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Glycosaminoglycan sulodexide decreases albuminuria in diabetic patients

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Abstract

Albuminuria is a dominant biochemical feature of developing diabetic nephropathy. A disturbed metabolism of heparan sulphate characterized by an increased loss of anionic charges in the basement membrane has been considered as one of the main factors causing an increased albumin output into urine. All therapeutic approaches inducing a reduction of the albumin excretion rate (AER) have a protective effect on renal function. The effect of glycosaminoglycan sulodexide on albuminuria was studied in a group of 53 diabetic patients (26 Type 1 and 27 Type 2) with micro and macroalbuminuria. Sulodexide (Vessel Due F) was administered intramuscularly in one daily dose (600 lipasemic units) for 3 weeks followed by a 6 week wash-out period. A significant decrease of AER was found in a total cohort of patients following just 1 week of sulodexide treatment (mean 162 μg/min, range 10–2708 μg/min vs mean 248 μg/min, range 20–3160 μg/min, $P < 0.001$). This effect lasted 3–6 weeks after drug withdrawal. Similar results were obtained if Type 1 and Type 2 diabetic patients were evaluated separately but a delay of the AER reduction was observed in the latter group. In all patients the mean AER was reduced to 60–65% of the initial values. A greater effect of sulodexide on albuminuria was observed in patients with AER above 200 μg/min than in those with microalbuminuria (a reduction to 47 vs 65% of the initial output). Sulodexide did not significantly reduce albuminuria in 28% of diabetic patients ('non-responders'). In conclusion, glycosaminoglycan sulodexide may reduce AER in patients with micro or macroalbuminuria and it could slow down development of diabetic nephropathy.

Keywords: Albuminuria; Sulodexide; Diabetes mellitus

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1. Introduction

Diabetic nephropathy represents one of the major long-term complications leading to progressive loss of the kidney function in diabetes mellitus [1,2]. Glomerular basement membrane thickening and the mesangium expansion are the consequences of disturbed metabolism in diabetes. Non-enzymatic glycation of the proteins as well as impaired glycosaminoglycan (GAG) metabolism are considered as the main biochemical changes contributing to diabetic nephropathy. Reduced content of heparan sulphate and its undersulphation are the critical points inducing the abnormal charge permselectivity of the basement membrane in diabetes [3–5]. An increased loss of albumin into urine is a typical consequence of the biochemical changes. Microalbuminuria as an early marker of incipient diabetic nephropathy has been widely used in the clinical practice in the last decade [6]. A lowering of albumine excretion into urine may delay the evolution of diabetic nephropathy and therefore it may have a great impact on diabetic patients.

Some experiments in diabetic rats demonstrated that daily administration of low molecular weight heparins (LMWH) could prevent the glomerular basement membrane thickening and the increased albumin excretion into urine [7,8]. A similar effect on the reduction of albuminuria has been observed in humans [9,10].

Sulodexide, which is composed of an 80% heparin fraction and 20% dermatan sulphate fraction, revealed an antiproliferative effect which may favorably slow down the progression of diabetic nephropathy [11]. It's administration positively influences negative electrical potential of the basement membrane possibly by increasing the anionic charge of the vessel wall glycosaminoglycans [7,8,12,26].

The previous findings demonstrated a decrease of albuminuria by glycosaminoglycans either in a small sample of persons or only in Type 2 diabetic patients [9,10,13,14]. This induced us to study the effect of sulodexide on albuminuria in a larger group of both Type 1 and Type 2 diabetic patients with micro and macroalbuminuria.

2. Patients and methods

A total of 53 patients (24 men and 29 women) of Type 1 ($n = 26$) and Type 2 ($n = 27$) diabetes mellitus were enrolled in this pilot multicentric study. Their characteristics are shown in Table 1. The patients were selected according to the presence of micro (20–200 μg/min) or macroalbuminuria (above 200 μg/min) proved at least in three random overnight urine samples examined prior to enrollment in the study. All patients with liver impairment, microhematuria or severe renal failure characterized by serum creatinine concentration above 150 μmol/l were excluded from the study. Only patients with arterial blood pressure up to 160/90 mmHg either spontaneously or pharmacologically achieved within 3 months before the study were selected for the evaluation. Antihypertensive therapy was performed in 21 patients. Calcium channel antagonists were administered in 17 patients, beta-blockers in seven, alpha-blockers in two and ACE inhibitors in six. Seven patients were treated with a combination of two drugs and three drugs were used for treatment of the remaining two persons. This pharmacological therapy was maintained for the 6 months prior to enrollment and during the course of the study. Patients with acute vitreoretinal bleeding, demonstrated by ophthalmological examination, were not included in the study. The ophthalmological status was not evaluated at the end of the drug administration

Table 1
Characteristics of 53 diabetic patients

	Diabetes mellitus	
	Type 1 ($n = 26$)	Type 2 ($n = 27$)
Male/female	16/10	17/10
Age (years)	42 (22–63)	54 (33–65)
Duration of diabetes (years)	20 ± 8	16 ± 12
Blood pressure (mmHg)		
Systolic	140 ± 12	143 ± 11
Diastolic	82 ± 6	83 ± 7
Number of micro-/macro-albuminuric patients	14/12	10/17

The results are expressed as means with ranges or SD.

because of its short duration. An informed consent was obtained from each of the patients.

Sulodexide (Vessel Due F, Alfa Wassermann, Italy) was administered intramuscularly in one daily dose of 600 lipasemic units corresponding to 60 mg of the drug for 5 days a week. Fifteen doses were administered to each patient over 3 weeks followed by a 6 week wash out period.

Blood samples for biochemical and hematological examinations were collected after an overnight fast before and at the end of treatment as well as after the wash out period. The blood count, plasma fibrinogen concentration, activated partial thromboplastin time, blood urea nitrogen, serum creatinine, creatinine clearance rate, aspartate and alanine aminotransferase activities, total plasma protein, blood glucose, cholesterol and triglyceride concentrations were evaluated before and after treatment with sulodexide by standard procedures on an automatic analyzer. Glycated haemoglobin (HbA_{1c}) was determined in all three samples by FPLC [15].

One overnight urine sample was collected before the treatment and at the end of weeks 1, 2, 3, 6 and 9 of the study. Albuminuria was evaluated by radioimmunoassay method using RIA kit [^{125}I]albumin (Immunotech, Czech Republic) and albumin excretion rate (AER) was then calculated in μg/min. The internationally accepted range of 20–200 μg/min has been used for microalbuminuria whereas values above 200 μg/min were considered as macroalbuminuria. The results of microalbuminuria were distinguished into those with lower (20–100 μg/min) and higher (100–200 μg/min) AER. Normal values of AER for our laboratory are 4.7 μg/min (1.9–11.8 μg/min) expressed as the mean and 2SD ranges. Urine samples were tested by routine methods and urinary infection was excluded in all patients.

Statistical evaluation of the results was performed by using Student's t-test for paired values. Albumin excretion rates (AER) were logarithmically transformed before statistical evaluation because log-normal distribution of their values was found. Analysis of variance (ANOVA) was used for comparison of subgroups. The results have been expressed as mean $\pm$ SD or means with 2SD ranges.

Table 2
Laboratory variables in the group of 53 diabetic patients before and after 3 weeks of sulodexide treatment

Variable	Before treatment	After treatment
fP-glucose (mmol/l)	11.3 ± 4.1	10.9 ± 3.6
HbA_{1c} (%)	8.4 ± 1.1	8.8 ± 1.2
fS-urea (mmol/l)	6.4 ± 2.0	6.8 ± 2.6
fS-creatinine (μmol/l)	105 ± 23	109 ± 27
fS-cholesterol (mmol/l)	6.3 ± 1.4	6.2 ± 1.6
fS-triglycerides (mmol/l)	2.4 ± 1.7	2.7 ± 2.2
fS-protein (g/l)	73.9 ± 6.8	74.3 ± 6.2
APTT (s)	31.4 ± 2.7	32.1 ± 3.9
fP-fibrinogen (g/l)	3.7 ± 1.0	3.8 ± 1.0
Creatinine clearance rate (ml/min)	101.8 ± 31.5	93.6 ± 38.8 *
Blood pressure (mmHg)		
Systolic	141 ± 12	142 ± 11
Diastolic	82 ± 7	84 ± 7

Statistical significance: * $P = 0.05$.

3. Results

Selected biochemical and haematological variables in the group of 53 patients were not changed by sulodexide treatment (Table 2). The same was true for blood pressure values as well as for diabetes control as measured by fasting plasma glucose and glycated haemoglobin. Only a borderline decrease of the creatinine clearance rate was observed at the end of therapy ($P = 0.05$).

The mean AER values in the cohort of 53 diabetic patients are shown in Table 3. A significant decrease of AER was observed during sulodexide administration and this effect was still apparent in the follow-up period of 6 weeks. AER reduction occurred after only the first week of sulodexide treatment with the average value decreased to about 60–65% of the initial albuminuria even after 3–6 weeks of drug withdrawal. There were 29 patients with AER exceeding 200 μg/min (whole group) whereas 'microalbuminuria' was present in the remaining 24 patients. The results in the whole group (patients with AER above 200 μg/min) were similar whereas the effect of sulodexide was attenuated in the 'microalbuminuric' group (Table 4). No response in

Table 3
Albumin excretion rate (AER) in diabetic patients before (week 0) and at the end of the first, second and third weeks of sulodexide treatment as well as after 3 and 6 weeks following its withdrawal

Week	Albumin excretion rate (μg/min)		
	Total group ($n = 53$)	Type 1 diabetes ($n = 26$)	Type 2 diabetes ($n = 27$)
0	249 (20–3155)	258 (22–3090)	241 (17–3366)
1	174 (10–2900)**	155 (9–2590)**	194 (11–3536)
2	162 (10–2708)*	162 (12–2220)**	160 (13–1893)*
3	165 (12–2300)*	150 (15–1550)*	170 (10–3130)***
6	151 (12–1970)*	170 (9–3360)***	129 (10–1680)*
9	166 (11–2550)**	167 (10–2770)**	155 (7–3390)

Statistical significance as compared with pretreated values: * $P<0.001$, ** $P<0.01$, *** $P<0.02$. AER is expressed as the mean and 2SD range.

the AER during sulodexide treatment was observed in patients with albuminuria lower than 100 μg/min (Table 4). The values of 2SD ranges at the baseline overlap those for micro or macroalbuminuria due to the statistical evaluation of logarithmically transformed data.

The results of AER in both Type 1 and Type 2 diabetic patients were compared (Table 3). A similar reduction of AER was found in both groups. An earlier decrease of albuminuria was present in Type 1 diabetic patients with the lowest mean AER at the end of sulodexide treatment whereas it was moderately delayed in Type 2 patients with the lowest value 3 weeks later. In addition, a small but significant decrease of AER was found in microalbuminuric Type 1 diabetic patients just after sulodexide treatment ($P < 0.05$) whereas no effect was observed in Type 2 patients with microalbuminuria.

Table 4
Albumin excretion rate (AER) in diabetic patients with lower range of microalbuminuria (AER 20–100 μg/min), higher microalbuminuria (AER 100–200 μg/min) and macroalbuminuria (AER above 200 μg/min)

Week	Albumin excretion rate (μg/min)		
	Low micro ($n = 12$)	High micro ($n = 12$)	Macro ($n = 29$)
0	46 (19–111)	148 (102–213)	619 (113–3400)
1	37 (5–320)	117 (29–470)	390 (40–3740)**
2	44 (6–312)	91 (28–289)**	347 (48–2500)*
3	46 (6–360)	78 (16–370)*	363 (52–2525)*
6	29 (2–386)	116 (28–483)	289 (36–2325)*
9	30 (3–362)	104 (23–472)	396 (53–2960)**

AER are expressed as the means and 2SD range. Statistical significance as compared with pretreated values: * $P<0.001$, ** $P<0.02$.

The patients were also subdivided into 'responders' ($n = 38$) and 'non-responders' ($n = 15$) to sulodexide treatment (Fig. 1). The mean value of albuminuria from the second, third and sixth weeks of the study was used for calculation of the sulodexide effect in each of the subjects. Patients with the mean AER from the above three measurements equal to 90% or higher of the initial value were considered as 'non-responders'. The average albuminuria in 'responders' decreased to 50% of the initial AER. We could not find any changes of other biochemical variables which explained the difference between 'responders' and 'non-responders'.

The tolerance of the drug was excellent, no side effects like skin reactions or bleeding were observed in our patients during the whole period of sulodexide treatment.

4. Discussion

The aim of this pilot study was to evaluate if glycosaminoglycan sulodexide could induce some improvement of micro or macroalbuminuria in diabetic patients. Previous studies have shown a decrease of AER due to LMWH administration

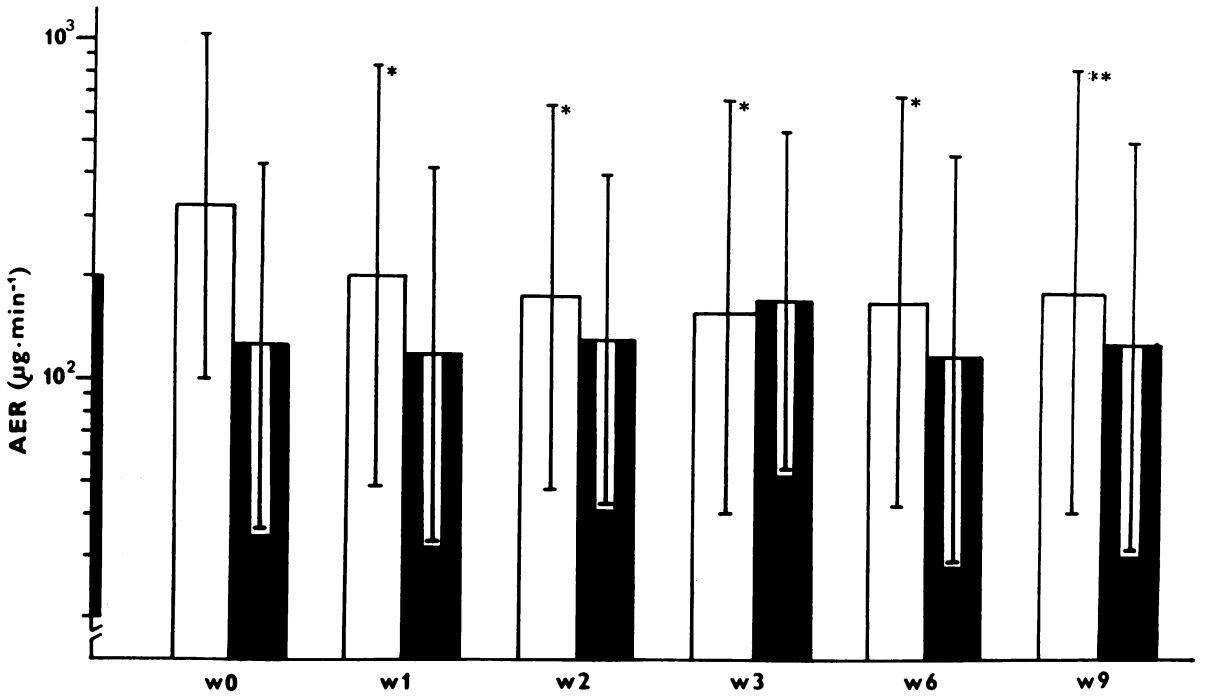

Fig. 1. Albumin excretion rate (AER) in 'responders' (white columns, $n = 38$) and 'non-responders' (black columns, $n = 15$) on sulodexide treatment. Statistical significance as compared with pretreated values: * $P < 0.001$, ** $P < 0.02$.

but in a small number of Type 2 diabetic patients [10,13,14]. We therefore selected a larger number of well defined Type 1 and Type 2 diabetic patients for sulodexide treatment. We did not use a placebo in our multicentric study but we did not demonstrate any changes either in diabetes control or in blood pressure values, two variables having the impact on albuminuria in diabetes. In addition, the previous pharmacological therapy for our patients was not changed during this study, so that the results might give some information on the effect of glycosaminoglycan on albuminuria.

We could demonstrate that by sulodexide treatment: (1) AER was reduced more in patients with albuminuria above 200 μg/min (to 47% of initial values) than in those with microalbuminuria (to 68%); (2) no significant effect on AER was observed in patients with the lower range of microalbuminuria (AER below 100 μg/min); (3) AER was reduced slightly earlier in Type 1 than in Type 2 diabetic patients; and (4) some patients (28%) had no significant reduction of albuminuria and were classified as 'non-responders'. Our results, which demonstrate reduction of albuminuria by sulodexide, are consistent with recent findings of other investigators who used either sulodexide or other LMWHs [9,10,13,14]. However, all these studies differ by designs and by doses and routes or duration of the drug administration. The results are therefore hardly comparable.

During the sulodexide treatment period we observed a decrease in AER after the first week of treatment which lasted into the wash-out phase of the study. The mean AER values in 6 weeks following the sulodexide administration did not reach the pretreatment results. This observation support an idea that sulodexide could have some influence on glomerular structure rather than an hemodynamic effect. Also, a borderline decrease ($P = 0.05$) of the creatinine clearance rate at the end of sulodexide treatment does not support a suspicion of significant hemodynamic changes

substantially influencing albuminuria during sulodexide administration. A weaker effect of sulodexide found in our 'low microalbuminuric' patients was also demonstrated by other investigators [13]. A more pronounced decrease of albuminuria is present in patients with higher values of AER, both within microalbuminuric and macroalbuminuric ranges.

Our results demonstrate the influence of parenterally administered sulodexide. There are only preliminary data on a reduction of AER by orally treated diabetic patients with sulodexide [14]. This was not found in animal studies in which orally administered LMWH had a negligible effect on glomerular basement membrane thickness in addition to albuminuria [16]. However, the LMWH was used without protection against gastric acid which may deteriorate its activity. It will be necessary therefore to evaluate the influence of orally administered sulodexide on AER, together with the estimation of an optimal daily dose and total duration of the drug treatment.

Diabetic nephropathy is characterized by glomerular basement membrane thickening and mesangial expansion. Heparan sulphate plays a central role in the morphological and functional properties of the basement membranes [17] as well as of the glycosaminoglycan layer in the luminal surface of endothelium [18]. An increasing loss of anionic charges due to glycosaminoglycan undersulphation causes the progression of albuminuria. In the glomerular basement membrane a negative correlation between its anionic charge, albuminuria and heparan sulphate concentration has been described [19,20]. An increased loss of glycosaminoglycans in urine preceding albuminuria was also observed in diabetes [21]. The reduction of albuminuria, a marker of diabetic nephropathy as well as of generalized microangiopathy [22,23], is of great importance because it may delay kidney failure development and protect structural and functional properties of the vessel wall. Different factors like improvement of diabetes control and blood pressure, including the use of ACE inhibitors, have impact on albuminuria in diabetes.

LMWHs bring new insights in this area because of their possible influence on basement membrane glycosaminoglycans. Although the proper mechanism of their action remains to be discovered, a direct antiproliferative effect on mesangial cells was previously demonstrated [24]. Heparin not only stimulates the synthesis of heparan sulphate in vitro but also increases its sulphation [25,26]. The long-term administration of glycosaminoglycans could prevent renal alterations in diabetic rats by normalizing the collagen gene expression and increasing glomerular [^{35}S]sulphate incorporation [27].

Sulodexide administration in diabetic nephropathy is very promising at present not only in reduction of albuminuria but by improving the integrity of the vessel wall. Further research on sulodexide treatment in albuminuric patients will be therefore necessary.

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