

*Original Article***A randomized, controlled study of sulodexide therapy for the treatment of diabetic nephropathy**I. Dedov<sup>1</sup>, M. Shestakova<sup>1</sup>, A. Vorontzov<sup>1</sup> and E. Palazzini<sup>2</sup><sup>1</sup>National Research Centre for Endocrinology, Moscow, Russia, <sup>2</sup>Medical Dept., Alfa Wassermann SpA, Bologna, Italy**Abstract**

**Background.** Glycosaminoglycans (GAGs) play an important role in the physiopathology of diabetic nephropathy; they are essential for the maintenance of glomerular charge selectivity and their administration can reduce albuminuria in diabetic patients.

**Methods.** Following a randomized block design, controlled *versus* placebo, we investigated, in insulin-dependent diabetic patients with micro- or macroalbuminuria, whether GAG therapy can influence an altered albumin excretion rate (AER). Thirty-six patients (18 micro- and 18 macroalbuminuric) were randomized to receive, during 5 days/week for 3 weeks, either a daily dose of 600 lipoproteinlipase releasing units (LRU) of sulodexide by the i.m. route (9 micro- and 9 macroalbuminuric patients), or a matching i.m. placebo (9 micro- and 9 macroalbuminuric patients). All patients were followed-up for further 6 weeks. AER was evaluated before treatment, weekly during it and every 3 weeks during follow-up.

**Results.** Seventeen of the 18 sulodexide-treated patients showed a trend towards decrease in AER, more evident and statistically significant in microalbuminurics ( $P < 0.01$  after the first week). At the end of follow-up, AER was still significantly reduced in microalbuminurics, while macroalbuminurics showed again increased values. Placebo-treated patients evidenced no AER variations during all the study period. No statistically significant differences vs baseline, concerning blood pressure, haematological, haematochemical, and coagulative tests, and urinalysis, were ever observed, apart from a clear-cut decrease in blood cholesterol and triglycerides at the end of treatment, in a subgroup of hyperlipidaemic, sulodexide-treated subjects. No adverse events were registered.

**Conclusions.** Our results suggest that the GAG sulodexide exerts a positive activity in type I diabetic patients with micro- and macroalbuminuria, by reducing the abnormally high AER levels.

**Key words:** albuminuria; diabetes mellitus; diabetic nephropathy; glycosaminoglycans

**Introduction**

Diabetic nephropathy (DN) is a chronic, severe complication of diabetes, characterized at the beginning by microalbuminuria and predicting subsequent progression to overt nephropathy and increased mortality due to uraemic and/or cardiovascular death [1,2]. Twenty-five to 50 per cent of diabetic patients, both insulin-dependent (type 1) and non-insulin-dependent (type II) suffer from this condition [3]. The pathogenesis of DN is still unclear, with both the haemodynamic hypothesis and a poor blood sugar regulation being unable to completely explain the causes of its development [4].

Among the anatomical hallmarks of DN are the thickening of the glomerular basement membrane (GBM) and the expansion of the mesangial matrix, with hyalinosis both in the mesangium and in the capillary lumen [5,6]. These alterations are associated with a loss of anionic sites as well as of size and charge selectivity on the GBM. An increased synthesis of collagen IV, the overexpression of TGF $\beta$ , and the reduction of a glycosaminoglycan (GAG), heparan sulphate (HS), from the glomerular extracellular matrix [7,8], are also evident.

GAG metabolism has been shown in experimental diabetes to be abnormal and susceptible to improvement upon exogenous GAG supply, with possible restoration of normal kidney function. Supporting these data, Gambaro *et al* [6,9] showed that administration of heparin or other GAGs prevents nephropathy in diabetic rats by maintaining a normal GBM thickness and anionic charge density with parallel prevention of the onset of albuminuria. A relationship between albuminuria and heparan sulphate content in the GBM of patients with diabetic nephropathy has been demonstrated [10]. A significant reduction of albuminuria has also been observed in diabetic patients with micro- and macroalbuminuria after treatment with GAGs [11–14].

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These data point to an important role of GAGs in the physiopathology of DN: an abnormal GAG metabolism has also been hypothesized to be the cause of such pathology [7,8,15].

The protective effect of GAGs is primarily due to their ability to modulate the function of different cell types, influencing the synthesis and composition of the extracellular matrix [8,15–18]; this action is not dependent on their antithrombotic or anticoagulant activity. GAGs are furthermore essential for the integrity of the glomerular charge selectivity, due to their properties of electrostatic repulsion [19,20]. Taking into consideration these factors and GAG ability to reduce albuminuria, we investigated whether sulodexide, a naturally occurring GAG containing a fast-moving heparin-like fraction by 80% and a dermatan fraction by 20% [21], is able to influence albumin excretion rate (AER) in type I diabetic patients with micro and macroalbuminuria.

## Subjects and methods

Thirty-six insulin-dependent (type I) diabetic subjects were randomly selected from the micro- and macro-albuminuric patients attending our outpatient clinic, to take part in this open, placebo-controlled study. All provided their written informed consent to participate. Eighteen patients had microalbuminuria (AER between 20 and 200  $\mu\text{g}/\text{min}$ ) and 18 macroalbuminuria (AER > 200  $\mu\text{g}/\text{min}$ ). More than three determinations in the previous 6 months confirmed this allocation. Among the inclusion criteria were diabetes duration longer than 7 years, evidences of diabetic nephropathy, blood pressure (measured in sitting position) lower than 160/90 mmHg, spontaneously or pharmacologically stabilized for more than 6 months; adherence to the established diet (carbohydrates 55–60%, lipids 25–30%, proteins 15–20%) as evaluated by a dietician, and maintenance of the usual insulin therapy that yielded a satisfactory metabolic control.

Exclusion criteria were neoplasms, secondary hypertension, severe liver, cardiac, or systemic disease, creatinine > 150  $\mu\text{mol}/\text{l}$ , hypersensitivity to mucopolysaccharides, HbA1c > 9.5%, symptomatic urinary tract infections, haematuria, previous nephropathy, and laboratory or sonography findings suggesting nephropathies other than the diabetic one.

Following a randomized block design, patients were distributed between treatment with sulodexide (Vessel Due F, Alfa Wassermann S.p.A., Bologna, Italy (18 patients, 9 with micro- and 9 with macroalbuminuria), at the dose of 600 LRU (60 mg) by i.m. route, once a day, 5 days/week, for 3 weeks; or with placebo (2 ml ampoules containing saline; 18 patients, 9 with micro- and 9 with macroalbuminuria), at the same posology. A follow-up period of 6 weeks was observed in all patients.

End-point of the study was AER monitoring: this was performed on a 24-h urine collection before treatment (T0), weekly during it (T1, T2 and T3), and at 3-week intervals during the follow-up (T6 and T9). AER was assessed using the nephelometric method on the biochemical analyser 'Abbot Spectrum' (Abbot Laboratories, USA).

Before the start and at the end of treatment, creatinine clearance, haematological and coagulative parameters (blood count, PTT), haematochemical tests (total proteins and their

electrophoretic fractions, glycaemia, azotaemia, uricaemia, blood creatinine, total cholesterol and triglycerides), glycosylated haemoglobin (by HPLC with an automatic analyser), and urinalysis were also monitored in all patients.

Statistical evaluation of data was performed by comparing the two groups of micro- and macroalbuminuric patients submitted to either treatment, taking as primary criterion for efficacy assessment the behaviour of AER levels. The distribution of AER values was normalized by log transformation. Comparability of the two treatment groups during the study period (T0–T9) was checked by analysis of variance for repeated measures, corrected by Bonferroni. Since the sample size was small, the differences between treatments and within groups were verified by means of non-parametric tests (Mann–Whitney U test and Friedman's ANOVA). Furthermore, by means of one-way analysis of variance, the homogeneity of variances of micro- and macroalbuminurics' data between treatments, at each control time, was verified.

The lower level for statistical significance was set at 5%. Values are presented as mean  $\pm$  SEM. All data analyses were performed using the commercially available statistical software SPSS for Windows (v. 6.1.3).

## Results

All patients completed the administration period showing a good compliance with treatments. One sulodexide-treated patient did not attend the last follow-up visit (T9). No adverse events were registered. All patients' data were eligible to undergo the provided analyses. Table 1 summarizes the baseline data of the two patient groups. On the whole the 36 type 1 patients (15 male and 21 female, aged on average  $32.1 \pm 1.8$  years) had a mean time of diabetes duration of  $14.9 \pm 1.07$  years, and the baseline mean value of glycosylated haemoglobin (HbA1c) was  $8.47\% \pm 0.04$ .

During the study period all subjects followed their usual diet, without variation of individual insulin daily requirement; they did not show any variation of metabolic control and creatinine clearance (Table 2). No other therapies were prescribed.

Taking into consideration the macroalbuminurics' population, in sulodexide-treated patients AER was found decreased at the end of the treatment (T3), but again increased during follow-up (Figure 1). By performing a global analysis of the behaviour of this parameter, a significant difference depending on treatment could not be evidenced. By analysing, conversely, the variations at each control time, statistically significant ( $P < 0.05$ ) changes from T3 to T9, due to the treatment-with-time interaction, are pointed out.

A different trend was registered in microalbuminurics (Figure 2), in whom a global evaluation of results pointed to statistically significant ( $P < 0.001$ ) variations in the sulodexide group, due to both treatment and the treatment-with-time interaction ( $P < 0.01$  from T1 to T9). The two groups of micro- and macroalbuminurics, when performing the comparison of treatments, were homogeneous at T0. Also the analysis, through non-parametric tests, of the observed variations led to similar conclusions.

In the 18 placebo-treated patients no statistically

Table 1. Patients' characteristics at recruitment

Patients	Treatment	Age (yrs) (Mean $\pm$ SD)	Sex (M/F)	Systolic blood pressure (mmHg) (Mean $\pm$ SEM)	Diastolic blood pressure (mmHg) (Mean $\pm$ SEM)	Hb (g/100 ml) (Mean $\pm$ SEM)	WBC ( $10^3/mm^3$ ) (Mean $\pm$ SEM)	Platelet count ( $10^3/mm^3$ ) (Mean $\pm$ SEM)	APTT (s) (Mean $\pm$ SEM)
	<i>Sulodexide</i>								
9	Microalbuminuria	27.9 $\pm$ 11.2	3/6	123.9 $\pm$ 3.5	80.6 $\pm$ 1.8	13.2 $\pm$ 0.8	6.6 $\pm$ 0.4	293.3 $\pm$ 10.5	11.7 $\pm$ 0.4
9	Macroalbuminuria	29.9 $\pm$ 7.4	3/6	120.6 $\pm$ 4.9	76.7 $\pm$ 3.2	12.2 $\pm$ 0.6	7.3 $\pm$ 0.5	305.0 $\pm$ 21.5	11.9 $\pm$ 0.5
	<i>Placebo</i>								
9	Micro-albuminuria	33.3 $\pm$ 2.5	6/3	126.7 $\pm$ 5.2	76.1 $\pm$ 2.3	13.6 $\pm$ 6.8	6.7 $\pm$ 0.3	283.4 $\pm$ 10.4	11.7 $\pm$ 0.4
9	Macro-albuminuria	35.3 $\pm$ 3.95	3/6	133.3 $\pm$ 4.5	77.2 $\pm$ 2.7	12.9 $\pm$ 7.1	7.2 $\pm$ 0.4	304.2 $\pm$ 19.1	11.4 $\pm$ 0.3

significant variations were observed during the study period both in the micro- and in the macroalbuminuric patients.

No statistically significant differences concerning blood pressure, haematological, haematochemical, and coagulative tests, and urinalysis, were observed after treatment with respect to basal values, within and between the two groups.

## Discussion

The rationale for this study was based on previous results, collected in experimental models, supporting the hypothesis that GAG metabolism might have a pathogenetic role in the onset of diabetic nephropathy and that GAGs are effective in ameliorating the natural history of a number of nephropathies [7,8,15]. Diabetes leads to a generalized reduction of negative charge in the extracellular matrix and plasma membrane, reflecting qualitative changes in the composition of the membrane itself [19,22]. On the other hand, GAGs and particularly HS [8] are involved in glomerular permeability for macromolecules; they are essential for the integrity of the glomerular charge selectivity, due to their property of electrostatic repulsion, that is responsible for the net negative charge [20] and prevents the filtration of the negatively charged albumin. It has been shown that the enzymatic removal of HS by heparinase, the neutralization of HS charge by cations, or the injection of a monoclonal antibody specific for GBM HS, all induce an increase of proteinuria [19,23,24].

The decreased charge selectivity of GBM may also be the consequence of undersulphation and/or of altered content of GAGs [7,8,25]: in fact, in diabetes, GAG sulphation is abnormal. Results of experimental diabetes models also showed that chronic GAGs administration can reduce albuminuria and prevent some renal morphological and functional alterations [9]. In human diabetic nephropathy, GMB alterations are markedly correlated with expansion of the mesangial area. On the other hand GAGs can inhibit mesangial cells growth, interfere with growth factors, and modulate synthesis of extracellular matrix proteins in cells of mesenchymal origin [8,15].

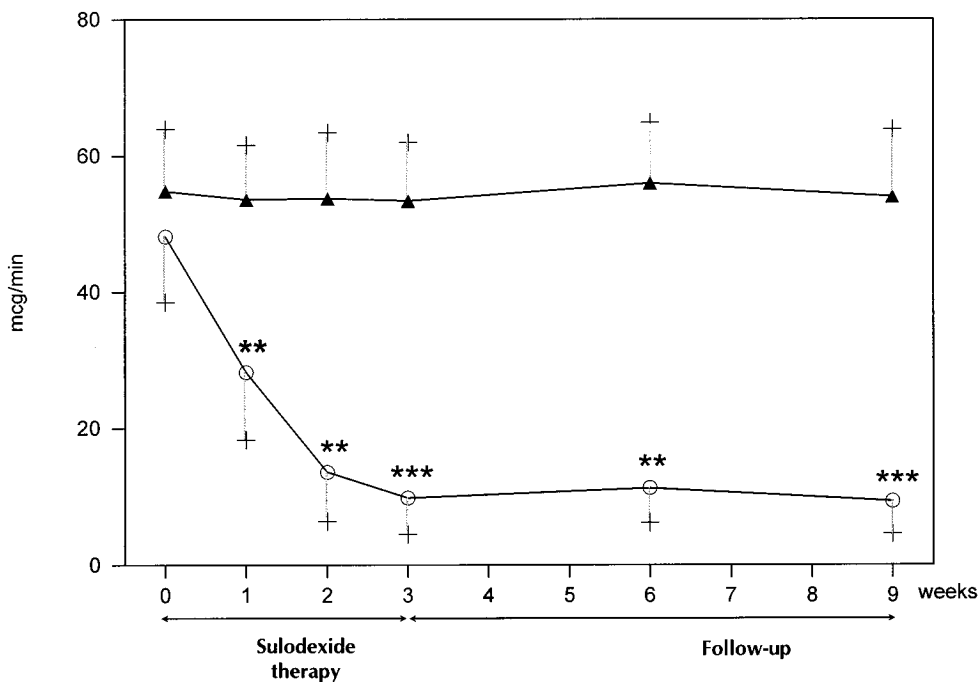
The above data may indirectly support the hypothesis that the derangement in GAG metabolism may have a pathogenetic role in the onset of diabetic nephropathy, through the induction of a biochemical derangement in GBM composition [26].

In diabetic patients with albuminuria, a favourable effect on AER has been recently reported by Myrup *et al.* [12] and by Tamsma *et al.* [13] with the use of low-molecular-weight heparins and by Solini *et al.* [11] and Velussi *et al.* [14] with sulodexide.

In our study sulodexide administration significantly reduced AER in type I diabetic patients both with micro- and with macroalbuminuria, while placebo-treated micro- and macroalbuminurics did not evidence AER variations. This behaviour was not related with

**Table 2.** Behaviour of some metabolic parameters during treatment period

Patients	Treatment	Creatinine clearance (ml/min) (Mean $\pm$ SEM)			HbA1c (%) (Mean $\pm$ SEM)		
		Before	After	<i>P</i>	Before	After	<i>P</i>
<i>Sulodexide</i>							
Microalbuminuria 9		98.1 $\pm$ 10.5	89.52 $\pm$ 8.3	NS	8.47 $\pm$ 0.7	8.45 $\pm$ 0.6	NS
Macroalbuminuria 9		122.7 $\pm$ 22.7	105.8 $\pm$ 17.4	NS	8.55 $\pm$ 0.08	8.6 $\pm$ 0.06	NS
<i>Placebo</i>							
Microalbuminuria 9		103.5 $\pm$ 10.3	102.2 $\pm$ 10.2	NS	8.5 $\pm$ 0.09	8.46 $\pm$ 0.07	NS
Macroalbuminuria 9		109.0 $\pm$ 11.8	108.7 $\pm$ 10.5	NS	8.36 $\pm$ 0.03	8.41 $\pm$ 0.05	NS

**Fig. 1.** Behaviour of AER ( $\mu\text{g}/\text{min}$ ) mean values ( $\pm$ SEM) in diabetic, macroalbuminuric patients treated with sulodexide ( $\circ$ ) or with placebo ( $\blacktriangle$ ) during treatment (weeks 1–3) and follow-up (weeks 4–9). \*\* $P < 0.01$ , \*\*\* $P < 0.001$  (one-way ANOVA between groups).

changes of metabolic control, blood pressure, and diet; neither plasma glucose nor glycated haemoglobin showed any variation during both placebo and sulodexide treatment. No symptomatic urinary tract infection, another parameter influencing AER, was ever observed during the study; at the same time the administration scheme for insulin therapy remained unmodified in all the patients.

Solini *et al.* [11] described in their small study group a different trend in microalbuminuric with respect to macroalbuminuric patients, the latter showing a higher AER decrease than the former. The opposite was registered in our study: the percentage of AER decrease in microalbuminuric patients was significantly higher than in macroalbuminurics. Such differing study results may be due to the different type of patients (type I diabetics in our study and type II in Solini's trial),

and/or to the different administration scheme adopted (parenteral sulodexide in our study and oral sulodexide in the Solini trial). In our study another difference between micro- and macroalbuminurics was that the AER reduction registered at the end of treatment in microalbuminuric patients was maintained during the 6 weeks of follow-up, while in macroalbuminurics AER was found in the end again appreciably increased, independently of metabolic control, systolic and diastolic blood pressure, sex distribution, and body weight, since all these parameters were initially similar and did not vary as a consequence of treatment.

It is, on the other hand, possible that depending on the severity of albuminuria, different GAG dosages must be administered, so that dosages suitable for microalbuminuric patients could be insufficient for macroalbuminurics. Furthermore, since an abnormal

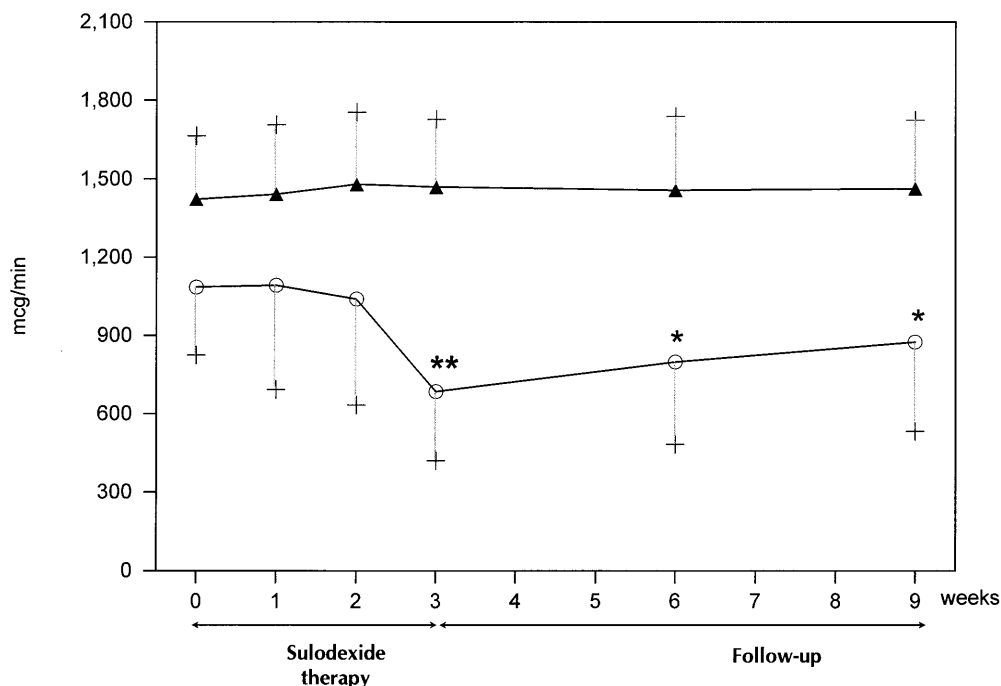


Fig. 2. Behaviour of AER ( $\mu\text{g}/\text{min}$ ) mean values ( $\pm\text{SEM}$ ) in diabetic, microalbuminuric patients treated with sulodexide ( $\circ$ ) or with placebo ( $\blacktriangle$ ) during treatment (weeks 1–3) and follow-up (weeks 4–9). \* $P < 0.05$ , \*\* $P < 0.01$  (one-way ANOVA between groups).

GAG metabolism is detectable not only at the kidney level but also on all the other vessel walls [28], in macroalbuminuric patients GAG synthesis may be greatly compromised not only in GBM but also at the vessels level, and the administered sulodexide could be captured by all the vessel surfaces and therefore attain a lower concentration at the GBM level.

During the treatment period all patients showed a good compliance with treatment and no significant adverse drug reactions were observed.

The good safety profile of sulodexide has also been pointed out after long-term (1 year) treatment in post-AMI patients [21], as well as in a multicentre, medium-term, controlled investigation involving patients affected by peripheral vascular disease (PVD) [28]. The reported adverse drug reactions—rare cases of haematoma after i.m. injection, and some episodes of gastralgia, nausea/vomiting after oral administration—were transitory and did not necessitate stopping the treatment. Also a recent meta-analysis performed on a number of clinical trials in patients with PVD evidenced a very good tolerability of intramuscular and oral sulodexide long-term administration [29].

Our results, together with those obtained in experimental models and in diabetic, albuminuric patients, support the hypothesis that an abnormal GAG metabolism is involved in the onset of anatomic-functional derangements in diabetic nephropathy, and justify the investigators' interest in GAGs therapy, and namely in sulodexide administration.

Trials in large groups of patients receiving long-term sulodexide treatments are needed to better ascertain the influence of this GAG on the morphological and

functional renal alterations that occur in diabetic nephropathy.

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