

# Randomized Trial of an Inhibitor of Formation of Advanced Glycation End Products in Diabetic Nephropathy

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## Key Words

Pimagedine · Advanced glycation end product inhibition · Diabetic nephropathy · Diabetic retinopathy

## Abstract

**Background/Aims:** Pimagedine inhibits the formation of advanced glycation end products and slows the progression of diabetic complications in experimental models. This study was undertaken to determine if pimagedine ameliorates nephropathy in type 1 (insulin-dependent) diabetes mellitus. **Methods:** This was a randomized, double-masked, placebo-controlled study performed in 690 patients with type 1 diabetes mellitus, nephropathy, and retinopathy. The patients received twice daily dosing with placebo, pimagedine 150 mg, or pimagedine 300 mg for 2–4 years. The primary end point was the time to doubling of serum creatinine; the secondary end points included evaluations of proteinuria, kidney function, and retinopathy. **Results:** Serum creatinine doubled

in 26% (61/236) of the placebo-treated patients and in 20% (91/454) of those who received pimagedine ( $p = 0.099$ ). The estimated glomerular filtration rate decreased more slowly in the pimagedine-treated patients with a 36-month decrease from baseline of 6.26 ml/min/1.73 m<sup>2</sup> as compared with 9.80 ml/min/1.73 m<sup>2</sup> in the placebo-treated patients ( $p = 0.05$ ), and pimagedine reduced the 24-hour total urinary proteinuria. (The mean reduction from baseline at month 36 was 732 mg/24 h at the low dose and 329 mg/24 h at the high dose as compared with 35 mg/24 h in the placebo group;  $p \leq 0.001$ .) Fewer pimagedine-treated patients with baseline and end point evaluations (31/324; 10%) as compared with those receiving placebo (16%; 28/179) experienced a three-step or greater progression of the retinopathy (Early Treatment of Diabetic Retinopathy Study) score ( $p = 0.030$ ). Three patients receiving high-dose pimagedine but none receiving low-dose treatment developed glomerulonephritis. **Conclusions:** While this study did not demonstrate a statistically significant beneficial effect of pima-

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gedine on the progression of overt nephropathy resulting from type 1 diabetes, it is noteworthy in providing the first clinical proof of the concept that inhibiting advanced glycation end product formation can result in a clinically important attenuation of the serious complications of type 1 diabetes mellitus.

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## Introduction

Diabetic nephropathy is the leading cause of end-stage renal disease (ESRD) in the United States [1]. Current treatment includes aggressive control of hyperglycemia, hyperlipidemia, and hypertension, preferably with angiotensin-converting enzyme inhibitors (ACEIs) or angiotensin receptor blockers (ARBs). The chemical interaction of glucose with proteins leads to the formation of early glycation products [2] as a consequence of nonenzymatic reaction of glucose with amino groups on long-lived structural proteins [3]. These products undergo chemical rearrangement/dehydration to form irreversibly protein-bound advanced glycation end products (AGEs). Diabetic patients form excessive amounts of AGEs which are thought to contribute to the development of chronic complications of diabetes mellitus, including nephropathy, retinopathy, and neuropathy [4–6]. Pimagedine has been shown in animal models to inhibit the formation of AGEs and to slow the progression of nephropathy [7–9]. The objective of the ACTION I trial (*A Clinical Trial In Overt Nephropathy of Type 1 Diabetics*) was to determine whether inhibition of AGEs by pimagedine would slow the progression of overt nephropathy in type 1 diabetes mellitus patients. This is the first report of a controlled study performed in man to assess whether inhibition of AGE formation can alter serious complications of diabetes mellitus, specifically nephropathy.

## Patients and Methods

The ACTION I Study was a randomized, double-masked, placebo-controlled clinical trial. The institutional review boards at each center approved the study protocol, and all patients gave written informed consent. The study complied with the Helsinki Agreement.

### Entry and Exclusion Criteria

Individuals aged 22–50 years were eligible, if they had type 1 (i.e., insulin-dependent) diabetes mellitus by clinical assessment and medical history with a disease duration >7 years and disease onset prior to age 30, diabetic retinopathy, and nephropathy with a 24-hour total

urinary protein concentration  $\geq 500$  mg and a creatinine clearance of 40–90 ml/min (0.67–1.50 ml/s) [10]. Exclusion criteria were unstable diabetes, nondiabetic nephropathy, abnormal liver function tests, antinuclear antibody titer  $\geq 1:80$ , active peptic ulcer disease, severe autonomic neuropathy, or clinical coronary artery disease. The study patients were maintained on tight glycemic, blood pressure, and dietary control as well as on ACEIs or ARBs, whenever possible in accordance with current therapeutic guidelines [11].

### Randomization and Treatment Plan

Eligible patients were randomly assigned to twice daily treatment with placebo, low-dose (LD; 150 mg) pimagedine or high-dose (HD; 300 mg) pimagedine for a 2- to 4-year treatment period to achieve an average of 3 years of therapy. The study drug was supplied as 50- or 100-mg pimagedine tablets and matching placebo tablets. All patients took up to 3 tablets twice daily adjusted for renal function to maintain serum pimagedine levels (predicted from subjects with normal renal function) within an estimated range. Stopping points for study treatment included dialysis, intolerable adverse events, and kidney/pancreas/islet cell transplantation. The patients were seen weekly for 6 weeks, at months 2 and 3, and every 3 months thereafter. Twenty-four-hour urine collections were obtained at baseline and at 3-month intervals. Standardized stereoscopic fundus photography was performed at baseline, at month 12, and at the end of the study. Retinal photographs were reviewed by the Wisconsin Fundus Photograph Reading Center with patient allocation masked to the reader. Photographs were graded to the Early Treatment of Diabetic Retinopathy Study (ETDRS) scale, where a three-step increase defined progression of retinopathy.

### Study End Points

The primary study end point was the time to doubling of baseline serum creatinine. The definition of a doubling event was based on comparison to the average of the serum creatinine levels obtained at baseline and randomization. Secondary end points included changes in total urinary protein, lipid parameters, glycosylated hemoglobin, blood pressure, change in ETDRS score, all-cause mortality, development of ESRD (maintenance dialysis or renal transplantation), and adverse event incidence.

### Statistics

The primary, prospectively planned analysis for all end points was the difference between the combined pimagedine dosage group and the placebo group; comparisons between each pimagedine dose group and the placebo group were considered secondary. The trial was powered on the expectation of a 50% decrease in the serum creatinine doubling event hazard rate, a magnitude of effect previously observed in patients treated with captopril versus non-ACEI controls in the 1993 collaborative study [12].

Demographic and baseline characteristics were compared using the Cochran-Mantel-Haenszel test stratified by center, the Van Elteren test, or a two-way analysis of variance. If continuous variables were nonnormally distributed, analysis of variance on ranks was performed instead. Kaplan-Meier survival estimates of the length of time to doubling of serum creatinine and time to ESRD or death were presented by treatment group, and treatment comparisons of these rates were made using the log-rank test. A multivariate repeated-measures analysis based on generalized estimating equations was used to analyze total urinary protein. The change from baseline in total urinary protein was also analyzed separately at each

**Table 1.** Baseline characteristics

	Placebo	Pimagedine	
		LD 150 mg b.i.d.	HD 300 mg b.i.d.
Total patients randomized	236	229	225
Age, years <sup>a</sup>	40 ± 7.6	39 ± 7.4	39 ± 7.4
Gender, % males	58	59	61
Race, % Caucasians	90	91	92
Body mass index, %			
<25.0 kg/m <sup>2</sup>	46	45	49
25.0–29.9 kg/m <sup>2</sup>	40	41	40
>30.0 kg/m <sup>2</sup>	14	14	0
Smoking status, % past or present <sup>b</sup>	53	47	45
Duration of diabetes, years <sup>a</sup>	25.9 ± 7.6	25.5 ± 7.4	25.3 ± 7.4
Blood pressure, mm Hg <sup>a, c</sup>			
Systolic	140 ± 21	140 ± 18	138 ± 20
Diastolic	82 ± 11	81 ± 11	82 ± 12
Serum creatinine, mg/dl <sup>a</sup>	1.53 ± 0.45	1.56 ± 0.45	1.58 ± 0.44
Measured creatinine clearance, ml/min <sup>a</sup>	60 ± 28.7	60 ± 28.3	59 ± 29.7
Estimated GFR <sup>a, d</sup> , ml/min/1.73 m <sup>2</sup>	49.9 ± 17.3	48.9 ± 16.5	49.2 ± 17.9
Proteinuria, g/24 h <sup>a</sup>	2.71 ± 2.42	2.69 ± 2.23	2.66 ± 2.22
Retinopathy present, %	95	95	94
HbA <sub>1c</sub> , % <sup>a</sup>	9.42 ± 1.49	9.27 ± 1.64	9.22 ± 1.48
Cardiovascular morbidity, %	83	84	82
Prior exposure to ACEI, %	80	78	73
Prior exposure to ARB, %	<5	<5	<5

<sup>a</sup> Data expressed as mean ± SD.

<sup>b</sup> Percentage based on number of patients for whom smoking data were present.

<sup>c</sup> Blood pressure measured after 5 min of rest in seated position.

<sup>d</sup>  $170 \times (\text{serum creatinine})^{-0.999} \times (\text{age})^{-0.176} \times (\text{blood urea nitrogen})^{-0.170} \times (\text{albumin})^{0.318}$  [ $\times 0.762$  if female,  $\times 1.180$  if black].

time point. Comparisons were adjusted by the Bonferroni method for multiple comparisons to the same placebo group. The number of patients exhibiting a three-step increase in the ETDRS score from baseline to end point was analyzed using a logistic regression model. Lipids, blood pressure, and glycated hemoglobin values were analyzed using a two-way analysis of variance. Serum creatinine and 24-hour creatinine clearance have recently been challenged as inadequate for estimation of the glomerular filtration rate (GFR) [13], so when analysis of serum creatinine revealed a wide scatter of points, data were reanalyzed as recommended by the Chronic Kidney Disease Clinical Practice Guidelines of the Kidney Dialysis Outcomes Quality Initiative [13] to obtain estimated GFR values (see table 1 for formula).

The prospectively defined intent-to-treat (ITT) analysis population encompassed all randomized patients. A second population, which was evaluated a posteriori, included patients with a baseline serum creatinine level <1.5 mg/dl (133 μmol/l). To protect against increasing the rate of type I error due to interim analyses, the Lan-DeMets group sequential method set the significance level in the primary analysis of time to doubling of serum creatinine at 0.04568. All statistical tests were two-sided.

## Results

A total of 690 patients were randomized at 56 centers in the United States and Canada. Of these, 236 patients were assigned to placebo, 229 to LD pimagedine, and 225 to HD pimagedine. The baseline demographic, laboratory, and disease characteristics of the groups were comparable (table 1). There were no differences among the groups in baseline diet, total urinary protein, ACEI use, or glucose control.

Table 2 summarizes the patient participation in the trial. The overall median exposure to study treatment was 2.49 years and did not differ appreciably among the three groups. A total of 472 patients (68%) completed the end-of-study visit, with a median follow-up period of 2.85 (range 1.88–4.55) years. Of the total population, 112 patients (16%) reached ESRD or died, with a median follow-up period of 1.59 (range 0.14–4.16) years. Fifteen

**Table 2.** Patient participation and exposure to ACEI/ARB

	Placebo (n = 236)	Pimagedine		Total population (n = 690)
		LD, 150 mg b.i.d. (n = 229)	LD, 300 mg b.i.d. (n = 225)	
Median exposure to study drug, years	2.47	2.53	2.47	2.49
End of study visit	166 (70%)	160 (70%)	146 (65%)	472 (68%)
ESRD or death	41 (17%)	37 (16%)	34 (15%)	112 (16%)
Discontinued participation	29 (12%)	32 (14%)	45 (20%)	106 (15%)
Exposure to ACEI or ARB during the study	93%	93%	92%	–

percent of the patients discontinued participation in the trial without reaching an end point of ESRD or death (median follow-up period 1.57 years). Of these 106 patients, survival and ESRD status at the time of censoring remained unknown in 64 cases. Over 90% of the patients were exposed to an ACEI or ARB at some time during the study.

#### *Serum Creatinine*

In the ITT population, a total of 152 patients experienced a doubling of serum creatinine: 61 of 236 (26%) in the placebo group, 45 of 229 (20%) in the LD pimagedine group, 46 of 225 (20%) in the HD pimagedine group, and 91 of 454 (20%) in the combined pimagedine treatment group ( $p = 0.099$  vs. placebo, log-rank test). Stratification for baseline serum creatinine distribution improved the statistical precision, although the associated risk reduction in the combined pimagedine group as compared with the placebo group remained not significant ( $p = 0.059$ ). Among the patients with a baseline serum creatinine concentration  $<1.5$  mg/dl ( $n = 367$ ), the number of doubling events was 22 of 130 (17%) in the placebo group, 13 of 122 (11%) in the LD pimagedine group, 11 of 115 (10%) in the HD pimagedine group, and 24 of 237 (10%) in the combined pimagedine group. The risk reduction in the combined pimagedine group for those with a baseline serum creatinine concentration  $<1.5$  mg/dl was not significant ( $p = 0.053$  vs. placebo, log-rank test).

The creatinine clearance revealed a wide scatter of values and no differences among the treatment groups. The estimation of the GFR showed a significantly slower rate of decline for pimagedine versus placebo patients (fig. 1), and these differences remained significant after adjustment for changes in blood pressure and urea excretion.

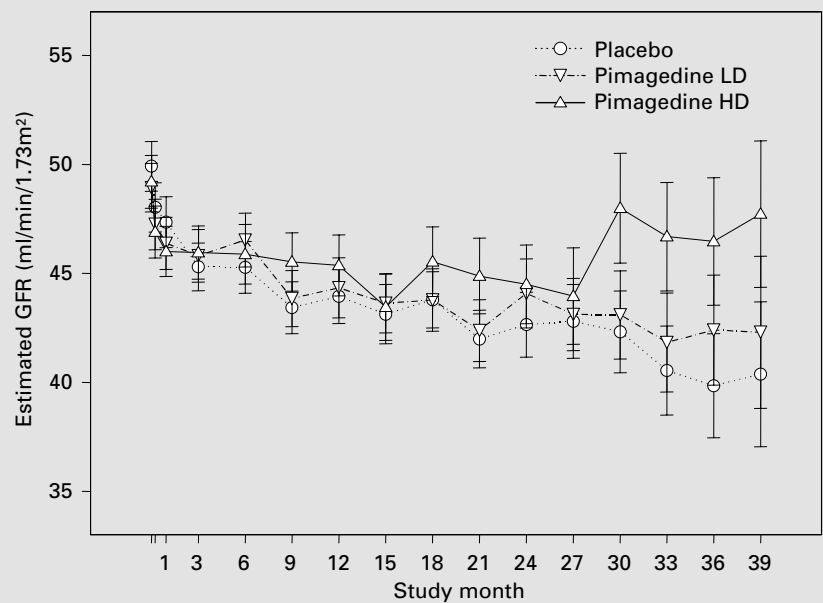
The effect of treatment on the end point of doubling serum creatinine was evaluated in subgroups defined by

the baseline covariates (table 1). Pimagedine was associated with a significant protection against doubling of creatinine in patients with a baseline protein excretion  $>2.0$  g/24 h ( $p = 0.02$  for combined dose group vs. placebo, log-rank test). Smoking was the only other baseline covariate that affected the magnitude of the risk reduction seen with pimagedine; smoking, past or present, was associated with a decreased therapeutic effect ( $p = 0.029$ ).

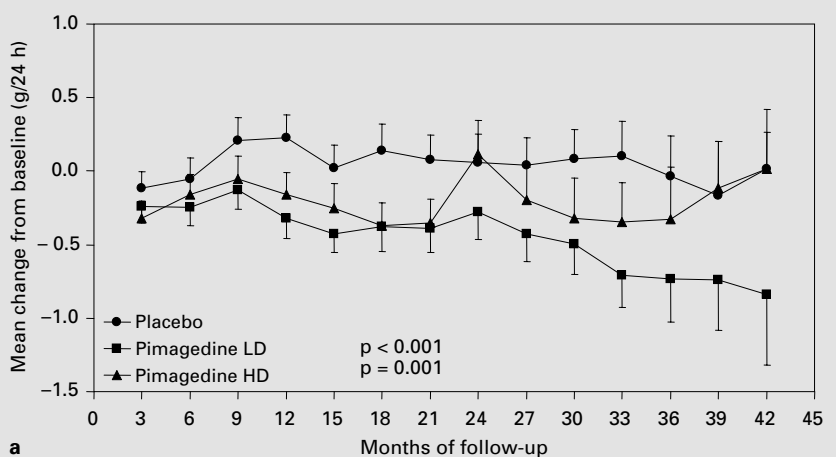
#### *Total Urinary Protein Excretion*

As compared with the placebo group, pimagedine treatment reduced the total urinary protein contents in patients exposed to both the LD ( $p < 0.001$ ) and HD ( $p = 0.001$ ) regimens (ITT population; fig. 2a). The reduction was more pronounced in patients randomized to the LD pimagedine than in those randomized to the HD pimagedine group (mean reduction from baseline at month 36: placebo 35 mg/24 h; LD 732 mg/24 h; HD 329 mg/24 h). In the population with a baseline serum creatinine concentration  $<1.5$  mg/dl (fig. 2b), the effect on proteinuria of LD pimagedine was greater ( $p < 0.001$ ) and exceeded the reduction observed in patients treated with HD pimagedine ( $p = 0.002$ ). The reduction in proteinuria in the combined pimagedine group remained significant after adjustment for changes in blood pressure and urea excretion.

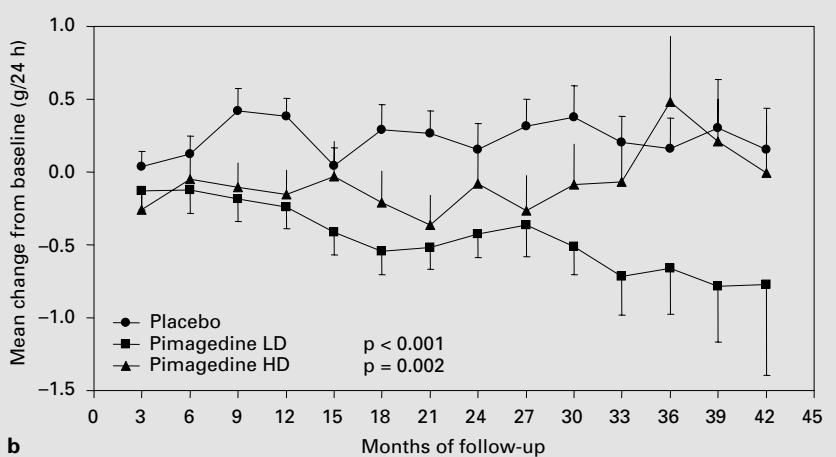
A posteriori analyses were performed to determine the predictive value of the change in proteinuria from baseline during the first 6 months after randomization for subsequent doubling of serum creatinine and progression to ESRD. Changes from baseline in proteinuria were grouped into quartiles using data from the ITT population. Patients with stable or decreased proteinuria experienced fewer instances of doubling of creatinine values or ESRD events ( $p \leq 0.002$ ), regardless of the treatment group. However, comparison of the placebo group to the



**Fig. 1.** Estimated GFR versus time for each treatment group (mean  $\pm$  SEM).



**a**



**b**

**Fig. 2.** Mean ( $\pm$  SEM) change in total urinary protein excretion from baseline. **a** ITT population. **b** Subjects with a baseline serum creatinine concentration  $<1.5$  mg/dl ( $<133$   $\mu$ mol/l).

**Table 3.** ESRD and death in the ITT population

	Placebo	Pimagedine		Combined
		LD 150 mg b.i.d.	HD 300 mg b.i.d.	
Number randomized	236	229	225	454
ESRD <sup>a</sup>	32 (14%)	30 (13%)	28 (12%)	58 (13%)
p vs. placebo <sup>b</sup>		0.691	0.511	0.541
Odds ratio (95% CI)		0.99 (0.57–1.72)	0.95 (0.72–1.25)	0.94 (0.59–1.51)
Deaths <sup>c</sup>	9 (4%)	7 (3%)	6 (3%)	13 (3%)
p vs. placebo <sup>b</sup>		0.676	0.472	0.509
Odds ratio (95% CI)		0.79 (0.29–2.18)	0.71 (0.24–2.02)	0.75 (0.31–1.79)

<sup>a</sup> Number of patients with ESRD event.

<sup>b</sup> Log-rank test, unstratified.

<sup>c</sup> Number of patients who died, excluding patients who died after ESRD.

pimagedine treatment groups revealed a further reduced risk for doubling of serum creatinine or ESRD. The treatment by baseline proteinuria quartile interaction was significant for both doubling of serum creatinine ( $p < 0.001$ ) and for ESRD ( $p \leq 0.009$ ).

#### *ESRD and Mortality*

In the ITT population, 112 patients developed ESRD or died (table 3). Treatment with pimagedine was associated with a statistically insignificant 13% risk-reduction of the combined end points of ESRD or death. Thirty-one events of ESRD or death occurred in the population with a baseline serum creatinine concentration  $< 1.5$  mg/dl: 16 (12%) among placebo patients and 15 (6%) in the combined pimagedine treatment group; the 51% risk reduction associated with pimagedine in this population was also not statistically significant.

#### *Diabetic Retinopathy*

In patients in the ITT population with baseline and end point evaluations, 59 experienced a three-step or greater progression of the ETRDS retinopathy score: 16% (28/179) in the placebo-group, 11% (18/170) in the LD pimagedine group, 8% (13/154) in the HD pimagedine group, and 10% (31/324) in the combined pimagedine treatment group ( $p = 0.030$ , combined pimagedine vs. placebo).

#### *Lipid Levels, Glycated Hemoglobin, and Blood Pressure*

The mean change in total cholesterol from baseline to the last available value was  $-11.6$  mg/dl in the placebo

group as compared with  $-21.9$  mg/dl in the combined pimagedine group ( $p = 0.008$ ), with comparable mean reductions in the two dosage groups. Treatment with LD pimagedine was associated with a larger mean decrease in triglycerides ( $-17.2$  mg/dl) and a larger mean increase in high-density lipoprotein cholesterol ( $4.4$  mg/dl) as compared with placebo treatment (mean changes of  $14.5$  mg/dl and  $2.0$  mg/dl, respectively;  $p < 0.001$  and  $p = 0.031$ ). The mean changes in triglyceride or high-density lipoprotein cholesterol levels in the HD pimagedine group were not significantly different from those in the placebo group.

The mean glycated hemoglobin levels dropped slightly in all treatment arms, but did not differ across the groups. Pimagedine treatment was associated with a slightly lower mean sitting diastolic blood pressure as compared with the placebo group ( $p < 0.05$ ), while there was no significant treatment difference for systolic blood pressure.

#### *Tolerability and Safety*

One or more serious adverse events were observed in 44% of the placebo-treated patients, in 46% of the LD pimagedine patients, and in 51% of the HD pimagedine patients. Treatment with pimagedine was associated with a higher percentage of permanent discontinuations from study treatment (table 4). Pimagedine-related adverse events included induction of autoantibodies, a transient flu-like syndrome, mild liver enzyme elevations, and anemia. The pimagedine-induced flu-like syndrome occurred between weeks 2 and 4 of treatment, signs and symptoms were fully reversible spontaneously or with drug discontinuation, and no long-term sequelae were observed. The

slight elevations in liver enzyme values were self-limited and resolved whether pimagedine was continued or stopped. A mild to moderate anemia of uncertain etiology occurred in all study patients, but was more pronounced in patients on pimagedine, particularly during the first few weeks of treatment. Crescentic glomerulonephritis was observed in 3 patients in the HD pimagedine group and was associated with induction of extremely high levels of antineutrophil cytoplasmic antibodies against myeloperoxidase. Two of the 3 patients required maintenance dialysis. After introduction of a monitoring program for autoantibodies, no further cases of crescentic nephritis were seen. Glomerulonephritis was not observed in any of the 229 ACTION I subjects receiving LD pimagedine for an average of 2.5 years.

## Discussion

The results of the ACTION I Study provide the first suggestive clinical evidence that AGE inhibition therapy may have a role in the treatment of complications associated with diabetes mellitus. In this study, a decrease in the primary end point of delay in time to doubling serum creatinine was not achieved, although a numerical, nonsignificant reduction in the risk of this end point relative to placebo was seen with pimagedine treatment. The study was powered by assuming a 50% decrease in the doubling-event hazard rate, an effect that was not achieved. The nearly universal use of ACEI/ARBs by the clinicians caring for the patients with diabetic nephropathy likely reduced the event rate in the placebo group as compared with the non-ACEI-treated controls upon which power calculations were based [12]. Although this study was limited because it was underpowered for its primary end point and although a substantial majority of the patients (>90%) received ACEI treatment at some time during the study, it did produce a clinically meaningful (although not statistically significant) primary end point risk reduction accompanied by significant effects on other complications of type 1 diabetes mellitus.

Although doubling of the serum creatinine has been accepted as the *sine qua non* for renal function clinical trial outcomes, serum creatinine is influenced by diet, age, sex, race, manner of food preparation, menstrual cycle, muscle mass, and multiple drugs [14]. The Chronic Kidney Disease KDOQI Clinical Practice Guidelines recommend that serum creatinine should not be used to estimate the GFR and suggest that factor-based estimation is more accurate [13]. We observed a wide scatter of serum

**Table 4.** Percentage of patients with permanent discontinuation of study medication as a result of adverse event by body system

Body system <sup>a</sup>	Placebo (n = 236)	Pimagedine	
		LD 150 mg b.i.d. (n = 229)	HD 300 mg b.i.d. (n = 225)
Any discontinuation due to adverse event	8	17	30
Body as whole	<1	3	13
Digestive	6	7	20
Hematopoietic	4	8	13
Urogenital	3	3	3
Musculoskeletal	<1	<1	4
Metabolic	<1	2	1
Cardiovascular	<1	1	2
Respiratory	0	<1	2

<sup>a</sup> Patients may have discontinued the study for an adverse event in more than one body system.

creatinine values in our patients. A post hoc analysis using the formula recommended in the Clinical Practice Guidelines yielded a significant protective effect of pimagedine on the GFR as compared with placebo.

Chronic hyperglycemia in diabetes accelerates AGE formation and tissue injury. In the case of the kidney, AGEs accumulate in basement membranes and microvascular endothelial beds [15, 16] and result in increased permeability to blood proteins and proteinuria [6, 17, 18]. In man, the risk of renal disease progression is highly associated with the level of proteinuria: in recently reported trials of kidney disease progression, the groups with reductions in proteinuria had slower rates of decline [19, 20, 21]. The beneficial effect of proteinuria interventions has led to the PARADE (proteinuria, albuminuria, risk, assessment, detection, elimination) initiative of the National Kidney Foundation [22], and drugs to lower proteinuria are now considered essential components in the treatment of chronic renal failure. In this study, pimagedine caused an important decrease in proteinuria which was independent of any effect on blood pressure reduction. This beneficial effect of pimagedine was associated with an improved renal survival above that obtained with ACEIs.

Renal clearance is an important route of elimination of AGEs. With renal dysfunction, low-molecular-weight AGEs accumulate. It is likely that pimagedine inhibition of AGE formation exceeds the overall rate of AGE accu-

mulation. The efficacy of pimagedine may be limited in patients with advanced kidney disease, such as our population with a serum creatinine concentration  $>133 \mu\text{mol/l}$  (1.5 mg/dl). The observation of a decreased effect of pimagedine in smokers is consistent with this hypothesis, since smoking causes systemic delivery of AGEs from burning tobacco [23]. Finally, the Diabetes Control and Complications Trial [24] showed a positive effect of tight glucose control on microalbuminuria which became manifest only after 3–5 years. Alterations in deposition of AGE proteins might be expected to follow a similar time course.

Although the trial was neither designed nor powered to detect an impact on progression of diabetic retinopathy, exposure to pimagedine was associated with a reduced progression of retinopathy, as determined by a three-step or greater increase in the ETDRS score. Moreover, exposure to pimagedine was associated with a reduction in the levels of total cholesterol and triglycerides and an increase in high-density lipoprotein cholesterol for the LD regimen: both of these effects are compatible with the theoretical mode of action of the drug. A possible impact on lipids consequent to decreased proteinuria was not examined.

The 300-mg twice-daily dosage regimen of pimagedine was associated with side effects, but minimal adverse effects were observed for the lower, 150-mg, twice-daily regimen. A small number of patients (3/225; 1.3%) receiving HD pimagedine developed autoimmune disease that was time dependent. Cessation of treatment in 1 HD patient rapidly led to abatement of antibody levels and resolution of nephritis, as described for hydralazine which is similar in structure to pimagedine and widely used for the treatment of hypertension [25, 26]; the remaining 2 of these patients developed ESRD. With the lower dose and prospective monitoring, no vasculitic events were observed.

The present trial provides, for the first time, proof of the concept that inhibiting AGE formation in man can result in clinically important therapeutic effects on the serious complications of diabetes mellitus. While the AGE inhibitor pimagedine failed to produce a statistically significant reduction in the primary end point of doubling of serum creatinine in this study, treatment with pimagedine did reduce proteinuria and had additional effects on diabetic retinopathy and circulating lipid levels in our diabetic study population – effects which are consistent with broad-spectrum activity of AGE formation on the pathogenesis of diabetic complications. The beneficial effects of pimagedine were apparent at the lower, 150 mg,

twice-daily dose, and this dose was well tolerated for a duration of exposure of up to 4.5 years. Toxicity observed with the higher dose of pimagedine was not noted with the lower dose of the drug. The impact of pimagedine was above that of current standard-of-practice therapies and suggests that the new class of pharmacologic agents able to block or disrupt AGE proteins may provide a clinically useful addition to existing therapies for treating this devastating disease.

## Appendix

Dr. Sharon Adler and Ms. Janine LaPage, Harbor UCLA Medical Center, Torrance, Calif.; Drs. Gerald Appel and C. Kunis, Columbia Presbyterian Medical Center, New York, N.Y.; Dr. Yousri Barri, University of Arkansas for Medical Sciences, Little Rock, Ark.; Dr. André Bélanger and Ms. Michelline Labbe, Centre de Recherche, Laval, Qué., Canada; Dr. David S.H. Bell, Endocrinology Research Unit, Birmingham, Ala.; Dr. Thomas C. Blevins, Center for Clinical Research, Austin, Tex.; Dr. Kline Bolton and Ms. Lori Ratliff, University of Virginia Medical Center, Charlottesville, Va.; Dr. John B. Buse, University of North Carolina at Chapel Hill and Diabetes Care Center, Chapel Hill, N.C.; Dr. Daniel Cattran, Toronto General Hospital, Toronto, Ont., Canada; Dr. Dana H. Clarke, Diabetes Health Center, Salt Lake City, Utah; Dr. George Dailey III, Scripps Clinic and Research Foundation, La Jolla, Calif.; Dr. Paresch Dandona, Millard Fillmore Hospital, Buffalo, N.Y.; Dr. Keith G. Dawson, Diabetes Research Centre, Vancouver, B.C., Canada; Dr. Diana G. Dills, University of Wisconsin-Madison, Madison, Wisc.; Dr. Andrew Drexler, Mt. Sinai Medical Center, New York, N.Y.; Dr. Mary Ann Emanuele and Ms. Diane Kernan, Loyola University Medical Center, Maywood, Ill.; Dr. Lisa H. Fish, International Diabetes Center, Institute for Research and Education Health Systems, Minneapolis, Minn.; Dr. Neal Friedman, Lovelace Scientific Resources, Albuquerque, N.Mex.; Dr. Suzanne Gebhart, Emory Clinic, Atlanta, Ga.; Dr. Barry J. Goldstein, Thomas Jefferson University Hospital, Philadelphia, Pa.; Dr. Douglas Greene, University of Michigan, Ann Arbor, Mich.; Dr. George Grunberger, Detroit, Mich.; Dr. Richard A. Guthrie, Mid-America Diabetes Associates, Wichita, Kans.; Dr. Bruce Henson, International Diabetes Center, Kansas City, Mo.; Dr. Kenneth Hershon, North Shore Diabetes, New Hyde Park, N.Y.; Dr. Irl B. Hirsch, University of Washington Medical Center, Seattle, Wash.; Dr. Carol Joyce, Health Sciences Centre, Metabolism Division, St. John's, Nfld., Canada; Dr. Frank P. Kennedy, Mayo Clinic, Rochester, Minn.; Dr. Kenneth Kleinman, Nephrology Medical Associates, Tarzana, Calif.; Dr. Philip Levy, Phoenix Endocrinology Clinic, Phoenix, Ariz.; Dr. Robert S. Mecklenburg, Virginia Mason Research Center, Seattle, Wash.; Dr. Janet McGill, Washington University, St. Louis, Mo.; Dr. Arshag D. Mooradian, St. Louis University, St. Louis, Mo.; Dr. James L. Neifing and Ms. Kelley Edwards, Portland Diabetes and Endocrinology Center, Portland, Ore.; Dr. Trevor J. Orchard, Diabetes Research Center of the Children's Hospital of Pittsburgh, Pittsburgh, Pa.; Dr. Philip Raskin and Suzanne Strowig, University of Texas Southwestern Medical Center, Dallas, Tex.; Dr. Robert E. Ratner and Ms. Samantha Toomey, Medlantic Clinical Research Center, Washington, D.C.; Dr. Michael L. Reeves, Chattanooga, Tenn.; Dr. Jane Reusch, Denver VA Medical



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