Effects of Perindopril on Renal Histomorphometry in Diabetic Subjects With Microalbuminuria: A 3-Year Placebo-Controlled Biopsy Study

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We conducted a 3-year randomized placebo-controlled double-blind study to determine the effects of the angiotensin-converting enzyme (ACE) inhibitor perindopril (PE) on the progress of renal function and histology in subjects with diabetes and microalbuminuria. Forty non–insulin-dependent (NIDDM) and insulin-dependent (IDDM) diabetic subjects, either normotensive or hypertensive, were randomly assigned to receive PE (n = 20) or placebo (n = 20). A percutaneous renal biopsy was performed initially in all patients and repeated in 29 patients after 3 years. The mean glomerular volume, glomerular basement membrane (GBM) thickness, interstitial fibrosis, sclerosed glomeruli, and volume fraction of capillary lumina were measured histomorphometrically. Before treatment, both groups had similar clinical characteristics, blood pressure, glycosylated hemoglobin (Hb), albumin excretion rate, glomerular filtration rate (GFR), serum creatinine, and renal structural damage. Blood pressure was well controlled in both groups. After 3 years' therapy, there was no significant change in renal function and albuminuria in the PE or placebo groups. The increase in GBM thickness in nine paired biopsies was significantly less in PE-treated subjects ($P = .0275$). Interstitial fibrosis tended to increase less in the PE group, although this did not reach statistical significance. This study indicates that long-term therapy with PE may decrease or delay the progression of structural glomerular damage in microalbuminuric diabetic subjects.
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IN DIABETES MELLITUS, kidney involvement is characterized by the development of both histological and functional abnormalities. The effects of angiotensin-converting enzyme (ACE) inhibitors on the progression of renal function have been extensively investigated. Studies in both insulin-dependent (IDDM) and non–insulin-dependent diabetics with nephropathy have shown that ACE inhibitors delay or prevent the progression of proteinuria and reduce the risk of end-stage renal failure in both normotensive and hypertensive patients.[1-4] Moreover, experimental studies have established that treatment with ACE inhibitors arrests or retards the progression of glomerular damage in various models of diabetic nephropathy (DN).[5-6] However, the effects of ACE inhibitors on the progression of structural renal damage in diabetic subjects with microalbuminuria have not been previously investigated.

We therefore conducted a 3-year randomized placebo-controlled double-blind study to determine the effects of perindopril (PE), a long-acting inhibitor of angiotensin 1–converting enzyme, on renal function and histology in microalbuminuric diabetic subjects.

SUBJECTS AND METHODS

Patients

Forty subjects with NIDDM or IDDM entered the study. The criteria for inclusion were as follows: age 18 to 65 years, microalbuminuria defined as urinary albumin excretion of 20 to 200 mg/L on two of three consecutive occasions, serum creatinine less than 120 μmol/L, and stable glycemic control. Patients were excluded if they had nondiabetic renal disease or other major disease. Patients were either normotensive or hypertensive at entry, but were excluded if they previously received treatment with ACE inhibitor. Hypertension was defined as a resting blood pressure greater than 140 mm Hg systolic (SBP) or 90 mm Hg diastolic (DBP). Subjects were evaluated prospectively for 3 years. Table 1 lists the clinical characteristics at entry.

Study Design

The study was approved by the Board of Medical Research and Ethics Committee of The Royal Melbourne Hospital, and patients provided written consent before starting the study. The subjects were randomly allocated to receive either 4 mg PE (n = 20) or placebo (n = 20) orally once per day in a double-blind manner. Unmarked formulations were provided by Servier Laboratory (Cedex, France). The supine and erect SBP and DBP were measured using a Dinamap (Critikon, USA) machine, initially weekly and then every 3 months. The dose of PE was not altered for blood pressure control; if blood pressure became or remained elevated, further antihypertensive medication was added.

Clinical and metabolic surveillance was maintained throughout the study. Blood pressure and weight were measured at 3-month intervals. The urine protein excretion, albumin excretion, serum creatinine, glucose, glycated hemoglobin (Hb), and glomerular filtration rate (GFR) were measured at baseline and at 12-month intervals over 3 years. A percutaneous renal biopsy was obtained from all subjects at entry. Thirty-one subjects continued the study for 3 years, and 29 underwent a second renal biopsy at the end of the study period.

Renal biopsy. Biopsies were performed percutaneously under ultrasound control. Tissue was divided for light microscopy, immunoperoxidase, and electron microscopy. Tissue for light microscopy was immediately fixed in mercuric-Formalin solution. Paraffin-embedded blocks were cut at 1 μm and stained with periodic acid-Schiff, silver methionine, and silver masson trichrome.

Histomorphometry. The mean glomerular volume and capillary volume were calculated by the method of Hirose et al[7] using the arithmetic mean profile surface area described by Bilous et al.[8] Point counting was performed at 400× magnification using a 25-μm eyepiece graticule. The distance between points corresponded to 25 μm. The glomerulus for point counting was defined as PAS-positive material and capillary lumen, but excluded Bowman's capsule and the space between glomerular segments and capillary loops. Sclerosed glomeruli were those in which all glomerular tufts were involuted or replaced by scar, and are expressed as a percentage of total glomeruli. The relative cortical interstitial area was calculated and expressed as a percentage of the total area. Interstitial fibrosis was estimated as points on the tissue between tubules but excluding glomeruli, arteries, and arterioles.

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0026-0495/98/4712-1004$3.00/0

Table 1. Clinical and Biochemical Characteristics of Diabetic Subjects With Microalbuminuria

Characteristic	PE (n = 20)	Placebo (n = 20)
Sex ratio (men/women)	16/4	16/4
Age (yr)	43 ± 3	49 ± 3
Duration of diabetes (yr)	16 ± 2	14 ± 2
Hypertensive (n)	10	7
IDDM (n)	12	8
Supine SBP		
Initial	144 ± 5	138 ± 4
Final	141 ± 7	147 ± 3
Supine DBP		
Initial	83 ± 3	83 ± 2
Final	80 ± 2	82 ± 2
Urinary albumin (mg/L)		
Initial	85 ± 18	84 ± 25
Final	101 ± 42	97 ± 43
Albumin excretion rate (μg/min; natural log)		
Initial	3.9 ± 0.2	4.4 ± 0.15
Final	3.2 ± 0.82	4.8 ± 0.66
GFR (mL/min)		
Initial	91 ± 7	96 ± 8
Final	82 ± 8	90 ± 7
Serum creatinine (μmol/L)		
Initial	97 ± 5	89 ± 4
Final	96 ± 6	87 ± 5
Glycated Hb (%)		
Initial	11.1 ± 0.9	11.3 ± 0.08
Final	10.00 ± 0.7	11.8 ± 0.6

NOTE. Data are expressed as the mean ± SEM.

In subjects in whom adequate tissue was obtained, glomerular basement membrane (GBM) thickness was measured by electron microscopy.

Statistical Analysis

Results are expressed as the mean ± SEM, or the median and range for nonparametric data. Analysis was performed on all fully documented patients. Homogeneity between groups was tested by the Student *t* test for independent samples and the Wilcoxon test for quantitative variables, and by a χ^2 test or the Fisher test for qualitative variables. Comparison between groups was performed using a two-way ANOVA (treatment × time) with repeated measurements on time, and a Student *t*-test and Mann-Whitney and Wilcoxon tests on the relative changes between baseline and final values. Values for the urinary albumin excretion rate were logarithmically transformed before analysis because of skewed distribution. Linear regression analysis and forward stepwise linear regression analysis were used to analyze data for the correlation between morphological parameters and microalbuminuria, urinary albumin, and the GFR. Correlation analyses were performed with absolute values measured at the initial visit and at the last visit. A *P* value less than .05 was considered significant.

RESULTS

Clinical Characteristics

Baseline data of the two groups are listed in Table 1. There were no statistical differences in initial characteristics between the PE and placebo groups. Nine patients were prematurely removed from the study: three PE subjects (two withdrew for personal convenience and one was lost to follow-up study) and six placebo subjects (three deaths, two intercurrent disease, and one poor compliance). The final analysis of matched data was therefore performed on 31 patients, of whom 17 received PE and 14 received placebo. Biopsy data were available on 29 subjects.

During the follow-up period, two PE-treated patients and six placebo-treated patients who were classified as normotensive at inclusion developed elevated blood pressure and were started on antihypertensive therapy. At the end of the study, 10 PE-treated patients and seven placebo-treated patients were receiving other antihypertensive drugs (calcium-channel blockers, β-blockers, α-blockers, or diuretics). In both groups, blood pressure was unchanged after 3 years' therapy. Glycated Hb and blood glucose levels were similar in the two treatment groups at baseline and after 3 years, and overall changes were not significantly different between the groups. The mean body weight was unchanged throughout the study. No side effects of the drug were reported.

Renal Function

There was no significant change in renal function in either the PE or placebo group after 3 years. The serum creatinine concentration remained unchanged throughout the study in both groups. The mean GFR in PE and placebo subjects did not change significantly over 3 years. There was no significant change in urinary albumin and albumin excretion rates during the study period and no difference between PE and placebo groups at the end of the study ($P = .666$ and $P = .492$, respectively).

Renal Histology

Initial histological characteristics in the PE and placebo groups were similar (Table 2). After 3 years' treatment, GBM thickness increased in the placebo group, whereas it remained

Table 2. Histomorphometric Features of Renal Biopsies in PE (n = 16) and Placebo (n = 13) Groups Initially and After 3 Years

Feature	First Biopsy	Second Biopsy
Sclerosed glomeruli (%)		
PE	13.9 ± 3.2	17.8 ± 3.6
Placebo	14.9 ± 3.7	22.5 ± 4.2
Volume fraction of capillary lumina (%)		
PE	0.429 ± 0.019	0.431 ± 0.021
Placebo	0.435 ± 0.018	0.428 ± 0.022
Index of interstitial fibrosis (%)		
PE	32.5 ± 2.7	36.4 ± 2.5
Placebo	27.9 ± 2.5	37.4 ± 2.4*
Mean glomerular volume ($\mu m^3 \times 10^6$)		
PE	1.42 ± 0.18	2.0 ± 0.28
Placebo	1.41 ± 0.17	1.69 ± 0.18
GBM thickness (nm)		
PE (n = 5)	419 ± 63 (359-505)	417 ± 60 (421-572)
Placebo (n = 4)	435 ± 39 (201-589)	517 ± 33† (229-571)

NOTE. Data are the mean ± SEM. The range is shown for GBM thickness.

*$P < .05$ *v* first biopsy.

†Change in GBM thickness, $P < .05$ *v* PE.

stable in the PE group. At the end of the study, GBM thickness was significantly higher in the placebo group versus the PE group ($P = .0275$). It should be noted, though, that only nine paired biopsies were available for analysis. In placebo-treated subjects, interstitial fibrosis increased after 3 years ($P = .0054$), whereas there was no change in PE-treated subjects ($P = .087$). There was no significant difference in the percentage of sclerosed glomeruli, mean glomerular volume, or volume fraction of capillary lumina between PE and placebo groups at study completion.

There was no significant correlation between the renal histomorphometry at initial biopsy and initial renal function. After 3 years' therapy in the PE group, the percentage of occluded glomeruli on second biopsy was negatively related to the GFR ($r = -.58$, $P = .028$). Neither the GBM thickness nor mean glomerular volume in second biopsy were significantly related to the final albumin excretion rate (actual or log-transformed) in either group.

DISCUSSION

This randomized controlled study describes the effects of long-term therapy with PE on clinical and histological parameters in diabetic subjects with microalbuminuria.

Although final analyses were performed on matched data, the power of the study was limited by the number of patients completing the study. Since there was no significant deterioration in renal function as measured by serum creatinine or the GFR or albumin excretion rates in either group, it is not surprising that we were unable to define a significant difference between the two groups in terms of renal function. This highlights the slow rate of progression of DN in the early stage in patients in whom blood pressure is well controlled.

ACE inhibitors have been shown to retard the rate of declining renal function in both normotensive and hypertensive patients with DN.[1,3,4] However, experimental data have been conflicting as to the capacity of ACE inhibitors to confer a specific advantage over other antihypertensive drugs in retarding or preventing glomerulopathy.[9,10]

Differences between studies on the effect of ACE inhibitor therapy on proteinuria in diabetic glomerular disease may be partly attributed to differences in treatment protocols and experimental populations, variable quality of study design, and highly variable duration of therapy. ACE inhibitors are known to decrease urinary protein excretion in patients with nondiabetic glomerulopathies,[11] and some of the "renoprotective" effects of ACE inhibition may be nonspecific. Renal biopsy is important to absolutely exclude nondiabetic renal disease before assessing the effects of ACE therapy in microalbuminuric diabetic subjects.

Cross-sectional studies in established DN have described several histological features correlating with renal function, including mesangial enlargement, glomerular size, GBM thickness, capillary filtration surface, obliteration of glomeruli, and increased interstitial fibrosis.[12-15] Whether these changes are observed in the early stage of diabetic glomerulopathy remains controversial.[16]

In our study, histomorphometry of the initial and paired biopsies showed that PE significantly reduced the progression of GBM thickening compared with placebo in nine biopsies available for analysis. This accords with the findings of Cooper et al,[6] who used a rat model to demonstrate that enalapril retards the development of GBM thickening in both normotensive and hypertensive diabetic rats. The increase in interstitial fibrosis tended to be smaller in PE-treated patients compared with the placebo group, although the difference did not reach the significance threshold (Table 2). Thus, this study suggests that long-term therapy with PE may modify glomerular and interstitial damage in microalbuminuric diabetic patients.

Consistent with the findings of other investigators, we found no correlation between GBM thickening and the level of albuminuria[13] either at baseline or after long-term therapy with PE. This suggests that GBM thickening per se is unlikely to be responsible for albuminuria in our patients.

In summary, the present study indicates that long-term therapy with an ACE inhibitor can delay or diminish the progression of structural renal damage in diabetic subjects with microalbuminuria.

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