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Effect of enalapril on urinary glycosaminoglycan, heparan sulphate and microalbuminuria in type II diabetic patients

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Recent studies in diabetic humans have shown that angiotensin converting enzyme (ACE) inhibitors may provide additional renal benefit above and beyond conventional antihypertensive agents. We investigated the effect of enalapril on urinary glycosaminoglycans (GAG), heparan sulphate (HS) and microalbuminuria (MAU) in diabetic patients. Urinary GAG and HS levels were determined in controls ($n = 16$, 41.3 ± 12.9 years old) and in type II diabetics ($n = 18$, 53 ± 9.6 years old) who were not using ACE inhibitors. Four of these patients had also hypertension. The duration of diabetes was 5.5 ± 3 years (mean ± SD). Microalbuminuria was detected in seven patients. The subjects were treated with enalapril (5–10 mg day^{-1}) for 6 weeks. The median values of GAG ($n = 18$, 2.8 mg uronic acid g^{-1} crea. day^{-1}) and HS ($n = 18$, 1.36 mg glycosamine g^{-1} crea. day^{-1}) in the pre-treatment group were significantly ($p < 0.01$) higher than the control group ($n = 16$, 1.98 mg uronic acid g^{-1} crea. day^{-1} and 0.87 mg glycosamine g^{-1} crea. day^{-1}, respectively. Before treatment, GAG and HS levels seemed to be not different between microalbuminuric and normoalbuminuric as well as hypertensive and normotensive patients. Following enalapril treatment, the median values of GAG ($n = 18$, 1.35 mg uronic acid g^{-1} crea. day^{-1}) and HS ($n = 18$, 0.99 mg glycosamine g^{-1} crea. day^{-1}) tended to decrease to the levels which were not significantly different from the control group. Following treatment, significant reduction in urinary albumin excretion (from 15.45 to 11.1 mg day^{-1}) ($p < 0.0005$) was also observed. When considering the pre- and post-treatment concentrations, there were positive correlations between urinary GAG and HS values ($p < 0.05$, $r = 0.6541$ and $p < 0.01$, $r = 0.5984$). These results suggest that measurement of urinary heparan sulphate may be another useful predictor of clinical diabetic nephropathy; and enalapril causes marked reduction in HS and GAG values in all patients independently by the presence of hypertension.

Keywords: heparan sulphate, glycosaminoglycan, microalbuminuria, diabetic nephropathy.

Introduction

Kidney disease is a significant problem in the diabetic population. Since diabetic nephropathy is the lethal microvascular complication in diabetics, it is the second leading cause of death in diabetic patients (Maher 1992). More than one-quarter of all cases of end-stage renal disease (ESRD) in the United States are the results of diabetes mellitus (DM). Among type II diabetics, the prevalence of diabetic nephropathy is not known but is estimated to be about 5–10 %. Despite this lower prevalence, the health care impact of renal disease caused by type II diabetes is greater, since type II diabetes is far more common (Gambaro *et al.* 1992). Several

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benefits could be derived from knowing early in the course of diabetes whether a patient is likely to develop clinical diabetic nephropathy. Determination of microalbuminuria has been suggested as an early predictor of diabetic glomerular disease (Reddi 1990, Hallab *et al.* 1993). But albumin excretion rates are altered by variations in blood pressure and exercise as well as blood glucose levels and there is an intra-individual variability during the evolution of albuminuria, and the day-to-day variation is known to amount to up to 40–50% (Schmitz *et al.* 1994). On the other hand microalbuminuria is seen in stage III diabetic nephropathy (Mogensen and Schmitz 1988). So, development of markers other than microalbuminuria are clearly warranted.

It is suggested that measurement of urinary heparan sulphate may be another useful predictor of clinical diabetic nephropathy, since it has been shown that increased urinary excretion of GAGs precedes microalbuminuria in diabetic human subjects (Reddi 1990).

Diabetic renal disease is usually accompanied by hypertension. The greatest impact on attenuating the progression of this disease appears to be adequate blood pressure control. Recent studies of animals in diabetic and/or hypertensive states suggest that the angiotensin converting enzyme (ACE) inhibitors and specific groups of calcium antagonists may provide additional renal benefit above and beyond conventional blood pressure control (Valentino *et al.*1991). One short-term trial (3 months) in normoalbuminuric patients revealed that ACE inhibitors modified renal parameters (fall in filtration fraction and albuminuria) without affecting blood pressure. Even in normotensive patients a reduction in microalbuminuria by ACE inhibitors has been demonstrated (Hallab *et al.* 1993, Capek *et al.* 1994, Lash and Bakris 1995). ACE inhibitors, have been shown to decrease proteinuria in diabetic animals and human subjects (Reddi *et al.* 1991). Since heparan sulphate proteoglycan (HSPG) confers a negative charge on the glomerular basement membrane (GBM), and either decreased synthesis or loss of this charge causes albuminuria in diabetic animals, it is possible that enalapril prevents albuminuria through glomerular preservation of heparan sulphate (Reddi 1991, Reddi *et al.* 1991,Van den Born and Berden 1995).

In the light of these aspects we observed the important components of GBM; HS and GAG to see if they are lost in urine at early stages in the progression of diabetic nephropathy and we also investigated the effect of ACE inhibitors, enalapril on urinary GAG, HS excretion and microalbuminuria.

Materials and methods

Study design

In this study, 16 (2 men and 14 women) healthy subjects aged between 25 and 67 years (mean ± SD: 41.3 ± 12.9) and who had no concurrent heart, liver, cerebral, or systemic diseases, or primary renal disorder were selected as controls. Eighteen type II volunteer diabetic outpatients (7 men and 11 women) aged between 33 and 68 years (mean ± SD: 53 ± 9.6) who were not using an ACE inhibitor were selected to enter the study and treated with enalapril (5–10 mg per day according to their blood pressure) for 6 weeks. The duration of diabetes was 5.5 ± 3.0 years (mean ± SD). At entry and then after 6 weeks of enalapril treatment, urinary GAG, HS, and microalbumin measurements of these patients were recorded, as well as their blood pressure.

Sample preparation

Non-infected urine samples were used for urinary GAG, HS, and microalbumin measurements. Urine samples without using a preservative were kept at +4 °C during the collection period. After the

determination of volume and density of the urine samples, they were centrifuged and creatinine and microalbumin levels were determined in the supernate. For the total GAG and HS measurements, the supernates were kept at –20 ° C until being processed. The specific gravity of the urine specimens were corrected to be below 1.020. Completeness of the urinary collections was confirmed by determining creatinine levels in each urine specimen.

Isolation of urinary GAGs

Urinary GAGs were precipitated with 5% cethyl trimethylammonium bromide (CTAB) according to Teller *et al.* (1962). A 1 ml sample of CTAB solution was added to 15–30 ml of urine after pH adjustment by 2 N HCl. Following refrigeration for one night at 2 °C, the precipitate was obtained by centrifugation at 2500 rpm for 20 min. The supernate was decanted and the precipitate was washed three times with 30 ml of 95 % ethanol saturated with NaCl in order to dissociate the CTAB from the GAGs and to remove other organic contaminants. The material that remained insoluble in ethanol was redissolved in 5 ml distilled water and separated from a small amount of insoluble residue by centrifugation; 4 ml of supernate was then separated for the determination of heparan sulphate.

Colour reaction for the determination of GAGs

Uronic acid is widely determined as one of the representative components of repeating unit of glycosaminoglycans in biological substances. This method is based upon the appearence of a chromogen when uronic acid is heated up to 100 °C in concentrated sulphuric acid/tetraborate complex treated with *meta*-hydroxydiphenyl (Blumenkrantz and Asboe-Hanson 1973). Absorbance measurements were made at 520 nm in a LKB-Biochrom Ultraspec II spectrophotometer and compared by the D-glucuronic acid reference solution and the results were expressed as micrograms of uronic acid in 200 ml of urine.

Isolation and determination of heparan sulphate (Smith and Gilkerson 1979)

The separated 4 ml supernate was passed through a 1 × 4 cm column of Dowex 50x2, H^+ form, packed and eluted with 5 ml distilled water. The acidic effluent was immediately neutralized with 0.5 N NaOH and lyophilized. After the lyophilization procedure, it was redissolved in 0.5 ml distilled water and 0.5 ml 1 N HCl was added. 200 µl of the mixture was placed in a small glass test tube with a teflon lined cap and heated at 110 °C for 2 h. Then the samples were cooled to room temperature in a water bath. A 400 µl sample of 2.5 % $NaNO_2$ was added, vortexed and allowed to stand at room temperature for 15 min, 200 µl of 12.5% ammonium sulphamate was then added, vortexed and allowed to stand at room temperature for 5 min. After liberation of the excess $NaNO_2$, 200 µl of 0.25 % MBTH (3- methyl-2-benzothiazolone hydrazone hydrochloride) was added, vortexed, and incubated at 37 °C for 30 min. A 200 µl sample of 0.5 % $FeCl_3$ was added and incubated at 37 °C for an additional 5 min. The samples were cooled to room temperature. The optical density was determined at 650 nm. The results were evaluated as µg glycosamine per 100 µl from a standard graphic by using glycosamine HCl.

Urinary creatinine measurements were made by the non-deproteinized kinetic Jaffé method. Urinary microalbumin measurements were made in a Technicon Dax Otoanalyser by using Boehringer Mannheim reagent, catalogue number 1203622. Systolic and diastolic blood pressures (SBP; DBP) were recorded with a mercury sphygmomanometer and are expressed as the mean of three determinations recorded in the supine position at 10 min intervals.

Statistics

Non-parametric statistics were used to evaluate the results. Post-hoc analysis of one way ANOVA for independent samples and Wilcoxon-Matched Pairs-Signed-Rank tests were used for comparison as appropriate.

Results

The data concerning urinary excretion of total GAGs, HS and microalbumin by type II diabetic patients are summarized in table 1. The statistical significance of the difference in blood pressures and urinary GAG and HS excretions between control, pre-treatment, post-treatment patient groups which was defined by 'One way Anova' and 'Student Newman–Keuls' test was one of the multiple-comparison procedures summarized in table 2. There is a significant difference in both systolic and diastolic blood pressures between control–pre-treatment and control–pre-

Table 1. Clinical data of the diabetic patients before and after enalapril treatment (Values are expressed as median , minimum and maximum).

Patient No	Sex	Age	SBP1	SBP2	DBP1	DBP2	MAU1	MAU2	GAG1	GAG2	HS1	HS2
1	M	62	120	120	70	65	337	270	4.2	1.9	1.3	1.1
2	F	62	140	140	90	90	4.3	30.2	8.0	1.1	2.6	0.5
3	F	48	180	135	100	90	10	8.5	5.1	3.1	2.6	1.8
4	F	40	120	110	80	80	7.5	3.6	3.2	1.9	2.4	1.6
5	M	66	135	130	85	80	7.8	5.9	4.9	1.1	3.2	0.6
6	F	43	150	135	80	80	9.4	8.2	3.6	3.1	0.9	2.8
7	F	52	130	135	70	70	14.3	12.0	2.4	2.2	1.7	1.7
8	F	33	110	130	80	70	58.9	3.2	3.9	1.0	3.1	0.9
9	F	56	110	140	80	100	16.6	9.6	1.9	0.9	0.9	0.8
10	M	59	100	130	80	85	5.0	3.4	1.7	0.6	1.2	0.5
11	F	47	150	120	100	80	21.4	12.6	1.6	0.4	0.8	0.3
12	F	46	140	125	95	80	228.9	274	2.3	1.3	1.4	1.2
13	M	56	100	140	60	95	7.2	57.3	1.33	0.6	0.9	0.5
14	M	62	140	140	80	80	6.6	8.9	1.4	1.4	1.0	1.1
15	F	60	170	140	90	85	37.6	31.3	3.3	1.4	1.6	0.5
16	M	48	135	135	90	85	34.9	10.2	1.4	0.9	0.4	0.4
17	F	50	120	135	80	85	32.6	18.2	1.5	1.9	1.3	1.5
18	M	68	170	150	90	85	423	340	8.9	5.7	2.1	1.2
Median		54	135	135	80	82.5	15.45	11.1	2.8	1.3	1.3	0.9
Min.		33	100	110	60	65	4.3	3.1	1.3	0.3	0.4	0.3
Max.		68	180	150	100	100	423.0	340	8.9	5.7	3.1	2.8

MAU1: Microalbuminuria levels (mg day^{-1}) before enalapril therapy.
MAU2: Microalbuminuria levels (mg day^{-1}) after enalapril therapy.
GAG1: Urinary glycosaminoglycan levels (mg g^{-1} crea. day^{-1}) before enalapril therapy.
GAG2: Urinary glycosaminoglycan levels (mg g^{-1} crea. day^{-1}) after enalapril therapy.
HS1: Urinary heparan sulphate levels (mg g^{-1} crea. day^{-1}) before enalapril tharapy.
HS2: Urinary heparan sulphate levels (mg g^{-1} crea. day^{-1}) after enalapril tharapy.
SBP1, DBP1: Systolic, diastolic blood pressures (mmHg) before enalapril therapy respectively.
SBP2, DBP2: Systolic, diastolic blood pressures before enalapril therapy respectively.

Table 2. Comparison of patient data in study groups (Values are expressed as median, min.-max.)

	Control group (n=16)	Pre-treatment group (n=18)	Post-treatment group (n=18)	Statistical significance
Systolic Blood Pressure (mmHg)	120*,** min. 90–max. 140	135** min. 100–max. 180	135* min. 110–max. 150	$P < 0.05$
Diastolic Blood Pressure (mmHg)	70*,** min. 60–max. 85	80** min. 60–max. 70	82.5* min. 65–max. 100	$P < 0.001$
Urinary GAG (mg g^{-1} crea. day^{-1})	1.98* min. 0.65–max. 3.10	2.8*,** min. 1.33–max. 8.90	1.35*,** min. 0.38–max. 5.70	$P < 0.005$
Urinary HS (mg g^{-1} crea. day^{-1})	0.87 min. 0.21–max. 1.50	1.36*,** min. 0.42–max. 2.80	0.99*,** min. 0.32–max. 2.80	$P < 0.01$

The statistical significance of the difference in blood pressures and urinary GAG and HS excretions between control, pre-treatment, post-treatment patient groups was found by using 'One way Anova' and 'Student Newman–Keuls' test.
* Between control and post-treatment groups, ** Between control and pretreatment groups.

treatment groups ($p < 0.05$). However, there is no significant difference between pre and post-treatment patient groups. Significant increased excretions of total GAGs ($p < 0.005$) and HS ($p < 0.01$) are also seen in pre-treatment group when compared with the control and the post-treatment groups. However, there is no significant

difference between control and pre-treatment group. The statistical significance of the difference in blood pressures and urinary GAG, HS and microalbumin excretions between pre- and post-treatment patient groups revealed by Wilcoxon-Matched-Pairs Signed-Ranks test are shown in table 3. Blood pressure levels seem to be not significantly different between pre-treatment and post-treatment patient groups. However, there is significant decreased excretions of microalbumin ($p < 0.001$), total GAGs ($p < 0.0005$) and HS ($p < 0.05$) by post-treatment patients when compared to the pre-treatment group (table 3, figure 1). Table 4 indicates that for the urinary GAG, HS and microalbumin excretions there are no significant differences between hypertensive and normotensive patients before and after the enalapril treatment. Table 5 shows that urinary GAG and HS excretions are not significantly different between microalbuminuric and normoalbuminuric patients before and after the enalapril treatment. Urinary GAG and HS concentrations

Table 3. Comparison of patient data between pre and post-treatment groups (Values are expressed as median).

	Pre-treatment group (n=18)	Post-treatment group (n=18)	Statistical significance
Microalbuminuria (mg day^{-1})	15.45	11.10	$P < 0.001$
Systolic Blood Pressure (mmHg)	135	135	$P > 0.5$
Diastolic Blood Pressure (mmHg)	80.0	82.5	$P > 0.5$
Urinary glycosaminoglycan (mg g^{-1} crea. day^{-1})	2.80	1.35	$P < 0.0005$
Urinary heparan sulphate (mg g^{-1} crea. day^{-1})	1.36	0.99	$P < 0.05$

The statistical significance of the difference in blood pressures, urinary glycosaminoglycan, heparan sulphate and microalbumin excretions between pre-treatment and post-treatment patient groups were revealed by Wilcoxon-Matched-Pairs Signet-Ranks test.

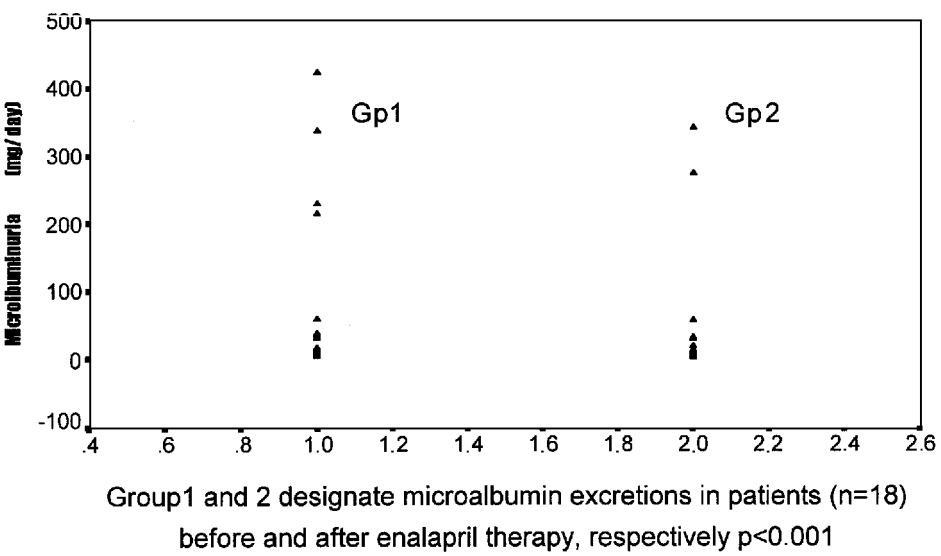

Figure 1. The effect of enalapril therapy on microalbumin excretions in type II diabetic patients. Group 1 designates the patient group before enalapril therapy. Group 2 designates the same patient group following 6 weeks of enalapril therapy at a dosage of 5–10 mg per day.

Table 4. Urinary glcosaminoglycan (GAG), heparan sulphate (HS) and microalbuminuria (MAU) levels (median) in hypertensive and normotensive patients before and after enalapril treatment.

	Hypertensive patients (n=4)	Normotensive patients (n=14)
GAG1	3.70 min. 1.6–max. 8.9	2.80 min. 1.33–max. 8.0
GAG2	2.20 min. 0.40–max. 5.70	1.25 min. 0.60–max. 3.10
HS1	1.75 min. 0.80–max. 2.60	1.30 min. 0.40–max. 3.20
HS2	1.20 min. 0.30–max. 1.80	0.85 min. 0.40–max. 2.80
MAU1	125.15 min. 10–max. 423	11.85 min. 4.30–max. 337
MAU2	143.3 min. 8.50–max. 340	9.90 min. 3.20–max. 270

GAG1: Pre-treatment GAG (mg uronic acid g^{-1} crea. per 24 h).
GAG2: Post-treatment GAG (mg uronic acid g^{-1} crea. per 24 h).
HS1: Pre-treatment HS (mg glycosamine g^{-1} crea. per 24 h).
HS2: Post-treatment HS (mg glycosamine g^{-1} crea. per 24 h).
MAU1: Pre-treatment microalbuminuria (mg per 24h).
MAU2: Post-treatment microalbuminuria (mg per 24h).

Table 5. Urinary glycosaminoglycan (GAG) and heparan sulphate (HS) concentrations (median) in microalbuminuric (30–300 mg/24h) and normoalbuminuric diabetic patients before and after enalapril therapy.

	Microalbuminuric (n=7)	Normoalbuminuric (n=11)
GAG1	3.30	2.40
GAG2	1.30	1.40
HS1	1.40	1.30
HS2	0.90	1.10

GAG1: Pretreatment GAG (mg uronic acid g^{-1} crea. per 24 h).
GAG2: Posttreatment GAG (mg uronic acid g^{-1} crea. per 24 h).
HS1: Pretreatment HS (mg glycosamine g^{-1} crea. per 24 h).
HS2: Posttreatment HS (mg glycosamine g^{-1} crea. per 24 h).

were positively correlated before ($p < 0.05$, $r = 0.654$) and after ($p < 0.01$, $r = 0.598$) enalapril treatment. A positive correlation was also observed between urinary GAG and microalbumin concentrations in the post-treatment diabetic patients ($p < 0.05$, $r = 0.573$).

Discussion

Persistent microalbuminuria predicts diabetic nephropathy and is thought to be an early sign of kidney disease (Reddi 1990, Hallab *et al.* 1993). Albumin excretion rates are altered by variations in blood pressure and exercise as well as blood glucose levels (Reddi 1990). Microalbuminuria is not specific for diabetes or early nephropathy alone but is considered to reflect generalized vascular damage (Konen and Shihabi 1993). As noted by Mogensen, the presence of microalbuminuria is indicative of stage III diabetes (Mogensen and Schmitz 1988). This suggests that

microalbuminuria is a late manifestation in the course of diabetic nephropathy, therefore development of markers other than microalbuminuria are clearly warranted.

Both biochemical and histological studies have shown that proteinuria is associated with decreased GBM polyanion concentration in diabetic and nondiabetic renal diseases (Reddi 1990, Van den Born 1997). The polyanion content is largely heparan sulphate proteoglycan (HSPG). Decreased synthesis and content of HSPG has been reported in diabetic animals and human subjects (Reddi 1990, Jensen 1997, Karasawa 1997).

On the other hand, ACE inhibitors have been shown to control blood pressure adequately and decrease proteinuria in patients with overt diabetic nephropathy (Bakris 1990, Konen 1993, Pavel 1993, Capek *et al.* 1994, Carella *et al.*). There are some papers indicating that ACE inhibitors preserve renal function to a greater extent than other antihypertensive agents by the presence of a multifactorial effect of these drugs (Bakris 1990, Hermans *et al.* 1992, Viberti *et al.* 1994, McCarty 1998). Although urinary GAG excretion changes with age, studies have shown that under normal conditions, after the age of 21, urinary GAG content does not change markedly (Rosenberg and Agostini 1992). In this study, we also found out that urinary GAG and HS contents do not change with age (table 1).

Increased excretions of total GAGs and HS have been reported in type I and type II diabetic patients (Reddi 1990).

Since urinary GAG levels correlated with urinary protein concentration, it is possible that the glomerular basement membrane is leaky for these substances. With time, this may result in total loss of anionic charge on the basement membrane, thus causing heavy proteinuria .

It has been proposed that the loss of HS in the urine is related to the extent of structural damage of the glomeruli (Mitsuhashi *et al.* 1993).

In-vivo studies revealed that removal of HS from the GBM resulted in albuminuria. It has been shown that removal of HS from the GBM of rats by means of heparinase treatment caused an increased permeability to ^{125}I-albumin (Reddi 1991, Garikiparthy *et al.* 1993). Intravenous injection of monoclonal antibodies directed against the HS moiety produced albuminuria in rats (Olgemöller and Schleicher 1993).

Bonavita found the urinary total GAG excretion in 22–63-years-old type II diabetic females to be 0.52–6.7 (2.7 ± 2.51) mg hexuronate per 24 h; and in 33–58-years-old type II diabetic males it was 0.92–4.66 (1.75 ± 1.06) mg hexuronate per 24 h (Bonavita *et al.* 1984).

In our study there was a significantly increased excretion of total GAGs ($p < 0.005$) by the pre-treatment group as compared with the controls (2.8 and 1.98 mg uronic acid g^{-1}crea. per 24 h, respectively) ($p < 0.005$) Urinary HS excretion by the pre-treatment group was also significantly increased as compared with the controls (1.36 and 0.87 mg glycosamine g^{-1} crea. per 24 h, respectively) ($p < 0.01$) (table 2).

Urinary HS measurement may be another useful predictor of clinical diabetic nephropathy, since it has been shown that increased urinary excretion of GAGs precedes microalbuminuria in diabetic human subjects (Reddi 1990).

High level of urinary excretion of GAGs is an early indicator of GBM involvement in diabetic patients with normoalbuminuria (Reddi 1990). However, in our study we did not find any statistically significant difference in urinary GAG

and HS concentrations between microalbuminuric and normoalbuminuric patients (table 5).

Almost equal proportions of both hypertensive and non-hypertensive subjects had raised albumin levels. This suggests that the high level of albumin was unlikely to be due to hypertension (Koh *et al.* 1993). In our study, urinary microalbumin excretions were not different between hypertensive and normotensive diabetic patients before and after enalapril therapy (table 4). Aggressive antihypertensive treatment has been shown to decrease urinary albumin excretion and slow the decline in GFR in patients with incipient nephropathy (Carella *et al.* 1994). In patients with diabetes, preferred antihypertensive agents are the α-antagonists, calcium-channel blockers, and the angiotensin converting enzyme (ACE) inhibitors (Pavel 1993, Carella 1994). Besides blocking the effect of the generation of angiotensin II, ACE inhibitors also reduce microalbuminuria. The mechanism for this effect is through a reduction in the shunting of protein through pores in the glomerular capillary membrane and restoration of the negative charge to the membrane. How this latter event occurs is not known. Thus, the protective effect of ACE inhibition is not due to a single factor (Lash and Bakris 1995). ACE inhibitors may improve proteinuria through other mechanisms, e.g. by reducing GBM thickening or by reducing glomerular pore size (Reddi 1991, Hallab *et al.* 1993). Since heparan sulphate proteoglycan (HSPG) confers a negative charge on the glomerular basement membrane (GBM), and loss of this charge causes albuminuria in diabetic animals and humans, it is possible that enalapril prevents albuminuria through glomerular preservation of heparan sulphate (Reddi 1991, Reddi *et al.* 1991, Van den Born and Berden 1995, Van den Born *et al.* 1997).

In our study we found a significant decrease in urinary GAG and HS concentrations after 6 weeks of enalapril treatment compared with the pre-treatment concentrations. The GAG median values decreased from 2.8 to 1.35 mg uronic acid g^{-1} crea.day^{-1} ($p < 0.0005$) (table 4, figure 1) and HS median values decreased from 1.36 to 0.99 mg glycosamine g^{-1}crea. day^{-1} ($p < 0.05$) (table 3).

We also observed that there was a significant difference in both systolic and diastolic blood pressures between control–pre-treatment and between control–post-treatment groups ($p < 0.05$) (table 2). However, there was no significant difference between pre-treatment and pre-treatment patient groups. These results suggests that enalapril reduced urinary GAG, HS and microalbumin excretions without affecting blood pressures.

Conclusion

Our data suggest that diabetes causes an increase in urinary excretions of GAGs, HS and microalbumin. In our data, enalapril treatment caused a marked decrease in urinary HS and GAG excretion both in hypertensive and normotensive as well as microalbuminuric and normoalbuminuric diabetic patients. This finding suggests that enalapril therapy improves albuminuria through preservation of glomerular HS and prevention of its urinary loss.

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