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Original Investigation

Sulodexide for Kidney Protection in Type 2 Diabetes Patients With Microalbuminuria: A Randomized Controlled Trial

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Background: Sulodexide, a heterogenous group of sulfated glycosaminoglycans, includes low-molecular-weight heparin (~80% ± 8%), high-molecular-weight heparin (~5% ± 3%), and dermatan (~20% ± 8%), with a mean molecular weight of ~9 kDa. The drug is absorbed orally and has no anticoagulant effect in the doses used. Small preliminary studies consistently showed sulodexide to be associated with decreased albuminuria in patients with diabetes.

Study Design: We conducted a multicenter placebo-controlled double-blinded study to determine the effect of sulodexide on urine albumin excretion in patients with type 2 diabetic nephropathy.

Setting & Participants: Patients with type 2 diabetes and urine albumin-creatinine ratios (ACRs) of 35-200 mg/g in men and 45-200 mg/g in women were enrolled. Serum creatinine level was <1.5 mg/dL. Blood pressure goal was 130/80 mm Hg. A maximum US Food and Drug Administration–approved dose of an angiotensin-converting enzyme inhibitor or angiotensin receptor blocker for a minimum of 4 months before randomization was required.

Intervention: The study drug was sulodexide, 200 mg/d.

Outcome & Measurements: The primary end point was normoalbuminuria (ACR <20 mg/g and a decrease >25%) or 50% decrease in baseline ACR.

Results: In 1,056 randomly assigned patients with a mean baseline ACR of 107.8 ± 83.7 mg/g, comparing the sulodexide versus placebo groups, the primary end point was achieved in 16.5% versus 18.4%; normoalbuminuria, in 7.9% versus 6.1%; and a 50% decrease in albuminuria, in 15.4% versus 17.6%. The relative probability of any given change in albuminuria was identical in both groups.

Limitations: We were unable to determine whether the administered sulodexide was absorbed from the gastrointestinal tract.

Conclusion: Sulodexide failed to decrease urine albumin excretion in patients with type 2 diabetic nephropathy and microalbuminuria.

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INDEX WORDS: Sulodexide; microalbuminuria; diabetic nephropathy; glycosaminoglycans; albuminuria.

Editorial, p. 692

A progressive increase in urine albumin excretion rate and decrease in glomerular filtration rate are characteristic clinical features of diabetic nephropathy. The initial clinical evidence of renal involvement in type 2 diabetes usually is the appearance of microalbuminuria, which has been defined as an albumin excretion rate of 20-200 μg/min. Patients with diabetes mellitus documented to have microalbuminuria are at high risk of developing overt progressive diabetic nephropathy.[1] In addition, patients with microalbuminuria have been shown to be at a significantly increased risk of experiencing an adverse cardiovascular event.[2] It has been proposed that microalbuminuria represents the clinical manifestation of systemic microvascular pathologic states.[3] Treatment and resolution of microalbuminuria have been reported to be associated with the prevention of progressive kidney injury[4] and a decrease in risk of a subsequent cardiovascular event.[5]

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In a series of small and underpowered studies, previous investigators have reported that sulodexide was capable of decreasing urine albumin excretion rates in this patient population.[6-14] Sulodexide is a glycosaminoglycan extract of porcine lung and liver; the primary components are low-molecular-weight heparin sulfate (80% ± 8%) and dermatan sulfate (20% ± 8%).[15] Pharmacokinetic studies have shown that sulodexide could be absorbed from the gastrointestinal tract in that orally administered deuterium-labeled sulodexide was measurable in plasma of healthy volunteers.[16] The characteristic biological activity of heparin compounds is their ability to interfere with blood coagulation. However, oral sulodexide administration has not been shown to achieve a plasma level capable of inhibiting the generation of factor Xa, the propellant of the terminal coagulation cascade. The achieved plasma oral sulodexide concentration is low, but components of sulodexide have an average molecular weight of ~9 kDa[17] and therefore are freely filterable at the glomerulus, exposing the structural components of the glomerular capillary wall to these anionic molecules.

The purpose of this study was to determine whether sulodexide was capable of normalizing or significantly decreasing urine albumin excretion rates in patients with type 2 diabetes and microalbuminuria who were receiving the highest US Food and Drug Administration (FDA)-recommended dose of an angiotensin receptor blocker (ARB) or angiotensin-converting enzyme (ACE) inhibitor.

METHODS

Participants

Eligible participants were men and women 18 years or older with type 2 diabetes. Participants were required to have a serum creatinine level <1.5 mg/dL and microalbuminuria, defined as albumin-creatinine ratio (ACR) of 35-200 mg/g in men and 45-200 mg/g in women while using an ACE inhibitor or ARB at the maximum FDA-recommended dosage for a minimum of 120 days. The difference in mean 24-hour creatinine excretion rates for males and females with type 2 diabetes entered into the Irbesartan Diabetic Nephropathy Trial was used to determine the ACR required for entry into this study. Patients had to have stable blood pressure ≤150/90 mm Hg. However, the systolic blood pressure goal was 130 mm Hg, and the diastolic goal, 80 mm Hg. Albuminuria at baseline and throughout the study was determined by first morning urine collections. Patients were advised that their physical activities on each day preceding the overnight urine specimen should be approximately the same and unusual alteration in physical activity should be avoided. Patients were excluded if they had glycated hemoglobin levels >12.0% or serum potassium levels >6 mEq/L. Other major exclusion criteria were unstable angina pectoris, New York Heart Association class III or IV congestive heart failure, active cancer, or abnormal liver function test results. Combination ACE-inhibitor/ARB therapy was prohibited, as was the use of aldosterone inhibitors and renin inhibitors.

All participants gave written informed consent before enrollment in the study. The study was approved by the appropriate local research ethics committee and performed in accordance with the Declaration of Helsinki of the World Medical Association at 99 sites in Europe/Israel, 75 sites in North America, and 26 sites in the Asia/Pacific region. The first patient was randomly assigned on October 2, 2005, and the last patient, on June 18, 2007. The study completion date was June 3, 2008.

Design and Study Procedures

The study was a multicenter, randomized, double-masked, placebo-controlled clinical trial and consisted of screening, run-in, maintenance, and wash-out periods. Screening assessment included complete medical history, safety laboratory assessment (blood chemistry, blood hematology, and urinalysis), and pregnancy test for women of childbearing potential.

Patients who met all study inclusion criteria and were already using a maximum approved dose of an ACE inhibitor or ARB for at least 120 days with optimal blood pressure control bypassed the run-in period and were allowed to proceed directly to establishing baseline parameters, followed by randomization. Patients who were not receiving a maximum approved dose of an ACE inhibitor or ARB or were not receiving adequate antihypertensive background therapy were either switched to the maximum approved dose of their current ACE inhibitor or ARB medication or started on treatment with irbesartan, 300 mg/d, or losartan, 100 mg/d, plus concomitant non-ACE/non-ARB antihypertensive therapy for a minimum 120-day run-in period to achieve optimal blood pressure control, with a goal blood pressure ≤130/80 mm Hg.

Patients who then met ACR inclusion criteria at the end of the run-in period proceeded to randomization. Patients were randomly assigned to treatment with placebo or sulodexide, 100 mg, twice daily (total, 200 mg) at a 1:1 ratio and instructed to take their medication orally with water 30 minutes before the morning and evening meals.

Patients attended the clinic 8, 16, 22, and 26 weeks after randomization. At the end of the 26-week maintenance period, study medication was discontinued and patients remained on their antihypertensive therapy and continued to be followed up during the washout period, with additional follow-up visits at 4 and 8 weeks after cessation of blinded study medication. At each follow-up visit, efficacy and safety parameters were evaluated. In addition, 3 consecutive first morning voided urine samples were collected before the day of each follow-up visit for ACR assessment.

Measurements

Albuminuria was determined at baseline and weeks 8, 16, 26, 30, and 34 as the geometric mean of the ratio of urine albumin concentration to urine creatinine concentration over 3 consecutive first morning voided urine collections during the 3 mornings before the visit and measured by immunoturbidimetry (Cobas Mira Plus; Roche, www.roche.com). Serum potassium and creatinine and other laboratory evaluations were performed on venous blood samples at screening, baseline, and 8, 26, 30, and 34 weeks thereafter. Serum creatinine concentration was determined by Jaffé reaction (Hitachi analyzer; Roche). Glycated hemoglobin was measured by ion-exchange high-performance liquid chromatography (Variant; Bio-Rad, www.bio-rad.com). All measurements were performed at a central laboratory in New York for the North American and Pacific basin sites and in Dublin for the European and Israeli sites. Urine samples are stored in −70°C freezers and shipped on dry ice. Office blood pressure was measured using a sphygmomanometer or automated blood pressure device in the sitting position after at least 10 minutes of rest. Three seated blood pressures were obtained 1 minute apart and averaged for the blood pressure of record.

Statistical Evaluation

The primary outcome of the study was therapeutic success, defined as either: (1) conversion from microalbuminuria to normoalbuminuria (ACR <20 mg/g) and at least 25% decrease in ACR relative to baseline at week 26 of therapy or (2) a >50% decrease in ACR relative to baseline at week 26 of therapy.

The primary analysis was performed by applying Fisher exact test to compare proportions of randomly assigned patients with therapeutic success between treatment groups. Treatment effect was expressed as the odds ratio of success for the sulodexide versus placebo group comparison with an exact 95% confidence interval constructed based on hypergeometric distribution. Secondary analyses were performed using the same methods to evaluate treatment effects on the separate components of the primary outcome (conversion from microalbuminuria and 50% decrease in albuminuria) at week 26 and on both the primary outcome and its 2 components at weeks 8 and 16, as well as weeks 20 and 34, at 4 and 8 weeks after discontinuation of therapy. Both the primary analysis and indicated secondary analyses were restricted to patients with nonmissing ACR values. In sensitivity analyses, each analysis was repeated with missing values imputed using a last-follow-up-carry-forward rule.

Further secondary analyses of covariance were used to compare the change in log-transformed ACR and percentage of change in serum creatinine levels from baseline to week 26 between the randomly assigned treatment groups while controlling for baseline level of the outcome variable. A similar approach was used to compare changes from baseline to week 26 in safety-related factors, including serum potassium and clinical blood and urine test results.

Finally, an additional secondary analysis was performed by applying a mixed-effects model with an unstructured covariance matrix to compare the mean slope in log-transformed ACR from baseline to week 26 (including ACRs at baseline and follow-up weeks 8, 16, and 26) between the sulodexide and placebo groups.

Assuming success of 15% in the placebo group and ~25% in the sulodexide group, rates we had encountered in our pilot trial, required a sample size of 1,000 patients total, with 500 in each treatment group, to achieve 90% power at the 0.0125 significance level (2 sided). This *P* value was requested by the FDA. All analyses in this report were performed using a modified intention-to-treat basis.

Safety analysis included changes in serum creatinine and potassium levels in addition to clinical blood and urine test results. Adverse events were classified according to the modified World Health Organization Adverse Events Scale. Frequencies of laboratory values in defined "notably abnormal" ranges were tabulated by treatment group. When relevant, frequencies were analyzed using Fisher exact test and Armitage trend test.

An independent Data Safety Monitoring Committee that included nephrologists, a statistician, a cardiologist, and an ethicist met twice yearly, and formal interim analyses were conducted when ~50% and 75% of total data were accrued.

RESULTS

Characteristics

A total of 1,056 patients were randomly assigned, 524 to the sulodexide group and 532 to the placebo group (Fig 1). Baseline characteristics of randomly assigned patients were similar between the 2 study groups (Table 1). More than 93% of patients were on ACE-inhibitor or ARB therapy at initial screening before entry into the study. The mean ACR of patients on maximal ACE-inhibitor or ARB therapy for a minimum of 4 months at baseline was 108.4 ± 87.5 mg/g. At entry, average seated blood pressure was 131 ± 12 mm Hg systolic and 76 ± 9 mm Hg diastolic.

Adherence to Protocol

Overall, a median of 96.4% patients showed treatment adherence (using the overall prescribed dose of blinded study medication for the study duration).

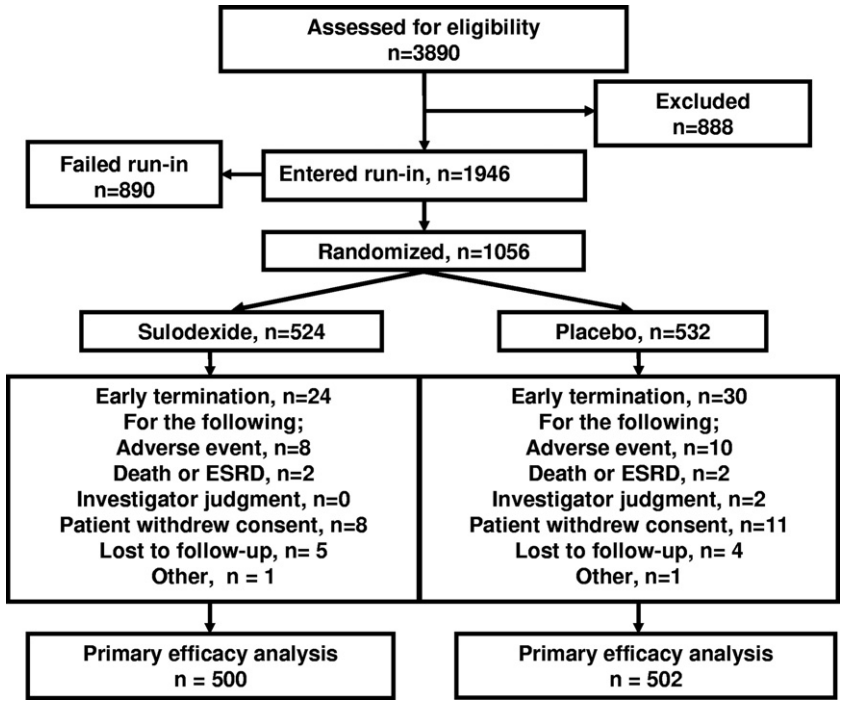

Figure 1. Flow of the trial. Abbreviation: ESRD, end-stage renal disease.

Table 1. Baseline Characteristics

Parameter	Placebo (n = 532)	Sulodexide (n = 524)	*P*
Age (y)	62.3 ± 9.9	62.0 ± 9.7	0.7
Men	408 (76.7)	394 (75.2)	0.6
Race			0.5
Asian	34 (6.4)	33 (6.3)	
Black	28 (5.3)	39 (7.4)	
White	451 (84.8)	440 (84.0)	
Other	19 (3.5)	12 (2.3)	
Ethnicity			0.7
Hispanic/Latino	31 (5.8)	28 (5.3)	
Non-Hispanic	501 (94.2)	496 (94.7)	
Height (cm)	170.2 ± 9.6	169.9 ± 9.6	0.6
Weight (kg)	92.8 ± 19.0	93.4 ± 17.7	0.6
BMI (kg/m^2)	31.9 ± 5.3	32.3 ± 5.7	0.3
Current smokers	100 (18.8)	100 (19.1)	0.9
Systolic BP (mm Hg)	131.4 ± 12.0	130.7 ± 12.6	0.4
Diastolic BP (mm Hg)	73.4 ± 8.5	73.7 ± 8.8	0.6
Elevated BP[a]	145 (27.3)	131 (25.0)	0.4
History of hypertension	491 (92.3)	496 (94.7)	0.1
SCr in men (mg/dL)	1.09 ± 0.24	1.09 ± 0.24	0.9
SCr in women (mg/dL)	0.90 ± 0.26	0.89 ± 0.25	0.9
eGFR[b] (mL/min/1.73 m^2)	77.9 ± 23.0	78.1 ± 21.9	0.9
ACR (mg/g)	108.4 ± 87.5	107.3 ± 80.0	0.8
HbA_{1c}	7.58 ± 1.24	7.54 ± 1.26	0.9

Note: Sulodexide dose was 200 mg. Continuous variables given as mean ± SD; categorical variables, as number (percentage). Conversion factors for units: SCr in mg/dL to mmol/L, ×88.4; ACR in mg/g to mg/mmol, ×8.84; eGFR in mL/min/1.73 m^2 to mL/s/1.73 m^2, ×0.01667.

Abbreviations: ACR, albumin-creatinine ratio; BMI, body mass index; BP, blood pressure; eGFR, estimated glomerular filtration rate; HbA_{1c}, hemoglobin A_{1c}; SCr, serum creatinine.

[a] Defined as systolic BP ≥140 or diastolic BP ≥90 mm Hg.

[b] Calculated using the Modification of Diet in Renal Disease (MDRD) Study equation.

Blood pressure was well controlled at an average of 131/74 mm Hg and remained essentially unchanged during the course of the study. Both systolic and diastolic blood pressures were virtually identical in both groups throughout the study. Administration of concomitant antihypertensive agents was balanced in both groups: α-adrenoreceptor antagonists (93, sulodexide group; 107, placebo group), selective β-blocking agents (182, sulodexide; 180, placebo), dihydropyridine derivatives (223, sulodexide; 227, placebo), selective β_2-adrenoreceptor agonists (24, sulodexide; 32, placebo), and thiazide diuretics (185, sulodexide; 185, placebo).

Urine Albumin Excretion

Of 1,056 randomly assigned patients, 986 (93.4%) completed the study with valid baseline and 26-week follow-up ACR data and were included in the primary modified intent-to-treat analysis (Table 2). Median overall adherence to using blinded study medication was 96.4%. A total of 172 (17.4%) patients achieved therapeutic success, 91 (18.4%) in the placebo group and 81 (16.5%) treated with sulodexide. Overall, 163 (16.5%) achieved at least a 50% decrease in ACR (87 [17.6%] in the placebo vs 76 [15.4%] in the sulodexide group compared with only 69 [7%] achieving normalization of microalbuminuria [30 (6.1%) in the placebo vs 39 (7.9%) in the sulodexide group]). The odds ratio comparing proportions of patients with therapeutic success between the sulodexide and placebo groups was 0.87 (95% confidence interval, 0.62-1.23; $P = 0.5$).

Proportions of patients achieving the composite outcome at the 8-week intervals during the maintenance phase of the study and during the 8-week wash-out phase after discontinuation of the study drug are shown in Fig 2. There was no statistically significant difference between treatment groups at any follow-up time. The distribution of changes in urine ACRs during the course of the study was essentially identical in both groups (Fig 3).

In a mixed-effects analysis incorporating ACR measurements at baseline and each follow-up visit through week 26, mean slopes of log-transformed ACR over time were similar between the sulodexide and placebo groups ($P = 0.9$), with increases in the geometric mean ACR of 1% per 6 months, with 95% confidence intervals ranging from a 6% decrease to an 8% increase in both groups.

Of 710 patients with baseline ACR <125 mg/g, 14.7% of those assigned to sulodexide compared with 15.0% of those assigned to placebo achieved the therapeutic success outcome (Fisher exact test, $P = 0.9$). Of 330 patients with a baseline ACR >125 mg/g, 20.3% assigned to sulodexide versus 25.8% assigned to placebo achieved success (Fisher exact test, $P = 0.3$).

Table 2. Primary Analysis

Outcome	Sulodexide (n = 492)	Placebo (n = 494)	Total (N = 986)	*P*
Therapeutic success	81 (16.5)	91 (18.4)	172 (17.4)	0.5
Normalization	39 (7.9)	30 (6.1)	69 (7.0)	0.3
50% ACR reduction	76 (15.4)	87 (17.6)	163 (16.5)	0.4

Note: Values given as number (percentage). The primary analysis included 1,056 randomly assigned patients and 986 with valid baseline and week-26 ACR measurements.

Abbreviation: ACR, albumin-creatinine ratio.

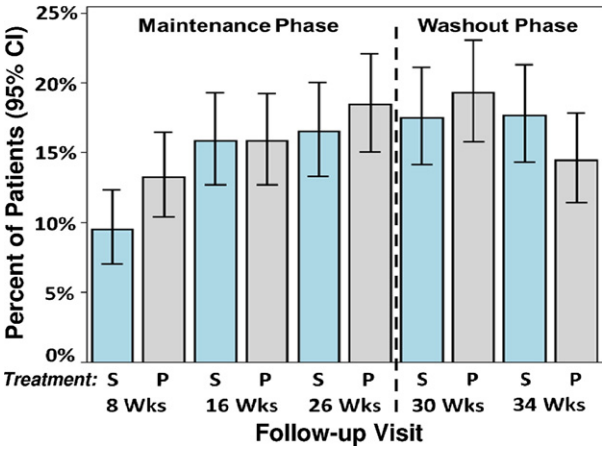

Figure 2. Percentage of patients achieving the primary end point during the course of 26 weeks on coded medication and 8 weeks of follow-up after cessation of coded medication. Abbreviation: CI, confidence interval.

Serum Creatinine

Mean serum creatinine levels at baseline were 1.04 mg/dL in the sulodexide group and 1.05 mg/dL in the placebo group. There was essentially no change in mean or median serum creatinine levels throughout the study in either group ($P = 0.5$).

Safety

There were no differences in severe adverse events (SAEs) between the 2 groups. A total of 57 (10.9%) SAEs were reported in the sulodexide group and 65 (12.2%) in the placebo group ($P = 0.6$). No SAE was believed to be related to the study medication. Two (0.4%) SAEs led to death in the sulodexide group, and 3, (0.6%) in the placebo ($P = 0.9$).

DISCUSSION

We conclude that the oral preparation of sulodexide used in this trial was ineffective in decreasing urine albumin excretion in the type 2 diabetic nephropathy population we studied. Our results fail to accord with reports of several investigators who have reported that in patients with diabetic nephropathy administered oral sulodexide, albumin excretion rates decreased.[6-14]

The working hypothesis for this study was that sulodexide was capable of interfering with the abnormal biochemistry of the glomerular capillary wall in the diabetic state, particularly with respect to heparan sulfate content. There is a substantial body of evidence implicating alterations in heparan sulfate metabolism as central to the alteration in glomerular capillary wall permeability characteristics in diabetes.[3] Diabetic nephropathy is associated with a decrease in glycosaminoglycan composition of the glomerular basement membrane, particularly heparan sulfate.[18-20] Increased urinary heparanase activity has been shown in patients with diabetic nephropathy, implying enhanced heparan sulfate degradation at the level of the glomerular capillary.[21] The notion has been advanced that heparanase inhibition may allow restitution of glomerular capillary wall heparan sulfate content and restore glomerular permeability to normal. Sulodexide has been shown in vitro to inhibit heparanase and thus restore the podocyte surface content of heparan sulfate.[22]

Previous reports, including our own pilot study, involved a small sample size and diminished power with respect to drawing a definitive conclusion.[6-14,23] Six of the 9 reported studies involved a total of 147 (range, 12-36) patients treated from 3 weeks to 2 months. Gambaro et al[13] carried out the largest study (the Diabetic Nephropathy and Albuminuria Study

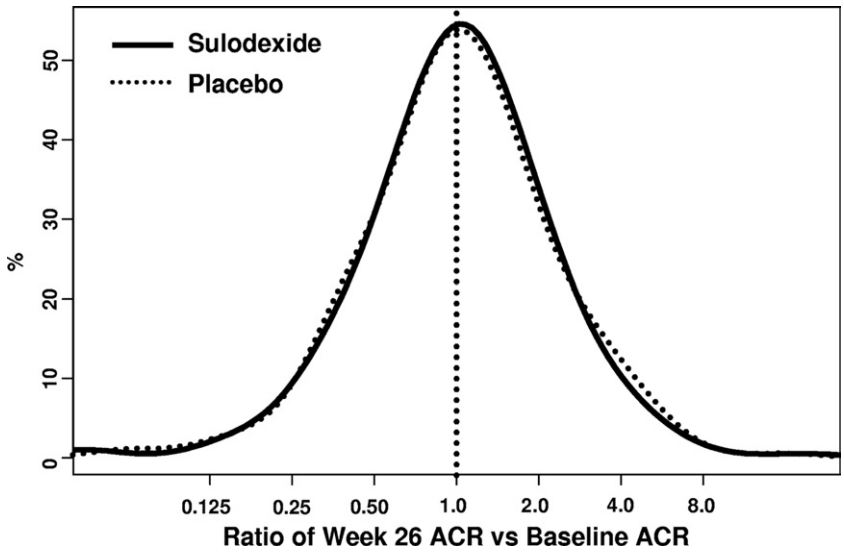

Figure 3. Change in albumin-creatinine ratio (ACR) from baseline to week 26.

[DiNAS]), which included 223 patients with type 1 or 2 diabetes and either microalbuminuria or overt nephropathy. The pilot trial that we conducted was underpowered and did not show a statistically significant difference in study end points between the placebo and sulodexide groups.[23] The placebo group achieved the primary end point of normalization or halving of baseline urine albumin excretion in 18.4% of cases. The group receiving 400 mg of sulodexide achieved the primary end point in 15.4% of cases ($P = 0.8$). Patients receiving 200 mg of sulodexide achieved a primary end point in 33.3% of cases ($P = 0.08$). There was no explanation for our finding that the higher dose of sulodexide was ineffective in achieving decreased albuminuria while the lower dose achieved significance. However, these findings raised the possibility that random changes in albumin excretion could explain results.

Undertaking a study of patients with microalbuminuria requires understanding of the remarkable variance in albumin excretion rate that can occur, particularly in patients with diabetes. The interassay coefficient of variation for the immunoturbidity assay measurement of low urine albumin concentrations used in this study would be expected to be 2%-4%.[24,25] However, during the conduct of our pilot trial,[23] variance among 3 first morning urine ACRs in a single patient averaged 35%. This agrees with other reports of intraindividual variance of 30% for morning ACRs and up to 85% for randomly collected specimens measuring ACR.[26] In addition, circadian variability and differences in urine albumin excretion between periods of activity and sleep are greater in patients with diabetes compared with nondiabetic patients.[24,25] The statistical powering of a study of microalbuminuria in patients with diabetes must take this biological variance into account.

Previous pharmacokinetic studies had shown oral absorption of sulodexide from the gastrointestinal tract at levels that did not alter hemostasis.[16,17] The inability to detect factor Xa generation–attendant oral administration of this drug makes pharmacokinetic studies difficult, if not impossible. It is possible that the low rate of sulodexide absorption from the gastrointestinal tract failed to provide enough agent to achieve an effect at the glomerular level. Sulodexide has been used as a therapeutic agent in Europe in a variety of settings associated with the treatment of occlusive vascular diseases. There has been no report of altered hemostasis attendant to the use of sulodexide in these settings.[27-29]

The chemical content of sulodexide is characteristic of heparin compounds, and the sulfate content, sulfate-carbonyl ratios, and in vitro antifactor Xa activity reported to the FDA for sulodexide was well within the required ranges.[15] The virtual identity in results between the control and sulodexide groups in the present study (Fig 3) not only raises the question of whether the therapeutic agent used was absorbed from the gastrointestinal tract, but also advances consideration of whether the agent had pharmacologic activity. The manufacturing requirements for sulodexide are complex.[15] There is no direct evidence that there was a lack of adherence to the required quality controls for the manufacture of the low-molecular-weight heparin–containing compound used in this study. However, failures in heparin manufacture have occurred in other circumstances, and this must be considered in the face of a negative clinical trial.[30-32]

In the present study, we observed a much larger patient population than other reports in the literature. In addition, all our patients were receiving the highest recommended dose of an ACE inhibitor or ARB. All patients enrolled in this study were receiving a consistent dose of ACE inhibitor or ARB throughout the study. In addition, blood pressure management was monitored carefully to limit variation in therapeutic measures that can alter urine albumin excretion. It is not clear that these rigorous management standards were undertaken in previous studies of sulodexide. It is likely that urine albumin excretion in the reported patient population was higher before the administration of agents that inhibit the renin-angiotensin system, implying that our patients had more advanced disease at baseline than had been reported in other studies of microalbuminuria. Before the use of ACE inhibitors or ARBs, microalbuminuria was considered to be the very first clinical manifestation of early diabetic nephropathy. Patients entered into this study with albumin excretion in the microalbuminuria range could have had more advanced glomerular pathologic states than patients previously defined as microalbuminuric. Nevertheless, this would not account for the negative results that we report.

The existing literature describing decreased albuminuria associated with glycosaminoglycan therapy is not limited to sulodexide. Parenteral administration of other heparin compounds also has been reported to decrease albumin excretion.[30-32] Parenteral administration of low-molecular-weight heparin compounds would ensure adequate delivery of the drug. The possibility that this latter parenteral approach may be clinically effective must remain open. Microalbuminuria appears to represent a clinical manifestation of diffuse microvascular pathology.[3] The therapeutic impact of an agent on the systemic microvasculature that is capable of decreasing urine albumin excretion therefore could have broad clinical consequences. Given the correlation between the presence of microalbuminuria and risk of serious renal and

cardiovascular events, efforts to decrease urine albumin excretion require continued pursuit.

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