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A Clinical Experience on Sulodexide in the Treatment of Patients with Diabetic Nephropathy

Sabeur Dakhli[1*], Adel Khedher[2], Zidi Borni[3], Abdellatif Achour[4] and Jamil Hachicha[5]

[1]*Centre Medical El Farabi, Ariana, Tunisia*

[2]*Internal Medicine Service M8, Hospital Charles Nicolle, Tunis, Tunisia*

[3]*Department of Endocrinology Military Hospital, Tunis, Tunisia*

[4]*Nephrology Service, Hospital Sahloul, Sousse, Tunisia*

[5]*Nephrology Service, Hospital Hedi Chaker, Sfax, Tunisia*

[*]**Corresponding author:** Sabeur Dakhli, Centre Medical El Farabi, Menzah 6, Ariana 2091, Tunisia, Tel: 0021671230220; E-mail: saberdakhli@yahoo.fr

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Abstract

Background: Diabetic nephropathy, characterized by albuminuria, is a severe complication of diabetes mellitus, leading cause of end-stage renal disease. The aim of the present study was to evaluate the efficacy and safety of sulodexide, alone or in combination with captopril, versus captopril alone in consecutive adult patients with diabetic nephropathy.

Methods: Patients aged ≥ 18 years, with type 1 or type 2 diabetes mellitus and albumin excretion rate (AER) ≥ 30 mg/24 h, without severe renal insufficiency, cardiac or hepatic insufficiency, or haematuria, were enrolled. Patients were treated with captopril 25 mg twice daily, sulodexide 25 mg twice daily, or a combination of captopril 25 mg twice daily + sulodexide 25 mg twice daily for 6 months. The primary endpoint was the evaluation of AER. Secondary endpoints included evaluation of arterial blood pressure, fasting glucose, HbA1c, serum creatinine and uricemia and safety.

Results: Globally, 123 patients were enrolled and treated with captopril alone (n=42), sulodexide alone (n=53) or sulodexide plus captopril (n=28). After adjustment for initial albuminuria, the AER reduction at T3 and T6 versus T0, although highly significant in all treatment groups, was higher in patients treated with the combination or with sulodexide alone than in patients given captopril alone (further decrease of 17.6% and 18.2% at T3 and of 29.3% and 19.8% at T6, respectively). In the whole population, serum creatinine and uric acid levels increased during the study, HbA1c and fasting glucose levels increased from T0 to T3 and remained stable thereafter, while blood pressure was constant throughout the study. Sulodexide was well tolerated.

Conclusions: Long term administration of sulodexide 50 mg/day, both in monotherapy or in combination with captopril, is effective and well tolerated in reducing proteinuria in diabetic patients and can be considered a valid therapeutical option in order to prevent major complications and reduce morbidity and mortality in this population.

Keywords: Sulodexide; Diabetic nephropathy; Macroalbuminuria; Microalbuminuria; Captopril

Introduction

Diabetes mellitus (DM) is characterised by specific changes in microvessels, thus causing diabetic microangiopathy, namely nephropathy and retinopathy, and macroangiopathy (including neuropathy) [1]. Diabetic nephropathy is a chronic and severe complication of, more frequent in type 1 (up to 40% of cases) than in type 2 (approximately 20% of cases). This complication is a leading cause of end-stage renal disease and has a significant impact on morbidity and mortality and on healthcare costs [2,3].

Diabetic nephropathy is initially characterized by microalbuminuria (defined as an albumin excretion rate (AER) of 30-299 mg / 24 hours or albumin/creatinine ratio (ACR) between 30 and 299 µg albumin / mg creatinine), with subsequent later progression to macroalbuminuria (defined as an AER ≥ 300 mg / 24 hours or ACR ≥ 300 µg albumin / mg creatinine) [2,4,5]. Without specific intervention, 20-40% of diabetic patients with microalbuminuria will progress to overt nephropathy with macroalbuminuria and, within 20 years, 20% of patients will develop end-stage renal disease. Albuminuria correlates both with risk of renal failure and cardiovascular events and mortality [1]. For these reasons, microalbuminuria and macroalbuminuria are not only good predictors of the progression of diabetic nephropathy to end-stage renal disease, but are also considered a target for treatment of the renal disease and an indicator of a drug's efficacy in preventing or delaying the onset of end-stage renal failure in diabetic patients [5].

The goal of treatment is to prevent the progression from micro- to macroalbuminuria, the decline of renal function in patients with macroalbuminuria and the occurrence of cardiovascular events with an early multi-pharmacological approach [4,6]. The main pathological features include desulfation and degradation of the glomerular matrix, thickening of the basal membrane and mesangial proliferation, and extra- and intracapillary hyalinosis [1,2]. Several mechanisms have been identified for explaining endothelial damage and proliferation.

Among them is the alteration of endothelial permeability due to the degradation and reduction of heparan-sulfate, a glycosaminoglycan component of the extracellular and basement membrane matrix. The decreased concentration of glycosaminoglycans causes a loss of negative-charged molecules from the glomerular basement membrane, a decreased activity of the anionic filtration barrier that prevents the passage of proteins from blood to urine, a greater synthesis of type II collagen and the activation of growth factors such as TGF-beta [1-3]. Glycosaminoglycans strongly influence thickness, integrity and permselectivity of the endothelial glycocalyx: the effect of exogenous administration of glycosaminoglycans on the sulfation and synthesis of proteoglycans and on the differential expression of type IV collagen concur in maintaining the normal structure and permeability of the glomerular basement membrane [2]. Other alternative hypotheses have been advocated to explain the favourable remodelling effect of glycosaminoglycans at renal level: downregulation of proteases, the inhibition of heparanase, the modulation of extracellular mesangial matrix synthesis, the inhibition of the TGFβ-1 gene, the modulation of angiotensin II mediated cellular signalling, the restoration of glomerular basement membrane anionic charges, and the inhibition of macrophage infiltration and activation [2,6].

Basically, endothelial dysfunction is the common starting point both for macro- and microangiopathy. The pattern of endothelial damage in diabetes is a complex phenomenon of abnormal augmentation of vascular permeability, gradual change of endothelial cells towards a secretory phenotype and enhanced cell proliferation [1]. The basis for the prevention of diabetic nephropathy is the treatment of its known risk factors (hypertension, hyperglycemia, smoking, and dyslipidemia) with an intensive blood glucose, blood pressure and lipid level control [4]. In this context, renin-angiotensin system blockade with angiotensin-converting enzyme inhibitors (ACE-I) or angiotensin receptor blockers (ARBs) confers an additional benefit on renal function. However, these strategies could not be effective in some patients with diabetes and novel and innovative therapeutic strategies are warranted.

Sulodexide (Vessel Due F®) is a specific, highly purified mixture of glycosaminoglycans (a fast-moving heparin fraction with affinity for antithrombin III and a dermatan sulphate fraction with affinity for heparin cofactor II), with a very high tropism for vessel endothelium (90% of the product is found in the endothelium and its concentration is about 20-30 times higher than in other organs) [7-11]. Among its several well recognized activities on blood and vasculature (anticoagulant and venous/arterial anti-thrombotic, hemorrheologic, antiatherosclerotic, antilipidemic and antiproliferative effect), sulodexide is able to guarantee the vessel wall normal permeability, preventing the heparin sulphate degradation, maintaining or restoring the normal electronegativity of vessel walls and inhibiting extracellular matrix expansion between the vessel wall cells, and exibits an anti-inflammatory activity [8,9,12,13]. Several experimental and human studies demonstrated the favourable anti-proteinuric effect of sulodexide therapy in diabetic patients with nephropathy [14-21].

The aim of the present study was to evaluate the efficacy and safety of sulodexide, alone or in combination with captopril, versus captopril alone in consecutive adult patients with diabetic nephropathy.

Subjects and Methods

Study design

This multicenter prospective open study was conducted in patients with diabetic nephropathy in 5 nephrology centers in Tunisia between October 2006 (first patient enrolled) and August 2008 (last patient completing the study). The study was in adherence with the Declaration of Helsinky.

The main inclusion criteria were: age ≥ 18 years, diabetes mellitus either type 1 or type 2, AER ≥ 30 mg/24 h. Exclusion criteria were: severe renal insufficiency (estimated creatinine clearance <30 mL/min), cardiac or hepatic insufficiency, haematuria, known hypersensitivity to mucopolysaccharides, haematuria haematuria or lactation.

Patients were enrolled in three groups, according to the investigator's judgment: captopril 25 mg twice daily, sulodexide (Vessel due F®, Alfa Wassermann, Italy) 25 mg (equivalent to 250 lipoprotein lipase releasing units) twice daily, or a combination of captopril 25 mg twice daily + sulodexide 25 mg twice daily. All the study drugs were administered for 6 months. Concomitant therapies, notably antidiabetic, antihypertensive, hypolipidemic and anticoagulant, were administered throughout the study as required and recorded in the case report form.

Patients were visited at baseline (T0) and after three (T3) and six (T6) months of treatment. Patient's history and demographic characteristics were recorded at T0. AER was evaluated through a single 24-hour urine collection at T0, T3 and T6; urine albumin was determined by turbidimetry. Arterial blood pressure, fasting glucose, glycosylated hemoglobin (HbA1c), serum creatinine and uricemia were also measured at three-month intervals. Patients were questioned for intercurring adverse events at post-baseline visits.

The primary endpoint of the study was the evaluation of AER. Secondary endpoints included evaluation of arterial blood pressure, fasting glucose, HbA1c, serum creatinine and uricemia as well as safety.

Statistical analysis

Quantitative data have been summarised using arithmetic means ± standard deviation (SD) if normally distributed or geometric means if log-normally distributed. Categorical data have been reported as proportions of non-missing data.

Baseline albuminuria was classified as microalbuminuria (30-300 mg/24 h) or macroalbuminuria (>300-2000 mg/24 h). Creatinine clearance was estimated using the Cockroft-Gault formula and adjusted for body surface area by the equation of DuBois and DuBois. Baseline characteristics were compared by analysis of variance (ANOVA) if continuous and by the chi-square test if categorical. Correlations between continuous variables have been expressed using Pearson's r.

Laboratory data including AER and blood pressure were analysed by ANOVA or analysis of covariance (ANCOVA), both for repeated measures. Model-derived 95% confidence intervals (95% CI) have been reported for each visit and for changes from T0 to T3 and T6. Only patients having a measure at baseline and at least one after baseline (T3 or T6) were included in the main analysis of each variable. To allow inclusion of subjects with a missing observation at either T3 or T6,

data were examined using repeated-measure mixed models with subjects as random effect; the structure of the covariance matrix was chosen according to the log-likelihood criterion [22,23].

AER values were analysed after logarithmic transformation. Fixed effects in the main analysis were time of visit, treatment, baseline albuminuria (micro- or macro-), DM type and DM duration, as well as the interactions of visit with treatment and with baseline albuminuria. ANCOVA models were also examined considering for inclusion, as additional fixed effects, baseline creatinine clearance adjusted for body surface area, age, sex, weight, body mass index (BMI), concomitant diseases, smoking, dwelling area, and their interactions with visit and baseline albuminuria, provided that they significantly improved the model. Sensitivity analyses were conducted in patients with complete observations at all visits rather than adjusting for missing values at either T3 or T6. Alternative analyses were carried out with observations at T3 and T6 as the dependent variable and including the baseline numerical value among the covariates.

Serum creatinine, uricemia, Hb1Ac, fasting glucose and blood pressure were analysed using mixed ANOVA or ANCOVA models for repeated measures including time of visit, treatment and their interaction; patients' characteristics were considered as additional fixed effects if they significantly improved the model.

Body weight changes from T0 to T6 of at least 2 kg were analysed using Wilcoxon's signed rank test to detect an overall trend in either direction or Kruskal-Wallis test to detect an overall difference due to treatments.

Incidence rates were calculated for adverse events, and were compared between treatment groups using risk ratios (RRs) with 95% CI and chi-squared p-values based on the Mantel-Haenszel method. Multiple logistic regressions were used to test the association between patients' characteristics and the incidence of adverse events.

P<0.05 has been considered as statistically significant. Sidak's correction was used when comparing treatments at each post-baseline visit (six comparisons); both uncorrected and Sidak-corrected p-values and CIs have been reported. Statistical analyses were carried out using the SAS® System version 8.2.

Results

Patients

Globally, 123 patients were enrolled and treated with captopril alone (n=42, 34%), sulodexide alone (n=53, 43%) or sulodexide plus captopril (n=28, 23%).

All patients were considered evaluable for safety as all had taken at least one dose of the study drugs and were visited at least once after T0. A patient treated with sulodexide alone was withdrawn because of nausea after 5 weeks. Another subject treated with sulodexide alone interrupted the study for unkwown reasons after T3.

The main demographic and clinical characteristics of the patients evaluated are reported in Table 1. Mean AER values were highest in the combination group and lowest in the sulodexide group (overall difference between treatments, p<0.0001; all pairwise differences corrected for multiple comparisons, p<0.012). The mean history of diabetes was shortest in the sulodexide group, while subjects with type 1 diabetes were preferably treated with the combination (overall difference, p=0.039). Almost all patients had normal (estimated creatinine clearance ≥ 80 mL/min) or mildly impaired (50-80 mL/min) renal function, with a similar distribution across treatment groups.

	All (123 patients)	**Captopril (42 patients)**	**Sulodexide (53 patients)**	**Combination (28 patients)**
Dwelling area, N (%)				
N	2	1	0	1
urban	91 (75)	28 (68)	42 (79)	21 (78)
rural	30 (25)	13 (32)	11 (21)	6 (22)
Age (years)				
min - max	21-74	33-70	26-74	21-71
mean ± SD	51.9 ± 9.9	52.5 ± 9.1	52.2 ± 9.7	50.3 ± 11.4
Sex, N (%)				
NA	2	0	1	1
female	40 (33)	16 (38)	15 (29)	9 (33)
male	81 (67)	26 (62)	37 (71)	18 (67)
Weight (kg)				
min - max	50-102	55-100	50-102	56-99
mean ± SD	74.8 ± 9.7	74.8 ± 10.3	74.5 ± 9.4	75.3 ± 9.5
BMI (kg/m^2)				
min - max	17.3-37.2	21.5-37.2	17.3-34.5	22.0-36.7
mean ± SD	26.9 ± 3.5	27.2 ± 3.6	26.6 ± 3.4	27.0 ± 3.4
Type of diabetes, N (%)				
NA	1	0	1	0
1	11 (9)	2 (5)	3 (6)	6 (21)
2	111 (91)	40 (95)	49 (94)	22 (79)
Duration of diabetes (years)				
min - max	0-26	1-24	0-24	5-26
mean ± SD	14.0 ± 6.0	14.8 ± 5.1	12.0 ± 6.6	16.4 ± 5.1
Creatinine clearance (*), N (%)				
NA	4	0	4	0
≥ 30 to 50 mL/min	2 (2)	1 (2)	1 (2)	0
≥ 50 to 80 mL/min	60 (50)	20 (48)	24 (49)	16 (57)
≥ 80 mL/min	57 (48)	21 (50)	24 (49)	12 (43)
AER, N (%)				
NA	4	0	3	1
30 to 300 mg/24 h	76 (64)	26 (62)	41 (82)	9 (33)

>300 to 2000 mg/24 h	43 (36)	16 (38)	9 (18)	18 (67)
AER (mg/24 h)				
min - max	30-2000	40-2000	30-1250	100-2000
geometric mean	295	320	173	702

NA=not available; SD=standard deviation; BMI=body mass index; AER=albumin excretion rate. (*) estimated using the Cockroft-Gault formula and adjusted for body surface area

Table 1: Patients' characteristics at entry.

Log-transformed AER at baseline was correlated with body-surface adjusted creatinine clearance (r= −0.29, p=0.0021), creatininemia (r=0.23, p=0.012) and diastolic blood pressure (r=0.28, p=0.0021), while there was no correlation (p>0.40) with systolic blood pressure, uricemia, HbA1c and fasting glycemia.

As shown in Table 2, the patients treated with sulodexide had fewest concomitant diseases (overall difference, p=0.0046), and all patients received one or more concomitant treatment, mainly oral antidiabetic agents, acetylsalicylic acid, calcium-channel inhibitors, insulin and beta-blockers.

	All (123 patients)	**Captopril (42 patients)**	**Sulodexide (53 patients)**	**Combination (28 patients)**
Smoking, N (%)				
no	74 (62)	25 (60)	34 (68)	15 (54)
yes	46 (38)	17 (40)	16 (32)	13 (46)
NA	3	0	3	0
Comorbidities, N (%)				
Previous cerebrovascular accident	1 (1)	0 (0)	0 (0)	1 (4)
Peripheral vascular disease	6 (5)	1 (2)	2 (4)	3 (11)
Coronary disease	13 (11)	10 (24)	2 (4)	1 (4)
Diabetic retinopathy	54 (44)	24 (57)	14 (26)	16 (57)
Diabetic neuropathy	23 (19)	7 (17)	7 (13)	9 (32)
Arterial hypertension	56 (46)	19 (45)	20 (38)	17 (61)
Dyslipidemia	14 (11)	6 (14)	3 (6)	5 (18)
Other disease	3 (2)	0 (0)	2 (4)	1 (4)
Any disease	76 (62)	29 (69)	29 (55)	18 (64)
Concomitant treatments, N (%)				
Insulin	31 (25)	9 (21)	10 (19)	12 (43)
Oral antidiabetic agents	96 (78)	33 (79)	46 (87)	17 (61)
Beta-blockers	20 (16)	10 (24)	6 (11)	4 (14)
Calcium-channel inhibitors	33 (27)	14 (33)	10 (19)	9 (32)
Diuretics	6 (5)	2 (5)	2 (4)	2 (7)
Other antihypertensive agents	2 (2)	0 (0)	1 (2)	1 (4)
Acetylsalicylic acid	46 (37)	18 (43)	17 (32)	11 (39)
Statins	6 (5)	3 (7)	2 (4)	1 (4)
Fibrates	6 (5)	2 (5)	3 (6)	1 (4)
Other drugs	6 (5)	2 (5)	3 (6)	1 (4)
Any treatment	123 (100)	42 (100)	53 (100)	28 (100)

Table 2: Smoking, comorbidities and concomitant treatments.

Albumin excretion rate

Seven patients lacking valid baseline or at least one post-baseline AER measure were excluded from this analysis, six in the sulodexide group (one withdrawal for adverse event, one not reported, four laboratory or reporting error) and one in the combination group (laboratory or reporting error). Patients lacking only one post-baseline measure were included. AER values observed throughout the study are reported in Table 3. Compared to baseline, average values (as geometric means) of AER at T3 and T6 were progressively lower in all groups; the time trend was therefore highly significant (p=0.0001). This reduction, however, was greater in microalbuminuric than in macroalbuminuric patients, both for the whole sample and for each treatment group. This difference must be accounted for when comparing treatment groups, as initial AER was not balanced among them. Unadjusted analysis of AER would favour sulodexide, the preferred treatment for initial microalbuminuria, over captopril and especially over the combination which was used mostly for macroalbuminuria. Table 4 shows the results of the main analysis of AER adjusted for initial albuminuria, and additionally for diabetes type and duration as they significantly improved the model. The AER reduction at T3 and T6 versus T0, although highly significant in all treatment groups, was greater in patients treated with the combination or with sulodexide alone than in patients given captopril alone, with a further decrease of 17.6% and 18.2% with the combination and of 29.3% and 19.8% with sulodexide respectively at T3 and T6. Although the AER relative reduction at T3 was similar with the combination and with sulodexide, the former did not achieve multiplicity-corrected statistical significance compared with captopril because of the smaller number of patients. At T6 the improvement with sulodexide versus captopril was similar to that observed at T3, but was no longer statistically significant (after correction for multiplicity) as the variability was greater. The AER relative reduction with the combination progressed further at T6 achieving multiplicity-corrected statistical significance versus captopril, while the comparison with sulodexide was not significant.

Treatment	Visit	Overall			Macroalbuminuria at T0		Microalbuminuria at T0	
		N	mg/24 h	% of T0	N	% of T0	N	% of T0
All	T0	116	297		42		74	
	T3	115	212	71 (66, 77)	42	82 (78, 86)	73	66 (60, 73)
	T6	113	163	55 (50, 61)	42	69 (63, 76)	71	48 (41, 56)
Captopril	T0	42	320		16		26	
	T3	42	261	81 (72, 92)	16	92 (86, 99)	26	76 (64, 89)
	T6	42	211	66 (56, 78)	16	84 (72, 96)	26	57 (44, 73)
Sulodexide	T0	47	170		8		39	
	T3	46	107	63 (56, 71)	8	77 (69, 85)	38	61 (53, 70)
	T6	45	81	48 (41, 56)	8	57 (47, 70)	37	47 (38, 58)
Combination	T0	27	702		18		9	
	T3	27	504	72 (62, 83)	18	76 (71, 81)	9	64 (48, 85)
	T6	26	368	52 (42, 65)	18	64 (56, 73)	8	34 (22, 54)

Table 3: AER at baseline (T0), after 3 months (T3) and after 6 months (T6) of treatment overall and stratifying by baseline albuminuria. AER (mg/24 h) and percent entries are geometric means (95% CI) estimated by repeated-measures mixed-effect ANOVA models on log-transformed data including visit (p<0.0001 overall), treatment (p<0.0001 overall) and their interaction (p=0.028 overall) and adjusting for missing values at either T3 or T6.

Treatment	Visit	% of T0	P-value
Captopril	T3	83.5 (75.5, 92.4)	0.0005
	T6	69.0 (59.3, 80.2)	<0.0001
Sulodexide	T3	68.3 (61.2, 76.3)	<0.0001
	T6	55.3 (46.9, 65.3)	<0.0001
Combination	T3	68.8 (60.5, 78.2)	<0.0001

	T6	48.8 (40.1, 59.3)	<0.0001
Combination / Captopril (*)	T3	82.4 (69.8, 97.2) (65.9, 103.0)	0.022 (0.13)
	T6	70.7 (55.0, 90.8) (50.4, 99.1)	0.0069 (0.041)
Combination / Sulodexide (*)	T3	100.7 (84.5, 120.0) (79.5, 127.5)	0.94 (1.0)
	T6	88.1 (67.5, 115.0) (61.6, 126.1)	0.35 (0.92)
Sulodexide / Captopril (*)	T3	81.8 (70.8, 94.5) (67.3, 99.4)	0.0067 (0.040)
	T6	80.2 (64.6, 99.6) (59.9, 107.4)	0.046 (0.25)

CI=confidence interval; ANCOVA= analysis of covariance; DM=diabetes mellitus; AER=albumin excretion rate. (*) p-values and CIs corrected for multiple comparisons are in square brackets

Table 4: AER after 3 months (T3) and after 6 months (T6) of treatment as ratios of baseline (T0). Percent entries are geometric means (95% CI) estimated by a repeated-measures mixed-effect ANCOVA model on log-transformed data adjusting for missing values at either T3 or T6. Model included visit (p<0.0001), treatment (p=0.017), baseline albuminuria (micro- or macro-, p<0.0001), DM type (p=0.0094), DM duration (p<0.0001), the interactions of treatment by visit (p=0.014) and baseline albuminuria by visit (p=0.0007).

Consistent results were observed in subsidiary analyses with other repeated-measures ANCOVA models introducing plausible alternatives to the main model, namely exluding patients with missing data, including other factors significantly and independently associated with AER, and considering baseline AER values as a covariate (data not shown).

Hematological variables and vital signs

Means and 95% CI of other hematological variables and blood pressure during the study, adjusted according to best-fit repeated-measures ANCOVA models, are shown in Figure 1. Overall, serum creatinine and uric acid levels increased, HbA1c and fasting glucose levels increased from T0 to T3 and remained stable thereafter, while systolic and diastolic blood pressure was fairly constant throughout the study. The increase in serum creatinine from T0 to T6 was significantly higher with captopril than with sulodexide (5.7 µmol/L and 0.6 µmol/L respectively, p=0.005 uncorrected, p=0.030 corrected for multiple comparisons). Other changes did not significantly differ across treatment groups after correction for multiplicity.

Eleven patients (four in the captopril group, four in the sulodexide group and three in the combination group) had a body weight decrease of 2-3 kg from T0 to T6 while two patients (one captopril alone and one captopril plus sulodexide) had a body weight increase of 2 kg. The trend toward weight reduction was statistically significant (p=0.007) with no difference due to treatments (p=0.76).

Safety

Twenty-three patients experienced one or more adverse events, mostly gastrointestinal (Table 5). The incidence of any adverse event was highest in patients treated with the combination and lowest in patients treated with sulodexide alone; the RR between these two groups was statistically significant (p=0.011) even after correction for multiple comparisons. No significant correlation was found between patients' characteristics and the incidence of adverse events. A patient interrupted the study because of nausea after 5 weeks of treatment with sulodexide alone.

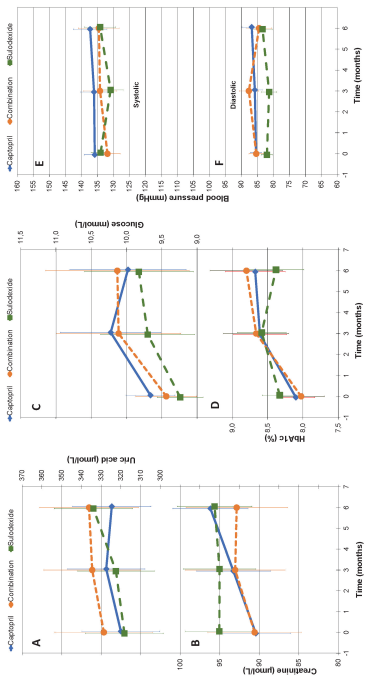

Figure 1: Plasma uric acid (A), creatinine (B), glucose (C), HbA1c (D), systolic (E) and diastolic (F) blood pressure during the study (ANCOVA-adjusted means and 95% CI).

	All (123 patients)	Captopril (42 patients)	Sulodexide (53 patients)	Combination (28 patients)
Gastrointestinal	20 (16)	7 (17)	5 (9)	8 (29)
epigastralgia	13 (11)	5 (12)	3 (6)	5 (18)
nausea	6 (5)	3 (7)	2 (4)	1 (4)

vomiting	2 (2)	0 (0)	1 (2)	1 (4)
diarrhea	4 (3)	1 (2)	1 (2)	2 (7)
Other	4 (3)	3 (7)	0 (0)	1 (4)
cough	3 (2)	2 (5)	0 (0)	1 (4)
leukopenia	1 (1)	1 (2)	0 (0)	0 (0)
Any adverse event	23 (19)	9 (21)	5 (9)	9 (32)

Table 5: Adverse events. Entries are N (%).

Discussion

This study was undertaken to compare the efficacy and safety of sulodexide, alone or in combination with captopril, versus captopril alone administered for 6 months in diabetic patients with persistent albuminuria. Increased urinary albumin excretion is an early and important finding of diabetic nephropathy, occurring in about 20-40% of diabetic patients, and associated with an increased risk of developing end-stage renal disease, as well as significant cardiovascular morbidity [1-4]. Pharmacological intervention on the renin-angiotensin-aldosterone system with ACE-I and ARBs has proven to be able to reduce albuminuria and protect the kidney, independently of their blood pressure reducing properties. Captopril, a well-known and used ACE-I, demonstrated to preserve the kidney function and to reduce proteinuria in diabetic nephropathy in several clinical trials and was chosen as comparator drug in this study [24-27]. Besides, Roozbeh et al. showed recently that combining captopril and another drug effective in improving diabetic nephropathy can lead to a greater reduction in proteinuria than captopril alone [28].

In our study, all the study drugs were able to significantly reduce proteinuria compared to baseline, but the reduction was higher in the groups treated with sulodexide (31.7% and 31.2% at month 3 and 44.7% and 51.2% at month 6 respectively for sulodexide and sulodexide plus captopril) than in the group treated with captopril alone (16.5% at month 3 and 31% at month 6). After correction for multiplicity, the intergroup comparison was statistically significant in favour of the monotherapy with sulodexide at month 3 and in favour of the combination of sulodexide plus captopril at month 6 with respect to captopril alone. Another important finding of our study was that in all the study groups the reduction was higher in patients with microalbuminuria than in patients with macroalbuminuria, suggesting the need of a pharmacological intervention in an early stage of the renal disease.

Our results are in agreement with previous clinical experiences carried out both in type 1 and type 2 diabetes patients, which highlighted the potential role of sulodexide. A retrospective analysis of 12 clinical trials enrolling approximately 600 patients with diabetes showed that sulodexide has anti-albuminuric effects and lower AER in patients with diabetes and either microalbuminuria or macroalbuminuria. In most of these studies it was demonstrated that the effect of sulodexide on reducing albuminuria was sustained up to four months after cessation of treatment, strongly suggesting that a biochemical-anatomic remodelling is potentially initiated in renal tissue by sulodexide [17].

The most important study performed in type 1 and 2 diabetic nephropathy with micro- and macroalbuminuria was the Di.N.A.S. (Diabetic Nephropathy and Albuminuria Sulodexide) trial [18]. Two hundred and twenty-three patients were randomly assigned to receive orally 50, 100 or 200 mg daily of sulodexide or matched placebo for 4 months, with a 4 month follow up period after drug suspension. At the end of the treatment, the reduction of AER was significantly different from placebo ($p<0.05$) and positively correlated with dose increments (30% for 50 mg/day, 49% for 100 mg/day and 74% for 200 mg/day). Very interestingly, sulodexide was similarly effective in both type 1 and 2 diabetic patients, in both micro- and macroalbuminuric patients and in patients with or without concomitant ACE-I therapy, showing that these drugs and sulodexide do not interfere with each other and have different mechanisms of action [18].

However, the Di.N.A.S. trial clearly demonstrated not only that the hypoalbuminuric effect of sulodexide is dose-related, but also that it increases over time. On this basis, another study evaluated the effects of a long-term administration (12 months) of sulodexide at low dosage (50 mg/day) on albuminuria in diabetic patients [19]. At 6 and 12 months, albuminuria was greatly reduced in patients treated with sulodexide and increased in the control group (-38.1%/-58.8% and +19.1%/+29.4% vs. baseline, respectively; $p=0.0001$). The same figures were observed in both type 1 and type 2 diabetes and in both micro and macroalbuminuric patients. The hypoalbuminuric effect of 50 mg/day sulodexide further increased from 6 to 12 months and was greater than that observed in the Di.N.A.S. trial (the difference may be explained by the longer duration of drug administration before albuminuria evaluation) [19].

These studies show that even the lowest sulodexide dosage is effective provided the drug is administered for as long as 6 months: this was the rationale of the DAVET (Diabetic Albuminuria Vessel Tunisia) Study, by Blouza and colleagues [20]. The administration of 50 mg/day of sulodexide for 6 months to 269 diabetic patients was able to significantly and progressively reduce the AER ($p<0.0001$): the geometric mean after 3 and 6 months was 63.7% (95% CI, 59.3%-68.4%) and 42.7% (95% CI, 37.8%-48.2%) of baseline, respectively, meaning a reduction of 36.3% and 57.3%. The reduction was similar in type 1 and type 2 diabetes and was slightly greater in macroalbuminuric than in microalbuminuric patients [21].

The results of our study provide a further confirmation of the efficacy of 6 months treatment with a low dosage of sulodexide in diabetic patients with nephropathy, both in monotherapy and combined with an ACE-I.

The safety profile of sulodexide evaluated by Weiss in 12 clinical studies suggests that both intramuscular and oral administered dose of sulodexide ranging from 50 mg to 400 mg is well tolerated and safe [17]: in our experience, approximately 19% of the patients experienced one or more adverse events, mostly gastrointestinal, but the incidence was higher in patients treated with captopril alone (21%) or with the combination (32%) than in patients treated with sulodexide alone (9%).

This study has some limitations. Firstly, treatment assignment was not randomised but was left to the choice of the investigators. Patients given the combination were mostly macroalbuminuric whereas patients given sulodexide were mostly microalbuminuric, with captopril in an intermediate condition. This difference reflects the physicians' need of new treatments for the management of the most severe patients, unresponsive to ACE-I alone. Although these differences are of interest in highlighting the criteria for treatment preference in clinical practice, they constitute a major limitation for the comparability of treatment effects in this study. The comparability issue

was made more compelling by the finding that for each treatment microalbuminuric patients responded better than macroalbuminuric patients in terms of percent reduction of baseline AER. Therefore any comparison between treatments must be carried out either by stratification (separate tests within microalbuminuric patients and within macroalbuminuric patients) or by multivariate analysis adjusting for baseline AER imbalance. The latter was preferred to avoid further splitting into treatment strata of less than 10 patients (sulodexide in macroalbuminuria and combination in microalbuminuria). Furthermore, multivariate analysis allows adjustment for possible covariate imbalances other than baseline albuminuria. The results obtained with different treatments within the multivariate analysis were therefore not affected by differences in baseline albuminuria, DM type and DM duration, as far as statistical balancing permits. Even so, it should be recognised that statistical techniques are a surrogate, not a substitute, for randomisation of the treatments being compared. Therefore, the results of this study should be interpreted cautiously. To reduce the risk of spurious outcomes, however, alternative analyses were performed that supported the same conclusions of the main analyses presented in this paper. Also, as each treatment was compared with the other two, p-values have been corrected for multiple comparisons, i.e., setting to 0.05 the cumulative probability of a false-positive error (to declare a difference between treatments when there is none) in any of the pairwise comparisons on the same variable. A subgroup analysis according to type 1 and type 2 diabetes was not performed due to the very limited numbers of type 1 diabetic patients enrolled in each group.

Long term administration of sulodexide 50 mg/day, both in monotherapy and in combination with captopril, was effective and well tolerated in reducing proteinuria in diabetic patients; the effect was greater in microalbuminuric patients. Together with previously mentioned studies, our results further support the role of sulodexide in diabetic nephropathy as valid partner of ACE-I/ARBs in difficult-to-treat cases or as single agent in case of patients with contraindications to the use of these agents (poor tolerability or co-morbidities). Furthermore, sulodexide has other pharmacological effects potentially useful in the diabetic patients, subject to a high probability of developing acute cardiovascular diseases: anti-thrombotic activity, decrease of oxidative stress, hypolipidemic effect, prevention of glucose toxicity, suppression of cellular inflammation and anti-atheromatous effects [6]. Several clinical studies demonstrated sulodexide efficacy in patients, diabetic and not diabetic, affected by vascular diseases associated with a thrombotic risk: peripheral occlusive arterial diseases [29,30], prevention of recurrent deep venous thrombosis [31,32], diabetic retinopathy [33], diabetic foot [34,35], cerebrovascular [36,37] and cardiovascular diseases [38,39] and management of chronic venous disease, including the more severe and complicated cases such as venous ulcers [40-42].

In conclusion, due to its several pharmacological effects, sulodexide can be considered a valid therapeutical option in the management of diabetic patient, in order to prevent major complications and reduce morbidity and mortality in this population.

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