Glycosaminoglycans Delay the Progression of Nephropathy in NIDDM

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OBJECTIVE — To determine the effect of oral administration of glycosaminoglycans on metabolic control and albumin excretion rate (AER) in NIDDM patients with increased urinary albumin excretion.

RESEARCH DESIGN AND METHODS — Twelve NIDDM hypertensive patients (age 52 ± 3 years, HbA_{1c} 7.7 ± 0.2%) on antihypertensive treatment were enrolled in a double-blind placebo-controlled study, assuming either placebo or sulodexide (100 mg/day) for 4 months; at the end of this period, a crossover was performed. We have evaluated routine biochemical parameters plus AER and coagulative function every 2 months.

RESULTS — Both plasma fibrinogen (from 4.15 ± 0.32 to 2.77 ± 0.47 mmol/l) and AER (from 128.3 ± 40.6 to 39.6 ± 11.9 μg/min) decreased significantly after treatment with glycosaminoglycans in respect to placebo; moreover, blood pressure control ameliorated, also in the absence of any variation of therapy.

CONCLUSIONS — Glycosaminoglycan therapy, likely in association with a satisfactory control of blood pressure values, seems to prevent the progression of diabetic nephropathy in NIDDM.

Increased urinary excretion of albumin, undetectable by standard tests (microalbuminuria), is considered by many investigators as an early marker in the clinical expression of the progression of diabetic nephropathy lesions. It may predict the eventual development of clinically recognizable proteinuria (1,2) and has been also recognized as an independent marker of cardiovascular risk in the general population (3). Its pathogenesis is still a matter of debate: in addition to increased intraglomerular pressure (4), loss of glomerular charge selectivity (5) and altered glomerular size selectivity with increased nonenzymatic glycation of structural proteins and formation of advanced glycosylation end products (6) seem to be present in type I diabetic patients with clinically advanced nephropathy. An additional basement membrane alteration that has been associated with diabetes is the decreased production of heparan sulfate proteoglycans (HSPGs) (7).

The presence of proteoglycans in the glomerular basement membrane (GBM) is essential for the integrity of the glomerular charge selective barrier due to properties of electrostatic repulsion and steric hindrance (7). Biochemical studies in kidneys of IDDM patients with end-stage diabetic nephropathy have shown a decreased content of proteoglycan molecules in glomerular basement membranes compared with nondiabetic subjects (8). Moreover, several studies of experimental diabetes in animals describe abnormalities of synthesis, sulfation, content, and/or proteoglycan matrix interaction of heparan sulfate molecules in diabetic GBM (9,10).

Supporting these observations, it has been shown that heparan can prevent albuminuria and thickening of glomerular basement membrane in streptozotocin-induced diabetic rats (11); it also inhibits mesangial cell growth and suppresses mesangial matrix expansion in experimental mesangioproliferative glomerulonephritis (12).

In agreement with these experimental evidences, there is a rationale in using these molecules in the prevention of several kidney diseases characterized by different degrees of mesangial expansion (extracellular matrix accumulation, mesangial cell hypertrophy). The aim of our study was to evaluate the effect of oral administration of sulodexide (a naturally occurring glycosaminoglycan extracted from pig intestinal mucosa and containing a fast-moving heparan-like fraction [80%] and a dermatan sulfate fraction [20%]) on kidney function and albumin excretion rate (AER) in microalbuminuric NIDDM patients.

RESEARCH DESIGN AND METHODS

— This double-blind placebo-controlled study was performed in the Department of Internal Medicine of the University of Padova. We selected 12 hypertensive micro- and macroalbuminuric NIDDM patients with mild essential hypertension, matched for age, sex, and diabetes duration. Clinical characteristics of study population are shown in Table 1. All patients were between 40 and 65 years of age, were of Caucasian origin, and were following an isocaloric diet containing 55% carbohydrate, 25% fat, and 20% protein, and ~150 mmol/day sodium chloride. They were all treated with insulin and had satisfactory metabolic control (Table 1). No variation was observed in the individual daily requirement of insulin during the 8 months of treatment.

All subjects gave their written informed consent. Exclusion criteria were neoplasms, secondary hypertension, cardiovascular or cerebrovascular diseases, plasma creatinine >180 μmol/l, impaired liver function, any therapies other than insulin, and antihypertensive agents. All patients were under antihypertensive pharmacological treatment from at least 1 year, and none had registered any change in the therapeutic scheme, either during the 6 months preceding enrollment in our trial or during the

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AER, albumin excretion rate; AT, antithrombin; GAG, glycosaminoglycan; GBM, glomerular basement membrane; GFR, glomerular filtration rate; G/P, patients who started with active treatment; HSPG, heparan sulfate proteoglycan; P/G, patients who started with placebo; M°, study enrollment (month 0).

Table 1—*Clinical characteristics of the patients before starting the treatment*

Age (years)	52 ± 3
BMI (kg/m^2)	26.1 ± 1.1
Diabetes duration (years)	7.3 ± 1.6
HbA_{1c} (%)	7.76 ± 0.20
Systolic blood pressure (mmHg)	155 ± 6
Diastolic blood pressure (mmHg)	81 ± 3
GFR (ml · min^{-1} · 1.73 m^{-2})	96.52 ± 8.32
AER (μg/min)	128 ± 40.6

Data are means ± SE.

study period. Ten out of twelve patients were treated with nicardipine (20 mg/daily) and two with enalapril (5 mg/daily); blood pressure control was satisfactory in all subjects (<150 and 90 mmHg).

Diagnosis of NIDDM was made when fasting plasma glucose was ≥7.8 mmol/l on two different occasions (13). Baseline blood pressure was measured three times in both arms after a 15-min supine rest. These measurements were made by the same person (A.S.), who used a Hawksley random-zero sphygmomanometer (Hawksley and Sons, Lancing, Sussex, U.K.) with two different cuff sizes (12 × 35 cm and 12 × 45 cm) for mid-arm circumference, respectively placed lower or higher than 32 cm. Three 24-h urine collections were requested from each patient to evaluate AER. This was considered normal if ≤15 μg/min. Microalbuminuria was defined as an AER >20 μg/min.

At time 0 (before starting the treatment), a recent anamnesis was recorded, anthropometric measurements and blood pressure values were registered, and a complete clinical examination was performed; moreover, blood sampling was drawn for the evaluation of routine laboratory parameters, including fibrinogen and antithrombin (AT) III, C reactive protein, complement fractions C3 and C4, total and HDL cholesterol, triglycerides, and plasma creatinine. The degree of metabolic control was evaluated by glycated HbA_{1c} and measured by high-pressure liquid chromatography (14) and fasting plasma glucose. AER was measured on three 24-h samples by radioimmunoassay (15), using the mean value for calculation.

Renal functional measurements were evaluated in all the patients as they were admitted to our study after an overnight 12-h fast. The subjects remained at rest in an armchair through the procedure. A Venflo cannula (1.1 mm outer diameter) was placed in an antecubital vein for tracer infusion. A second similar cannula was inserted into an antecubital vein of the contralateral arm. The cannula was filled with 0.5–0.7 ml of isotonic saline, and the first 1 ml of blood was subsequently discarded at each sample time. At 8.00 A.M., a 30-ml blood specimen was taken for routine biochemical parameters. A baseline sample was collected just before tracer injection for background radioactivity measurements. Glomerular filtration rate (GFR) was then assessed by plasma clearance of ^{51}Cr-EDTA (Amersham, Buckinghamshire, U.K.) (16).

Each 3- to 4-ml blood sample was immediately mixed with 0.1 ml of heparan, kept in crushed ice until the end of the study, and spun down. The supernatant was used to measure glucose, sodium, potassium, and radioactivity of ^{51}Cr-EDTA in a gamma counter (Cobra 5002 CPM, Camberra Packard, Milan, Italy). Energy windows were set to 240–400 KeV for ^{51}Cr. A standard curve was used to correct each sample from residual radioactivity overlapping. Further details have been provided elsewhere (16).

After the enrollment, patients were randomly assigned to placebo (n = 6) or glycosaminoglycan (GAG) therapy (n = 6, oral

Table 2—*Main biochemical parameters in the six patients starting the protocol with GAG (G/P) and in the six patients starting with placebo (P/G) at enrollment (month 0) and after 2, 4, 6, and 8 months of treatment*

	Month 0	Month 2	Month 4	Month 6	Month 8
Fasting plasma glucose (mmol/l)					
G/P	9.6 ± 1.37	7.5 ± 0.45	7.05 ± 0.4	6.92 ± 0.23	6.13 ± 0.34
P/G	11.2 ± 2.57	11.13 ± 1.8	9.0 ± 1.6	13.5 ± 2.48	8.85 ± 1.77
HbA_{1c} (%)					
G/P	7.63 ± 0.12	7.28 ± 0.20	7.2 ± 0.21	7.33 ± 0.14	7.35 ± 0.23
P/G	7.88 ± 0.38	8.2 ± 0.44	8.8 ± 0.6	8.75 ± 0.41	8.6 ± 0.19
Total cholesterol (mmol/l)					
G/P	4.88 ± 0.43	4.92 ± 0.3	5.0 ± 0.25	4.98 ± 0.35	4.65 ± 0.24
P/G	5.88 ± 0.6	5.71 ± 0.48	5.32 ± 0.45	6.04 ± 0.78	5.53 ± 0.58
HDL cholesterol (mmol/l)					
G/P	1.61 ± 0.16	1.74 ± 0.09	1.6 ± 0.19	1.75 ± 0.26	1.95 ± 0.12
P/G	1.93 ± 0.32	1.74 ± 0.31	1.82 ± 0.24	2.09 ± 0.28	1.80 ± 0.23
Triglycerides (mmol/l)					
G/P	1.23 ± 0.19	1.20 ± 0.19	1.57 ± 0.29	1.71 ± 0.20	1.69 ± 0.27
P/G	1.68 ± 0.64	0.89 ± 0.36	1.81 ± 0.60	1.85 ± 0.58	1.92 ± 0.45
Fibrinogen (g/l)					
G/P	4.66 ± 0.38	3.47 ± 0.30	2.47 ± 0.27*	3.18 ± 0.28	2.59 ± 0.54†
P/G	4.63 ± 0.46	4.2 ± 0.38	4.28 ± 0.33	3.9 ± 0.36	3.29 ± 0.51
AT III (mg/l)					
G/P	0.98 ± 0.05	1.05 ± 0.06	1.08 ± 0.04	1.06 ± 0.03	1.03 ± 0.03
P/G	1.13 ± 0.03	1.13 ± 0.04	1.12 ± 0.03	1.12 ± 0.07	1.16 ± 0.05

Data are means ± SE. *P < 0.001 with respect to P/G; †P = 0.005 respect to month 0.

administration of sulodexide 100 mg/day) following a 4-month treatment; at the end, there was a crossover: the six patients initially treated with placebo were shifted on sulodexide treatment, while the other six were assigned to placebo.

All patients were evaluated after 2, 4, 6, and 8 months. At each control session, a physical examination was done, and blood tests and AER were determined, while GFR measurement was repeated only at the end of the follow-up.

Statistical analysis

Data are expressed as means ± SE. Statistical analysis was performed using Student's *t* test for paired data and analysis of variance for repeated measurements. A P value <0.05 was considered statistically significant.

RESULTS — Compliance was adequate for all 12 patients. None of them reported any side effects during the treatment.

Values of the main biochemical parameters at the enrollment and after placebo and drug treatment are reported in Table 2. A satisfactory metabolic control was maintained throughout the whole study period in all patients; plasma total and HDL cholesterol and triglycerides as well as AT III did not show significant variation. Flogosis indexes like C3, C4, and C reactive protein did not modify during either placebo or drug administration (data not shown).

Plasma fibrinogen at enrollment (M°) was 4.63 ± 0.46 in the patients who started with placebo (P/G) and 4.66 ± 0.38 mmol/l in those who started with active treatment (G/P). After the first 4 months of treatment, plasma fibrinogen significantly decreased to 2.47 ± 0.27 in G/P, while it was 4.28 ± 0.33 mmol/l in P/G ($P <$ 0.001). After shifting to glycosaminoglycans at the end of the 8th month, P/G showed a reduction to 3.29 ± 0.51, while G/P maintained a mean value similar to the first period (2.59 ± 0.44 mmol/l, $P = 0.005$ compared with M°).

Indexes of kidney function remained constant throughout the entire study period; particularly, plasma creatinine was 93.8 ± 6.8 at M°, 89.3 ± 4.4 at month 4, and 91.6 ± 5.9 μmol/l at month 8 in P/G and 90.3 ± 4.5 at M°, 89.5 ± 8.6 at month 4, and 93.1 ± 6.9 μmol/l at month 8 in G/P (NS). Mean GFR was 96.5 ± 8.3 at M° and 94.1 ± 6.7 ml · min^{-1} · 1.73 m^{-2} at the end of the study.

At enrollment, AER was 135.4 ± 37.9 in G/P and 119.7 ± 82.9 μg/min in P/G. AER significantly decreased to 35.9 ± 13.7 after the first 4 months in G/P ($P = 0.033$ vs. M°), while it was 220.4 ± 150.9 μg/min in P/G. At the end of the treatment, AER showed a decrement to 44.1 ± 22 μg/min in P/G, while in G/P, the reduced AER, with respect to basal state, was maintained during the placebo period (47.7 ± 19.1 μg/min). Individual values of AER at enrollment and during placebo and GAG administration are showed in Fig. 1.

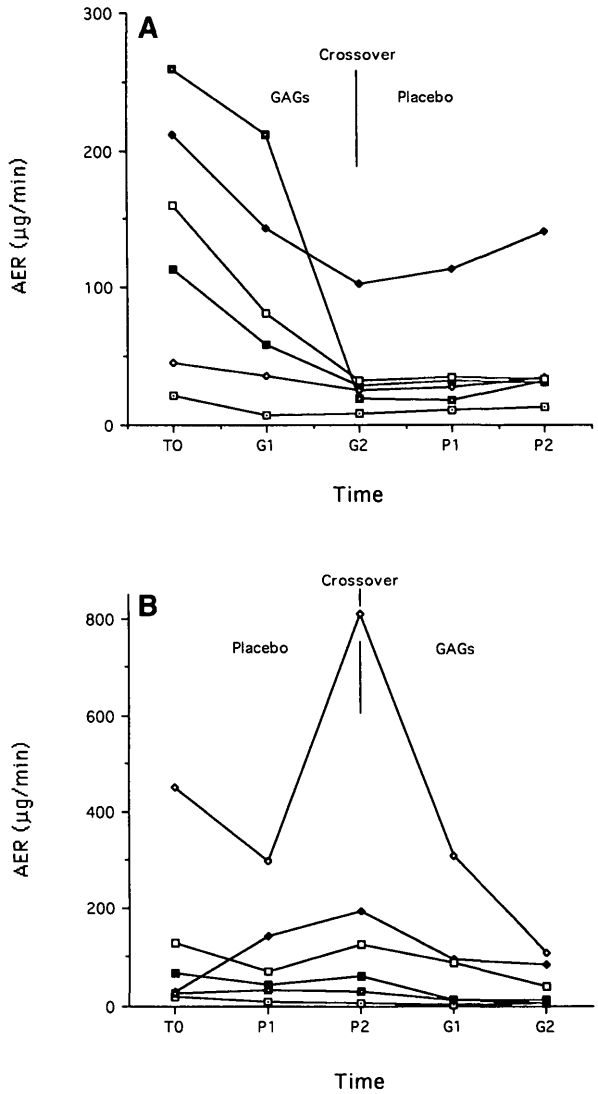

Figure 1—*AER (μg/min) in the six NIDDM patients who started the study with glycosaminoglycans* (A) *and in those who started with placebo* (B) *during the 8 months of the protocol.*

In addition to the beneficial effect on albumin excretion, we observed a relevant trend to an improvement of systolic blood pressure values during GAG treatment with respect to the placebo period (Fig. 2) in absence of any variation of the antihypertensive therapy during the study.

AER was then reevaluated in all subjects 3 months after the end of the study; we observed a persistent reduction of its values with respect to baseline (50.78 ± 9.31 μg/min), without rebound effects in any patient.

CONCLUSIONS — Proteinuria in diabetic nephropathy may arise primarily from disturbance of the charge barrier of the GBM (17,18); persistent decrease in HSPG should thus be a cause of structural abnormality of the extracellular matrix of the GBM, likely inducing irregularity of its meshwork structure, with detachment of endothelial cells and podocytes; an increased urinary albumin excretion may indeed result from both charge barrier disturbance and additional

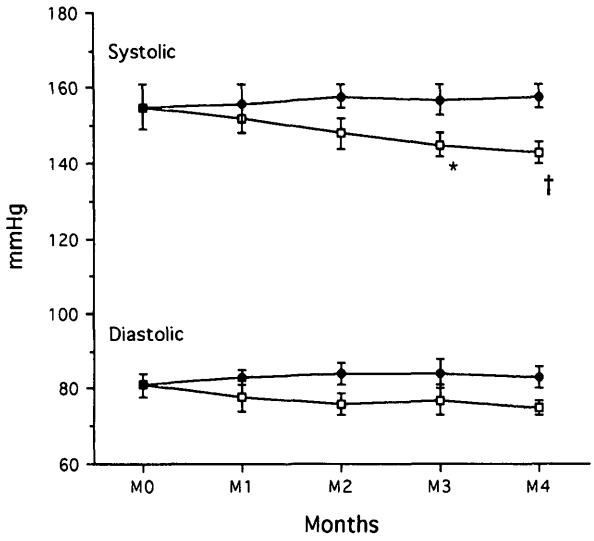

Figure 2—*Blood pressure values during placebo (●) and GAG administration (□) in all NIDDM patients. *P = 0.025 with respect to placebo; †P = 0.0019 with respect to placebo.*

damage of the size barrier because of structural change in the GBM.

Previous studies have reported that in streptozotocin-induced diabetic rats, chronic administration of GAG from the onset of diabetes is able to prevent some of the morphological and physiological alterations that occur in experimental diabetic nephropathy (11). Furthermore, GAG prevents the onset of albuminuria in experimental diabetic nephropathy without affecting GFR and metabolic control of the disease (11,19,20). The mechanism of GAG activity on diabetic nephropathy is probably complex and is still not clear. GAG may act through anticoagulant properties, hemodynamic effects, downregulation of several proteases, a putative role on nonenzymatic glycation, and mechanical restoration of glomerular charge; an antiproliferative effect of GAG has also been described (21,22).

To our knowledge, our study is the first demonstrating, with a double-blind placebo-controlled design, a reduction of albumin excretion rate in both micro- and macroalbuminuric NIDDM patients treated with long-term therapy with GAG. Interestingly, different from previous reports (23), our data do not show any rebound of AER to baseline values, at least in a short-term observation. It is important to outline that our patients were treated with a combination of iduronylglycosaminoglycan sulfate and dermatan sulfate; dermatan sulfate is located primarily within the mesangium and is decreased in glomeruli of diabetic rats (10). There is experimental evidence showing that dermatan sulfate per se is able to decrease capillary permeability to plasma proteins (24); an ability to prevent GBM thickening, glomerular anionic charge reduction, and the onset of albuminuria in diabetic rats has also been demonstrated (11). We may suppose that this compound could play a role in determining a prolonged reduction in albumin excretion in NIDDM.

Some studies have previously shown that improved metabolic control can prevent renal lesions in diabetic rats (25,26). Evidence suggests that poor blood sugar regulation plays an important role in the development of albuminuria (27), and several studies, including the Diabetes Control and Complications Trial (DCCT), have recently shown that a strict long-term metabolic control may reduce the progression of diabetic nephropathy as well as other diabetes complications (28,29). Nevertheless, the correlation between metabolic control and the development of persistent albuminuria is weak (27), and impaired metabolic control seems to be a necessary, but not sufficient, condition to cause the progression of albuminuria. In our study, neither plasma glucose nor glycated hemoglobin showed any significant variation during both placebo and drug treatment; furthermore, daily insulin dose was the same throughout the entire study period. We did not observe any correlation between AER and indexes of metabolic control; however, we agree that a trend toward an improved metabolic control during the study could have positively influenced our results.

We have no data regarding the nonenzymatic glycation of GBM proteins, but, considering that it is somehow mirrored by hemoglobin glycation, we believe that the positive effect exerted by GAG does not depend on an effect on protein glycation because of the already described absence of relevant variations in glycated hemoglobin values.

It has also been reported that the progression of diffuse diabetic glomerular lesion is closely related to an increase in fibronectin accumulation in GBM and mesangial matrix (30) and that heparan administration may suppress its urinary excretion (31). In addition, heparan has an indirectly inhibitory effect on renal kallikrein production, which may lead to decreased production of renin, followed by a fall in blood pressure (31) and by a reduction in glomerular hyperfiltration (32). In our study, we have observed a statistically significant trend toward a reduction of blood pressure values during GAG administration compared with placebo administration, in absence of any variation of antihypertensive therapy. These data confirm that heparan-like substances might play a role in normalizing the altered hemodynamics in diabetic patients with impaired kidney function.

Our study also confirms previous reports (33–35) showing that GAG may be able to reduce fibrinogen plasma levels in diabetic subjects, strongly contributing to control this cardiovascular risk factor. It is known that sulodexide, containing a fast-heparan and dermatan sulfate, can modulate the coagulation and fibrinolytic system working by inhibiting both factor Xa and thrombin. The fibrinogen plasma levels reduction may be ascribed to an antithrombotic effect more than to a direct influence on fibrinogen synthesis.

In conclusion, combined therapy with GAG and ACE inhibitors, possibly restoring GBM charge and size selectivity to albumin molecules, as well as reducing glomerular capillary pressure, may be useful in preventing progression from incipient to overt nephropathy in NIDDM. Further studies should confirm our observations and clarify the putative mechanisms underlying the effect of these compounds on kidney function in vivo.

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