Effects of sulodexide in patients with type 2 diabetes and persistent albuminuria

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Abstract

Background. Urinary albumin excretion frequently persists in diabetic patients who are treated with angiotensin-converting enzyme inhibitors (ACEI) or angiotensin receptor blockers (ARB). Sulodexide, a glycosaminoglycan mixture of 80% heparan sulfate and 20% dermatan sulfate, has been hypothesized to reduce persistent albuminuria. We have conducted a multi-center randomized double-blind pilot study in order to determine the effect of 6 months’ therapy with sulodexide on urinary albumin excretion and to address logistical issues for a full-scale trial.

Methods. A total of 149 patients with type 2 diabetes and an albumin:creatinine ratio (ACR) between 20 and 300 mg/g were randomized with equal allocation to either placebo, 200 mg of sulodexide or 400 mg of sulodexide. The primary endpoint was the achievement, at 6 months, of either 3(1) return to normoalbuminuria (ACR < 20 mg/g with a decrease of at least 25%) or (2) a decrease in ACR of at least 50% from the baseline value. All patients used a maximum tolerated recommended FDA approved dose of an ACEI or ARB for at least 60 days and had stable blood pressure prior to randomization.

Results. The primary efficacy endpoint was achieved in 25.3% of the patients in the two sulodexide groups combined versus 15.4% of the placebo-treated patients (P = 0.26). The primary endpoint was achieved in 33.3% (P = 0.075 for the comparison to placebo) in the sulodexide 200 mg group and 18.4% (P = 0.781) in the sulodexide 400 mg group. (No consistent patterns of side effects were observed.

Conclusion. Based on the experience gained in this pilot study, one full-scale trial is currently being conducted to evaluate the effects of sulodexide on change in ACR in patients with persistent microalbuminuria, and a longer-term trial is underway to evaluate the effects of sulodexide on long-term renal disease progression in patients with overt proteinuria.

Keywords: diabetes; microalbuminuria; randomized clinical trial; sulodexide

Introduction

Type 2 diabetes mellitus (DM) is a public health concern of epidemic proportion. Over 170 million people worldwide have diabetes and this number is expected to double by 2025 [1]. Increased urinary albumin excretion is an early and important finding of diabetic nephropathy that occurs in ~40% of patients with diabetes [2]. It is associated with a higher risk of developing end-stage renal disease (ESRD) and significant cardiovascular morbidity when compared to patients with lower levels of albumin excretion [3,4].

Pharmacologic interventions in the renin–angiotensin–aldosterone system (RAAS) with either angiotensin-converting enzyme inhibitors (ACEI) or angiotensin receptor blockers (ARBs) have proven to reduce albuminuria and protect the kidney, independent of their blood pressure reducing properties. The Bergamo Nephrology Diabetes Complication Trials (BENEDICT) and Irbesartan in Patients with Type 2 DM and Microalbuminuria Trial (IRMA-2) have demonstrated the value of early intervention with ARBs in patients with normal and microalbuminuria [5,6], Large-scale prospective trials in patients with type 2
diabetes and overt nephropathy, the Reduction of Endpoints in NIDDM with the Angiotensin II Antagonist Losartan (RENAAL) and the Irbesartan in Diabetic Nephropathy trials (IDNT), have shown that intervention with an ARB is effective in preventing the transition from overt nephropathy to ESRD [7,8].

Despite the use of ACEI or ARB, many patients have persistent albuminuria [9]. Additional treatments are urgently needed. Sulodexide, a mixture of heparan and dermatan sulfate (80/20%), targets metabolic defects in matrix and basement membrane synthesis as well as endothelial cell function. It has been shown to reduce proteinuria in animal models of diabetic nephropathy [10–13]. Several small-scale clinical studies in humans with diabetic nephropathy have demonstrated a consistent trend for the reduction of urinary albumin excretion in DM [14–23]. The DiNAS study demonstrated increasing reduction of urinary albumin in patients with both type 1 and 2 DM, with increasing doses of sulodexide 50 mg/day, 100 mg/day and 200 mg/day [17]. This study included patients with and without concurrent ACEI treatment.

We report here the results of a pilot study that was undertaken to explore the activity of sulodexide in the treatment of patients with diabetes and persistent microalbuminuria and to address logistical issues in advance of a prospective long-term clinical trial of sulodexide. This trial is now underway to determine the efficacy and safety of daily sulodexide in patients with DM who continue to show persistent albuminuria despite treatment with ACEI or ARB.

Subjects and methods

Participants

Eligible participants in the pilot study were men and women aged 18 years or older with type 1 or 2 DM. Participants were required to have microalbuminuria, which was defined as an albumin-to-creatinine ratio (ACR) between 20 and 200 mg/g in patients already taking an ACEI or ARB at the maximum FDA recommended dosage or an ACR between 30 and 300 mg/g if not on a maximal FDA recommended dose of ACEI or ARB. In order to be randomized, patients had to demonstrate stable blood pressure ≤150/90 mmHg. All patients included in the study had been on a maximal dosage of an ACEI or ARB at least for 60 days prior to randomization. Patients were excluded if they had HbA1c >11.0% or serum potassium >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 evidence of hepatic dysfunction.

All subjects gave written informed consent prior to enrolment in the study. The study was approved by the appropriate local research ethics committee and was performed in accordance with the Declaration of Helsinki of the World Medical Association.

Design and study procedures

The sulodexide pilot study was a multi-center, randomized, double-masked placebo-controlled study and consisted of screening, run-in, maintenance and washout periods. A screening assessment was performed in all patients. It included a complete medical history, safety laboratory assessment (blood chemistry, blood haematology and urinalysis) and pregnancy test for women of childbearing potential.

Patients who were not receiving a maximum approved or tolerated dose of an ACEI or ARB were either switched to the maximum approved dose of their current ACEI or ARB medication, or begun on treatment with irbesartan 300 mg/day for a 60-day run-in period. These patients did not have to re-qualify at the end of the run-in period. Patients, who met the study inclusion criteria and who were already on a maximum approved or tolerated dose of an ACEI or ARB, bypassed the run-in period and were allowed to proceed directly to randomization.

Those patients who met the inclusion criteria proceeded to randomization and were randomly assigned, based on a computer-generated randomization schedule, to treatment with placebo, sulodexide 200 mg or sulodexide 400 mg at a 1:1:1 ratio. Patients were provided identical capsules containing 100 mg or 200 mg of sulodexide and instructed to take their medication orally with water 30 min prior to the morning and evening meals.

Patients attended the clinic 8, 16 and 24 weeks after randomization. At each follow-up visit, efficacy and safety parameters were evaluated. In addition, three first morning voided urines were collected prior to the day of each follow-up visit for ACR assessment. At the end of the 24-week maintenance period, study medication was discontinued and patients were maintained on their antihypertensive therapy and continued to be followed for 8 weeks after cessation of blinded study medication.

Measurements

Albuminuria was determined as the geometric mean of three consecutive first morning voided urine collections obtained the three mornings prior to the visit and measured by immunoturbidimetry (Cobas Mira Plus; Roche, Montclair, NJ, USA). Serum potassium and creatinine levels, glycosylated haemoglobin concentration and other laboratory evaluations were performed on venous samples at baseline and every 8 weeks, thereafter. The serum creatinine concentration was determined by the Jaffe reaction (Hitachi Analyzer, Roche, Montclair, NJ, USA). Glycosylated haemoglobin was measured by ion-exchange high performance liquid chromatography (Variant; Bio-Rad, Richmond, CA, USA). All measurements were performed at a central laboratory in New York for the North American sites and in Dublin for the Israeli site.

Office blood pressures were taken using a sphygmomanometer or an automated blood pressure device in the sitting position after at least 10 min rest. Three seated blood pressures are taken 1 min apart and the average was used for calculation.

Statistical analyses

This study was exploratory in nature and designed as a pilot study for a subsequent definitive trial. The objective of the data analyses is primarily descriptive, and the planned total
sample size of 135 was determined based on administrative and logistical considerations. Statistical significance tests and confidence intervals are provided herein to facilitate the interpretation of the results, but the sample size was not intended to be sufficient to reach definitive statistical conclusions.

The primary efficacy analysis evaluated the proportion of randomized patients who achieved therapeutic success at their 24-week visit. Therapeutic success is a binary composite endpoint defined as either (i) conversion to normoalbuminuria (ACR < 20 mg/g) and a 25% reduction in ACR from the baseline level, or (ii) a 50% reduction in ACR from baseline. Additional analyses summarized the percent change in the geometric mean ACR from baseline and the rates of the therapeutic success composite and its individual components at each follow-up visit. Fisher’s exact tests were used for therapeutic success and other binary outcomes to evaluate the following contrasts between the randomized treatment groups: (i) 200 mg sulodexide versus placebo, (ii) 400 mg sulodexide versus placebo and (iii) the combination of the 200 and 400 mg sulodexide groups versus placebo. Exact 95% confidence intervals for odds ratios corresponding to Fisher’s exact test statistics were constructed to represent the precision of the results.

In accordance with the intent-to-treat principle, all analyses compared patients according to their randomized treatment assignment irrespective of their adherence to the interventions. However, the analyses presented in this report were conducted using observed data only, without imputation of missing values. Sensitivity analyses using the last value carried forward (LOCF) rule produced similar results (data not shown). Analysis of covariance, adjusting for the baseline level of the factor being analyzed, was used to evaluate the effects of treatment interventions on changes in coagulation parameters and secondary outcomes including plasma fibrinogen, serum creatinine, reciprocal of the serum creatinine, serum albumin and log-transformed ACR. Additional safety analyses included comparisons between treatment groups of the rates of adverse events (AE) classified according to the Modified World Health Organization Adverse Events Scale.

A Lan-DeMets spending function was used to conduct three formal interim analyses in addition to the final analysis of the primary therapeutic success outcome. The two-sided critical value for the primary efficacy analyses at the completion of the study was 0.043. All other hypothesis tests were conducted using a two-sided significance level of 0.05, without adjustment for multiple comparisons.

Results

Patient disposition and baseline characteristics

During 2004, 149 patients with type 2 diabetes were randomly assigned with equal allocation to treatment with placebo, sulodexide 200 mg/d or sulodexide 400 mg/d. The disposition of these patients is summarized in Figure 1. Of the 149 randomized patients, 130 (87.2%) completed the study and provided a valid urine albumin:creatinine ratio (ACR) determination after 24 weeks of therapy.

The 149 randomized patients included 76 (51.0%) who entered the baseline phase while not on the maximum recommended dose of ACEI or ARB therapy. Therefore, they had their ACEI or ARB dose titrated upwards during the run-in phase. Demographic and clinical baseline characteristics were comparable among the three treatment groups except for total cholesterol (Table 1). As a result of the random allocation, total cholesterol was slightly lower in patients treated with sulodexide 200 mg/day.

A concurrent 24-h urine collection and three consecutive first morning voided urine collections at their baseline assessment were available in 143 randomized patients. The Pearson and Spearman correlation coefficients relating the 24-h urine ACR to the geometric mean of the three first-morning spot urine ACRs were 0.78 and 0.84, respectively.

Clinical parameters and tolerability

Every effort was made to control the blood pressure at the level obtained at randomization since changes in blood pressure could affect the albumin excretion and prevent the accurate efficacy evaluation of sulodexide. Systolic and diastolic blood pressure remained stable throughout the course of the trial and no significant differences among groups could be documented (Table 2).

Efficacy analyses

Proportion of patients with therapeutic success

The proportion of patients reaching the therapeutic success composite endpoint is displayed over the 24-week treatment period in Figure 2 and presented in greater detail for the 24-week assessment in the top panel of Table 3. At 24 weeks the composite endpoint was achieved by 15.4% of the patients assigned to placebo, 33.3% of the patients assigned to sulodexide 200 mg/day and 18.4% of the patients assigned to sulodexide 400 mg/day. The odds ratio for the comparison of the composite endpoint between the two sulodexide groups combined versus the placebo group was 1.86 (95% CI 0.65 to 6.10, \( P = 0.26 \)). The odds ratio was 2.75 (CI 0.84 to 9.83, \( P = 0.075 \)) for comparison of the sulodexide 200 mg/day group versus placebo and was 1.24 (CI 0.35 to 4.68, \( P = 0.78 \)) for the comparison of the sulodexide 400 mg/day group versus placebo.

A total of 17.2% of the patients in the combined sulodexide groups both achieved the primary endpoint at the 24-week time point and confirmed therapeutic success after the 8-week washout period. The corresponding percent of patients with confirmed therapeutic success in the placebo group was 7.9%, and the odds ratio comparing the rates of therapeutic success between the combined sulodexide groups and placebo was 2.43 (CI 0.62 to 13.86, \( P = 0.27 \)).

Percentage change in ACR

At baseline, the distribution of ACR values was positively skewed with an arithmetic mean of 101.9 mg/g, and 10th, 50th and 90th percentiles of 23.5 mg/g, 76.2 mg/g and 186.4 mg/g, respectively. The percent change in ACR varied substantially between patients during follow-up (Figure 3), with a nonsignificant trend for a steeper rate of decline observed throughout the treatment.
Fig. 1. Patient flow diagram. A total of 19 of the 149 randomized patients did not provide a valid measure of the change in ACR from baseline to the Week 24 visit. These include 10 patients who were lost to follow-up prior to the Week 24 visit, and 9 additional patients who failed to provide a valid baseline or a valid Week 24 ACR.

Table 1. Baseline characteristics\textsuperscript{a}

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Placebo (N = 47)</th>
<th>Sulodexide 200 mg/day (N = 50)</th>
<th>Sulodexide 400 mg/day (N = 52)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean ± SD, median (interquartile range), or N (%)</td>
<td>Mean ± SD, median (interquartile range), or N (%)</td>
<td>Mean ± SD, median (interquartile range), or N (%)</td>
</tr>
<tr>
<td>Age (years)</td>
<td>60.3 ± 12.1</td>
<td>64.1 ± 9.2</td>
<td>61.1 ± 12.2</td>
</tr>
<tr>
<td>Gender, male</td>
<td>33 (70.2%)</td>
<td>36 (72.0%)</td>
<td>38 (73.1%)</td>
</tr>
<tr>
<td>Race</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Black</td>
<td>10 (21.3%)</td>
<td>5 (10.0%)</td>
<td>6 (11.5%)</td>
</tr>
<tr>
<td>Caucasian</td>
<td>37 (78.7%)</td>
<td>45 (90.0%)</td>
<td>45 (86.5%)</td>
</tr>
<tr>
<td>Other</td>
<td>0</td>
<td>0</td>
<td>1 (1.9%)</td>
</tr>
<tr>
<td>Ethnicity</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hispanic/Latino</td>
<td>6 (12.8%)</td>
<td>4 (8.0%)</td>
<td>3 (5.8%)</td>
</tr>
<tr>
<td>Non-hispanic</td>
<td>41 (87.2%)</td>
<td>46 (92.0%)</td>
<td>49 (94.2%)</td>
</tr>
<tr>
<td>Height (cm)</td>
<td>172.9 ± 9.2</td>
<td>171.5 ± 12.3</td>
<td>173.1 ± 10.1</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>100.5 ± 27.5</td>
<td>94.8 ± 17.9</td>
<td>99.7 ± 24.4</td>
</tr>
<tr>
<td>BMI (kg/m\textsuperscript{2})</td>
<td>33.4 ± 8.0</td>
<td>32.2 ± 5.5</td>
<td>32.7 ± 5.8</td>
</tr>
<tr>
<td>S. creatinine (mg/dL)</td>
<td>1.14 ± 0.53</td>
<td>1.15 ± 0.47</td>
<td>1.13 ± 0.43</td>
</tr>
<tr>
<td>24-h urine albumin (mg/24 h)\textsuperscript{b}</td>
<td>97.8 (60.5–247.1)</td>
<td>124.8 (64.4–251.0)</td>
<td>134.1 (69.9–229.0)</td>
</tr>
<tr>
<td>First morning ACR (mg/g)\textsuperscript{b}</td>
<td>75.2 (34.0–160.0)</td>
<td>86.2 (43.5–137.0)</td>
<td>67.7 (37.4–116.5)</td>
</tr>
<tr>
<td>HbA1c (%)</td>
<td>7.5 ± 1.5</td>
<td>7.5 ± 0.9</td>
<td>7.8 ± 1.3</td>
</tr>
<tr>
<td>T. cholesterol (mg/dL)</td>
<td>183.8 ± 46.0</td>
<td>160.2 ± 33.3</td>
<td>177.8 ± 40.1\textsuperscript{a}</td>
</tr>
<tr>
<td>Triglycerides (mg/dL)</td>
<td>174.6 ± 137.7</td>
<td>169.0 ± 102.2</td>
<td>214.4 ± 238.0</td>
</tr>
</tbody>
</table>

\textsuperscript{a}P-values for treatment group comparisons were > 0.05 for all baseline factors except total cholesterol, for which P = 0.01.
\textsuperscript{b}Shown are median levels and inter-quartile ranges.
Table 2. Blood pressure levels throughout the trial

<table>
<thead>
<tr>
<th>Treatment group</th>
<th>Baseline</th>
<th>Week 8</th>
<th>Week 16</th>
<th>Week 24</th>
<th>Post-treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Systolic blood pressure (mmHg) (mean ± SE)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Placebo</td>
<td>128 ± 12</td>
<td>128 ± 11</td>
<td>129 ± 16</td>
<td>130 ± 17</td>
<td>129 ± 16</td>
</tr>
<tr>
<td>Sulodexide 200 mg</td>
<td>130 ± 12</td>
<td>126 ± 14</td>
<td>132 ± 18</td>
<td>130 ± 16</td>
<td>131 ± 14</td>
</tr>
<tr>
<td>Sulodexide 400 mg</td>
<td>129 ± 14</td>
<td>128 ± 15</td>
<td>130 ± 14</td>
<td>132 ± 15</td>
<td>129 ± 15</td>
</tr>
<tr>
<td>Diastolic blood pressure (mmHg) (mean ± SE)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Placebo</td>
<td>73 ± 9</td>
<td>74 ± 9</td>
<td>74 ± 13</td>
<td>73 ± 12</td>
<td>71 ± 11</td>
</tr>
<tr>
<td>Sulodexide 200 mg</td>
<td>73 ± 10</td>
<td>72 ± 10</td>
<td>74 ± 12</td>
<td>71 ± 8</td>
<td>71 ± 11</td>
</tr>
<tr>
<td>Sulodexide 400 mg</td>
<td>75 ± 9</td>
<td>73 ± 10</td>
<td>74 ± 11</td>
<td>76 ± 9</td>
<td>73 ± 10</td>
</tr>
</tbody>
</table>

*There were no significant differences (at P < 0.05) between either of the sulodexide groups and the placebo group at baseline or any follow-up time point.

Fig. 2. Shown are the proportions of patients reaching the therapeutic success composite endpoint at 8-week intervals during the 24-week treatment period. There were no significant differences at the P < 0.05 level in the rate of therapeutic success between either of the sulodexide groups and the placebo group at any of the follow-up time points.

Table 3. Patients achieving therapeutic success, N and (%)

<table>
<thead>
<tr>
<th></th>
<th>Placebo</th>
<th>Sulodexide 200 mg</th>
<th>Sulodexide 400 mg</th>
<th>Sulodexide 200 and 400 mg combined</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of patients available for analysis at 24 weeks</td>
<td>39</td>
<td>42</td>
<td>49</td>
<td>91</td>
</tr>
<tr>
<td>Therapeutic success at 24 weeks</td>
<td>6 (15.4%)</td>
<td>14 (33.3%)</td>
<td>9 (18.4%)</td>
<td>23 (25.3%)</td>
</tr>
<tr>
<td>Regression to normoalbuminuria</td>
<td>3 (7.7%)</td>
<td>7 (16.7%)</td>
<td>5 (10.2%)</td>
<td>12 (13.2%)</td>
</tr>
<tr>
<td>50% decline in baseline ACR</td>
<td>5 (12.8%)</td>
<td>12 (28.6%)</td>
<td>8 (16.3%)</td>
<td>20 (22.0%)</td>
</tr>
<tr>
<td>Number of patients available for analysis at 32 weeks</td>
<td>38</td>
<td>41</td>
<td>46</td>
<td>87</td>
</tr>
<tr>
<td>Therapeutic success at 24 weeks confirmed at 32 weeks</td>
<td>3 (7.9%)</td>
<td>9 (22.0%)</td>
<td>6 (13.0%)</td>
<td>15 (17.2%)</td>
</tr>
<tr>
<td>Regression to normoalbuminuria</td>
<td>1 (2.6%)</td>
<td>4 (9.8%)</td>
<td>2 (4.4%)</td>
<td>6 (6.9%)</td>
</tr>
<tr>
<td>50% decline in baseline ACR</td>
<td>2 (5.3%)</td>
<td>7 (17.1%)</td>
<td>5 (10.9%)</td>
<td>12 (13.8%)</td>
</tr>
</tbody>
</table>

*P-values for comparisons of therapeutic success at 24 months were sulodexide 200 mg versus placebo = 0.075; sulodexide 400 mg versus placebo = 0.78; sulodexide 200 and 400 mg combined versus placebo = 0.26.

period in the sulodexide 200 mg/day group compared to the other two treatment groups. In analysis of covariance, while adjusting for the baseline level the adjusted geometric mean (95% confidence interval) ACR changed by +12.7% (−12.1% to +44.7%) in the placebo group, by −15.1% (−33.5% to +8.3%) in the sulodexide 200 mg group and by +5.8% (−15.8% to 32.8%) in the sulodexide 400 mg group. The geometric mean changes did not differ significantly between the treatment groups (P = 0.11 and 0.71, respectively, for the comparisons of the 200 mg and 400 mg sulodexide groups versus placebo).

Safety monitoring

Laboratory measurements Since sulodexide belongs to the same family as heparin and heparinoids, coagulation parameters were monitored throughout the study (online appendix Table 1A). No significant changes in mean fibrinogen
levels, APTT, INR and prothrombin time were observed over time and no significant differences were observed between the treatment groups.

**Adverse events** A total of four patients reported at least one serious adverse event (SAE) in the placebo group, as did 16 in the sulodexide 200 mg group and four in the sulodexide 400 mg group. An AE was classified as an SAE if the medical occurrence was life threatening at the time of the event, required hospitalization or prolongation of hospitalization or resulted in death. None of the SAEs were reported by the investigators as possibly or probably related to the study drug. A total of eight patients had an AE that was not classified as serious but was indicated as possibly related to study medication. The incidence of possibly related events was similar in the three treatment groups (four, five and four patients, respectively, in the placebo and the 200 mg and 400 mg sulodexide groups).

Two deaths occurred. One patient receiving 200 mg died of terminal pneumonia 2 months after his last dose of study medication. This patient had previously accounted for six prior SAEs related to infections, congestive heart failure, acute renal failure and stroke. The other, assigned to 400 mg sulodexide, died suddenly, apparently due to a previously unobserved cardiac arrhythmia. One additional patient withdrew from study drug due to the requirement for warfarin. Ten patients discontinued study medication temporarily due to an SAE.

**Discussion**

This pilot study was undertaken to evaluate preliminary efficacy and safety of sulodexide as well as procedures and to identify logistical difficulties in preparation for a full-scale prospective trial of sulodexide in diabetic patients who have persistent albuminuria despite treatment with full doses of an ACEI or ARB. The sample size was small and not designed to definitely test efficacy or safety of the intervention. Consequently, the magnitudes of any plausible treatment effects in comparison of groups are substantially smaller than the widths of their respective confidence intervals. This then reflects the uncertainty of results in such a small pilot study. Even so, a trend was observed for an increased rate of therapeutic success for sulodexide compared to placebo. The composite outcome was achieved by 25.3% of patients assigned to sulodexide compared with 15.4% of placebo-treated patients. Importantly, this pilot study redefined run-in period and entry qualification.

Many previous studies have shown a strong, consistent association between albuminuria and poor renal outcomes in patients with DM. Increased amounts of urinary albumin excretion are associated with an increased risk for the progressive loss of renal function [4,24]. These findings have led to the hypothesis that albuminuria is nephrotoxic, and a powerful surrogate marker for the loss of renal function. Furthermore, post hoc analysis of the IDNT and RENAAL trial showed that the albuminuria-reducing effect of losartan and irbesartan explains a substantial part of its renoprotective effect [4,24]. The degree of initial albuminuria reduction was the most important predictor for renal events in the long term.

RAAS inhibition has consistently shown reduction in albuminuria that exceeds the effect related to blood pressure reduction alone. Clearly a part of the answer lies in the preferential reduction of transcapillary pressure gradients. An additional important fact is the link of RAAS to other mechanisms related to progressive disease, for example local increases in kidney TGF-β [25].

Sulodexide apparently alters glomerular permeability and effectively reduces proteinuria by a non-blood
pressure, non-RAAS related mechanism. As a result, sulodexide could effectively increase therapeutic options in those patients who fail to respond to RAAS inhibition. Moreover, it may add further reduction in those patients who have partial response with RAAS inhibition. Such novel or additive effects are essential. After all, in the IDNT trial with overt proteinuria only 40% of patients treated with irbesartan achieved a 50% reduction in proteinuria [24]. Here we examined patients who had persistent microalbuminuria despite RAAS inhibition.

Sulodexide is a preparation of low-molecular weight porcine glycosaminoglycan (GAG) polysaccharides comprised of fast-moving heparin (∼75–80%), and dermatan sulfate (20%) with a mean molecular weight of 11,000–15,000 Da. The drug is absorbed orally [26]. Although it was first evaluated as an anti-thrombotic drug it has no anticoagulant effect after oral administration [27]. The drug has been shown to improve blood flow by lowering viscosity [28,29], to reduce cardiovascular events and to improve vascular disease-associated skin ulcers [30–32]. Gambaro first observed a relation between GAG and diabetic proteinuria in animal models and the suggested use of sulodexide in the treatment of diabetic nephropathy. Then, beginning in 1997, several small clinical studies demonstrated that sulodexide reduced albuminuria in patients with diabetic nephropathy (for review see [33]).

The largest renal study of sulodexide in humans was reported by Gambaro in the DiNAS study [17]. This multi-center placebo-controlled study included 223 type 1 or 2 diabetic patients with nephropathy and abnormal albumin excretion rate (AER) in either the microalbuminuria (AER = 30–<300 mg/day) or macroalbuminuria (>300 mg/day) range. Patients were enrolled with and without RAAS-inhibition therapy. Sulodexide was increasingly effective with doses escalating from 50 to 200 mg, in lowering albuminuria in patients with both type 1 and type 2 DM. Overall, at the 200 mg dose it lowered AER in micro- and macroalbuminuric patients by ∼60–70%. In addition, the diminished AER could be documented for up to 4 months after cessation of therapy. Many of the patients did not have prior or ongoing ACEI or ARB treatment. In this pilot study we examined the effects of sulodexide on urinary albumin excretion in patients on a maximum tolerated dose of an ACEI or ARB.

The precise mechanism of sulodexide nephroprotective effects remains unknown. In brief, one hypothesis links the use of therapeutic GAG to various GAG species that are among the biochemical components residing in the matrix of the GBM structure. Studies of proteoglycan sulfate incorporation have implied that the diminished GAG content is, at least, the result of a diminished rate of biosynthesis of heparan sulfate proteoglycan [34,35]. A simple explanation of the efficacy of sulodexide and related compounds is that they restore the anionic heparan sulfate charges on the glomerular basement membrane. Another possibility is that repair or restoration of glycoprotein content in diabetic nephropathy may ultimately be renoprotective. A third possibility is that the beneficial effects of GAGs are partially related to downregulation of TGF-β expression [10]. Lastly, studies also suggest that sulodexide may block heparanase-1 activity [36]. Heparanase-1 is unregulated in renal epithelial cells under high glucose conditions normally present in diabetes and has been implicated in playing an important role in the development of proteinuria in several nephropathies [37,38].

This pilot study provided several significant insights that affected the design of the full-scale trial. To begin with, albuminuria in 24-h urine collections was compared with the geometric mean ACR in three sequential first morning voided urine specimens. Results from matched 24 h and first voided morning urine samples were highly correlated. As a result, a change in the geometric mean ACR of first voided morning urines is used as a primary outcome parameter in the full-scale trial. Variation in the measurement of this outcome parameter in this trial helped the authors to better calculate the necessary sample size in the main trial.

Approximately 15% of the pilot study patients assigned to the placebo group achieved therapeutic success, raising the possibility that some patients who titrated their ACEI or ARB dose during the baseline period may have experienced residual post-randomization declines in ACR due to changes in ACEI or ARB dose during baseline. In the full-scale study design patients will be required to have been on maximum treatment with ACEI or ARB for 120 days prior to their final baseline visit. They must re-qualify at that time before they proceed to randomization. Moreover, additional time may be used so that patients meet blood pressure criteria at entry.

Also, this trial was not powered to determine the dose–response relationship between sulodexide and the outcome. Even so, there was no apparent trend for an increased effect with the 400 mg/day dose compared to the 200 mg/day dose. Due in part to this result, the main trial was designed with a single sulodexide dose of 200 mg/day. A dose response effect was seen in previous studies with 50–200 mg/day of sulodexide. It may be that efficacy plateaus at 200 mg/day. Alternatively, a lack of dose response with sulodexide 400 mg/day could conceivably be due to a bio-feedback mechanism regarding the restoration of anionic heparan sulfate charges on the glomerular basement membrane at higher sulodexide levels. However, as noted, it is entirely possible that the absence of a stronger trend for a sulodexide effect in the 400 mg/day dose group was due to a chance variation in this small pilot study.

The pilot study was also not planned to evaluate drug safety. As a result, no reliable statement regarding safety emerges from this study. Most side effects were seen with sulodexide, and SAEs were skewed to sulodexide 200 mg. However, treating physicians considered SAEs to be unrelated to study drugs. Side effects with high-dose sulodexide were substantially lower than with low-dose sulodexide and no more frequent than with placebo. A pattern could not be discerned. Moreover, previously published trials do not indicate significant patterns of SAEs. Furthermore, there is a well-established safety record for this drug since it has been used for the treatment of peripheral vascular disease in Europe for many years. Since sulodexide is largely composed of heparan sulfate, one might expect significant effects on hemostasis. In this regard, we found no significant changes in serial laboratory evaluations of haemostasis. This information allowed patients to be enrolled in the main
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trial, who had been excluded from this pilot study due to a risk of bleeding.

Two full-scale trials are currently being undertaken to evaluate whether sulodexide reduces urinary albumin excretion and slows renal disease progression. The sulodexide microalbuminuria trial (SUN-Micro Trial) is being conducted in 1000 patients with type 2 diabetes, hypertension and persistent microalbuminuria. It tests the hypothesis that 26 weeks of therapy with sulodexide will increase the fraction of patients experiencing either (i) a conversion to normoalbuminuria and at least 25% reduction in ACR or (ii) a 50% or greater decrease in the ACR. The sulodexide overt nephropathy trial (SUN-Macro Trial) is a 4-year trial in ~2240 patients with type 2 diabetes, hypertension and ≥ 900 mg of proteinuria per 24 h. It will examine whether sulodexide increases the time to the first occurrence of the composite endpoint of ESRD or a doubling of serum creatinine.

Overall, this pilot study led to improved study plans and procedures for the future sulodexide trials. It showed a trend for an increased rate of therapeutic success in patients treated with sulodexide 200 mg/day. If confirmed, this is of potential importance because a substantial number of patients show lack of therapeutic success with full inhibition of RAAS, normalization of blood pressure and good glucose control. Sulodexide, a drug with additional potential beneficial effects on the cardiovascular system, does not involve alterations in blood pressure or the RAAS system. It is therefore a promising new therapy that may confer additional renal and cardiovascular protection upon established therapies for diabetic nephropathy. Results from full-scale clinical trials are needed to provide the definitive answer whether sulodexide affords renal protection.

Appendix A. The Collaborative Study Group—Pilot Study

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Israel: Hadassah University Hospital, Jerusalem—Itamar Raz, MD.

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Conflict of interest statement. H.L.H. is a PhD fellow at the University Medical Centre Groningen, part of his job being a project coordinator of the sulodexide trials. T.G., J.B.L., I.R., R.D.R., L.G.H. and T.B.W. are investigators in the Keryx sponsored clinical trials.

Supplementary material

Supplementary material is available online at http://ndt.oxfordjournals.org

References


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