significantly reduced from baseline at any time point, despite incremental decreases in BP after the addition of amlodipine after week 4. Of importance, the greatest percentage

reduction in UACR achieved with eplerenone 50 mg (41%) was not particularly enhanced when the eplerenone dosage was doubled to 100 mg (48%). Collectively, these observations suggest that selective aldosterone blockade produces its antialbuminuric effect substantively by mechanisms that are independent of BP reduction. Importantly, our study demonstrates that the antialbuminuric effect can be achieved readily with eplerenone (50 or 100 mg) and clearly is additive to the antialbuminuric effects of an ACE inhibitor.

In contrast to the duration of effect of spironolactone (21), studies that used ambulatory BP monitoring demonstrated that eplerenone produces a sustained BP-lowering effect throughout a 24-h period (37). Consequently, the determination of BP at the end of the dosing interval in this study should not have an effect on our conclusion that there were no significant differences in BP between treatment regimens.

The selection of the ACE inhibitor dosage and the implications for interpreting the additivity of effect of eplerenone warrants explanation. Because patients were eligible for enrollment either with or without hypertension, we selected a dosage of enalapril that is known to produce an antialbuminuric effect without unwanted concomitant hypotension. Indeed, Keilani *et al.* (38) previously demonstrated that a low dosage of an ACE inhibitor (1.25 mg of ramipril orally once daily) clearly was efficacious in producing an antialbuminuric effect without concomitant hypotension.

The incidence of hyperkalemia that is associated with low-dose eplerenone treatment in this study merits comment. Previously, a forced-titration study with eplerenone in hypertensive patients with type 2 diabetes demonstrated a reduction in proteinuria at dosages of 200 mg/d, both as a monotherapy and when co-administered with enalapril 10 mg (27). However, this high dosage of eplerenone was associated with an increased risk for hyperkalemia in this population. Therefore, our study investigated whether lower dosages of eplerenone, co-administered with enalapril, would produce a similar antialbuminuric effect in these patients without producing the hyperkalemia that was observed previously. Indeed, in our study, the substantial reduction in UACR in the EPL50/enalapril treatment arm was not accompanied by significant increases in the incidences of either sustained or severe hyperkalemia compared with placebo/enalapril treatment. Whereas the incidences of sustained and severe potassium elevations with EPL100 treatment were not significantly higher than those with EPL50 treatment, in some cases, they were numerically higher, and, furthermore, there did not seem to be an incremental benefit for reduction of albuminuria with this higher eplerenone dosage. Consequently, a dosing regimen of EPL50 with an ACE inhibitor may confer the desired antialbuminuric benefit while reducing the risk for hyperkalemia. It should be noted, however, that the majority (90%) of the study patients had a baseline eGFR >50 ml/min per 1.73 m^2 at entry (eGFR at entry varied widely from 34 to 153 ml/min per 1.73 m^2). Consequently, these results cannot yet be extrapolated to patients with type 2 diabetes and an eGFR <50 ml/min per 1.73 m^2.

The impressive absence of progestational and antiandrogenic events in patients who received eplerenone also warrants comment. Although spironolactone is an effective MR-blocking agent, its widespread use is limited by its adverse effects (33). At standard dosages, it can cause both impotence and gynecomastia in men and menstrual disturbances in women (39). These adverse effects are due to the promiscuous binding of spironolactone to progesterone and androgen receptors, and they constitute an important cause of drug discontinuation (39). Indeed, in the Randomized Aldactone Evaluation Study (RALES) (40), 10% of male patients who were treated with spironolactone 50 mg/d reported gynecomastia or breast pain. Eplerenone has >100-fold lower affinity for progesterone and androgen receptors than nonselective MR blockers (41). This study confirms and extends previous suggestions that this structural difference results in substantively fewer progestational and antiandrogenic adverse events.

The possibility that eplerenone-induced decrements in eGFR may have mediated the antialbuminuric effects should be considered. As noted in Figure 5, the initial decrement in eGFR was most marked at week 4 and reverted toward baseline by week 12. Because the maximal reduction in UACR was observed at week 12 and because there was a temporal dissociation between the return of GFR toward baseline and the concomitant reduction in UACR, it is unlikely that the reduction in UACR was mediated primarily by changes in eGFR.

The possibility that the concomitant medications may have confounded the observed changes in UACR merits consideration. Although it has been reported that HmG-CoA (HMG-CoA) reductase inhibitors reduce proteinuria in patients with established proteinuria (20,42), this did not seem to be the case in our study. As noted in Table 1, the number of patients who received HmG-reductase inhibitors did not differ among the three groups. In addition, although dihydropyridine calcium antagonists have been proposed by some authors to exacerbate or amplify proteinuria (43), Table 1 shows that dihydropyridine usage also did not differ among the three treatment groups.

There are several potential mechanisms whereby eplerenone may reduce albuminuria. As detailed in recent reviews (9,10,32,44), aldosterone may promote fibrosis and target-organ dysfunction in the hypertensive patient *via* plasminogen-activator inhibitor expression and consequent alterations of vascular fibrinolysis (45), stimulation of TGF-β (46), stimulation of reactive oxygen species (47), and upregulation of AngII receptors (48,49). Another probable mechanism relates to the potential proinflammatory effects of AngII and aldosterone. In concert, the robust preclinical database provides compelling data demonstrating that the proinflammatory effects of aldosterone constitute major determinants of both the albuminuria of aldosterone and its reversal with eplerenone. By blocking the effects of aldosterone at its receptor, eplerenone thus would mitigate or attenuate these adverse effects, thereby mediating a reduction in albuminuria in patients with type 2 diabetes and hypertension.

Our study has several important clinical implications for establishing new treatment algorithms for patients with incipient nephropathy. Because full doses of ACE inhibitors and ARB attenuate but do not abrogate progression of renal disease,

additional therapeutic strategies that are additive to renin and AngII blockade are necessary. Because we have demonstrated that add-on aldosterone blockade reduces albuminuria, it suggests that long-term outcome studies that encompass hard end points should be conducted to test the hypothesis that add-on aldosterone blockade may constitute an additional therapeutic strategy for retarding progression of renal disease. Although theoretical considerations may suggest that this patient cohort is more susceptible to hyperkalemia, a recent study by Ravis *et al.* (50) in patients with CKD (creatinine clearance ranged from <30 [hemodialysis] to 80 ml/min) demonstrated that eplerenone-induced hyperkalemia was surprisingly infrequent. Serum potassium values increased modestly in all renal failure patients (range 0 to 0.50 mmol/L), but there were no discontinuations as a result of clinically relevant hyperkalemia.

Finally, we suggest that this treatment paradigm potentially may have a role in a wide array of clinical disorders in addition to CKD. Aldosterone has been shown to be a determinant of both cardiovascular and renal disease, and cardiovascular complications are inordinately overrepresented in patients with CKD. Consequently, our proposed intervention with low-dosage aldosterone blockade potentially may constitute a focused and powerful strategy for simultaneously attenuating both progression of renal disease and possibly its attendant cardiovascular complications.

Conclusion

Our study demonstrates that eplerenone, in doses of 50 or 100 mg added to the ACE inhibitor enalapril, results in a substantive and statistically significant reduction in albuminuria, as measured by UACR, in patients with type 2 diabetes. A dosing regimen of EPL50 with an ACE inhibitor may be preferable to EPL100 with an ACE inhibitor, because it confers the desired antialbuminuric benefit with a lesser risk for hyperkalemia. An appropriate caveat would be not to extrapolate these findings to patients with an eGFR <50 ml/min per 1.73 m^2, or to patients who are older than 66 yr, until additional studies are conducted in this patient cohort. This study supports the hypothesis that the role of aldosterone in promoting albuminuria in diabetes can be dissociated from the effects of renin and AngII in the RAAS. However, additional long-term studies are required to ascertain whether the antialbuminuric effect of eplerenone is sustained and whether it leads to long-term attenuation of the progression of renal disease.

Acknowledgments

Financial support for this study was provided by Pharmacia, Inc. (currently Pfizer Inc.), Skokie, IL. Editorial support was provided by Jennifer L. Alexander, MSc, at Medesta Publication Group and was funded by Pfizer Inc.

Preliminary findings were presented at the annual meeting of American Society of Nephrology; November 12 through 17, 2003; San Diego, CA.

M.E. and G.H.W. were paid consultants for Pharmacia, Inc. (currently Pfizer Inc.).

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