Proteinuria-Lowering Effect of Heparin Therapy in Diabetic Nephropathy without Affecting the Renin-Angiotensin-Aldosterone System

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Angiotensin-converting enzyme inhibitors and angiotensin II (AngII) type 1 receptor blockers lower proteinuria and preserve renal function in diabetic nephropathy (DN). The antiproteinuric effects are greater than their blood pressure reduction, involving the sieving properties of the glomerular filter. In DN, glomerular staining for heparan sulfate proteoglycans is decreased. AngII inhibits heparan sulfate synthesis. Also, heparins modulate AngII signaling in glomerular cells, inhibiting aldosterone synthesis and lowering proteinuria in DN. Is the antiproteinuric effect of heparins due to its interference with the renin-angiotensin-aldosterone system? Ten volunteers each with DN and glomerulonephritis and control subjects were examined before and after low-dosage enoxaparin. Renal hemodynamics were determined with $^{99m}$Tc-DTPA and $^{131}$I-hippurate clearance. Glomerular filtration rate (GFR), effective renal plasma flow, mean arterial pressure, and heart rate were measured at baseline and during AngII infusion before and after enoxaparin while on normal salt and salt restriction. Enoxaparin did not lower aldosterone levels. GFR remained stable in all groups. AngII caused a significant decrease in effective renal plasma flow, whereas mean arterial pressure and heart rate increased significantly. Enoxaparin did not influence the AngII-induced changes of renal hemodynamics during normal salt intake or salt restriction. All groups showed identical responses to AngII before and after enoxaparin. In patients with diabetes, enoxaparin caused a significant decrease in proteinuria.

It is concluded that the antiproteinuric effect of heparins in DN cannot be explained via interaction with the renin-angiotensin-aldosterone system. The absence of hemodynamic changes combined with reduced proteinuria point to intrinsic alterations in the glomerular filter. The effects were seen only in DN, not in glomerulonephritis.


Proteinuria is a hallmark of diabetic nephropathy (DN) (1). It strongly affects renal prognosis (2) and is the most powerful predictor of associated cardiovascular disease (3). The renin-angiotensin-aldosterone system (RAAS) is a key mediator of renal damage in DN. Interference with the RAAS both by angiotensin-converting enzyme (ACE) inhibitors (4) and angiotensin II (AngII) type 1 receptor (AT1R) blockers (5,6) decreases proteinuria and retards progression to ESRD in patients with DN that is caused by type 1 and 2 diabetes.

AngII increases glomerular capillary pressure predominantly at the efferent arterioles, which is believed to be pivotal in the progression of renal damage (7). AngII stimulates extracellular matrix protein synthesis through potent induction of TGF-β, a suspected key promoter of glomerulosclerosis (8). The nonhemodynamic effects of AngII also include the induction of proinflammatory cytokines (9,10). Recent studies suggested that AngII can induce oxidative stress, resulting in the generation of reactive oxygen species, such as superoxide, and, via the generation of hydrogen peroxide, hydroxyl radicals (11).

Besides these potentially harmful hemodynamic and nonhemodynamic effects, AngII attenuates both production and sulfation of heparan sulfate proteoglycans (HSPG) in cultured human mesangial cells (12). AngII also reduces HSPG expression in human podocytes, as could be demonstrated by a recent study from our group (13). In line with these in vitro findings are in vivo data showing that the ACE inhibitor enalapril improves albuminuria by preventing glomerular loss of HS in diabetic rats (14). Therefore, the antiproteinuric effect of ACE inhibition in DN may be explained at least partially by structural effects on the glomerular basement membrane (GBM); i.e., qualitative and quantitative preservation of HSPG improving glomerular permselectivity.

Not only RAAS-intervention strategies but also heparins may influence DN favorably. Heparins prevent and cure experimental DN in streptozotocin-induced diabetic rats (15,16). Also in humans, several phase 2 studies showed that heparins lower proteinuria in both type 1 (17) and type 2 diabetes (18). Several hypotheses to explain the renoprotective effects of heparins have been discussed: Downregulation of proteases and TGF-β cascade, modulation of mesangial matrix synthesis and restoration of GBM anionic charges (reviewed in reference [19]).

Relatively little is known about a putative interaction be-
tween heparins and the RAAS. Heparins are known to inhibit aldosterone production (20). In 1990, Zaragoza et al. (21) demonstrated that heparins suppress AngII-stimulated intracellular calcium mobilization in vascular smooth muscle cells. Another in vitro study from our group showed that heparins modulate Ca\(^{2+}\)-dependent AngII signaling in human mesangial cells (22).

Because heparins are renoprotective in DN and the RAAS is a key mediator in its pathogenesis, we wanted to clarify whether there is an interaction between heparins and the RAAS in vivo. Therefore, we studied the influence of heparin therapy on aldosterone levels, on the acute AngII-induced systemic and renal hemodynamic effects, and on proteinuria in patients with DN and nondiabetic renal disease.

**Materials and Methods**

**Patients**

A total of 30 men and women were recruited for the study. Patients with hypertensive BP values >160/90 mmHg at rest, instable coronary heart disease, or diabetes with poor glycemic control (glycosylated hemoglobin >8.5%) were excluded from the study. Pregnancy and age <18 or >80 yr were additional exclusion criteria. The participants were studied in three groups and admitted sequentially.

**Group 1.** Group 1 consisted of 10 patients with diabetes (three of 10 type 1, seven of 10 type 2) and DN. Diagnosis of DN was based on the presence of macroalbuminuria or overt proteinuria (confirmed by three consecutive 24-h urine collections) together with nonproliferative diabetic retinopathy. They ranged in age from 43 to 61 yr (55.2 ± 5.55). The duration of diabetes ranged from 8 to 21 yr (15.1 ± 4.65). Age of onset of diabetes ranged from 19 to 52 yr (38.5 ± 9.40). Body mass index (BMI) ranged from 21 to 41 kg/m\(^2\) (30.1 ± 5.61). They had acceptable metabolic control by the inclusion criterion of glycosylated hemoglobin <8.5% (range 5.30 to 8.50; 7.34 ± 1.07). Serum creatinine ranged from 0.81 to 2.78 mg/dl (1.42 ± 0.68), reflecting renal function that ranged from normal to moderately impaired. Arterial hypertension had been diagnosed in all patients previously. All of them were on long-term ACE inhibitor treatment for at least 6 mo, except for one patient who was on stable ACE inhibition therapy for exactly 8 wk before the study. Mean systolic office cuff BP was 153 ± 15 mmHg. Mean diastolic office cuff BP was 77 ± 6.80 mmHg. None had instable cardiovascular disease.

**Group 2.** Group 2 consisted of 10 patients without diabetes, seven with biopsy-confirmed IgA glomerulonephritis (IgA-GN) and three with membranous GN. They ranged in age from 28 to 71 yr (45.6 ± 11.7). BMI ranged from 19 to 39 kg/m\(^2\) (28.1 ± 6.11). Serum creatinine ranged from 0.73 to 2.39 mg/dl (1.33 ± 0.55), reflecting renal function that ranged from normal to moderately impaired. There were six of 10 in whom arterial hypertension had been diagnosed previously. All of them were on stable ACE inhibitor treatment for at least 8 wk. Mean systolic office cuff BP was 126 ± 16.8 mmHg. Mean diastolic office cuff BP was 77 ± 7.29 mmHg. None of them had instable cardiovascular disease.

**Group 3.** Group 3 consisted of 10 healthy control subjects. They ranged in age from 27 to 66 yr (49.8 ± 10.3). BMI ranged from 20 to 34 kg/m\(^2\) (25.5 ± 3.75). Serum creatinine ranged from 0.66 to 1.07 mg/dl (0.89 ± 0.16), reflecting normal renal function. Arterial hypertension had been diagnosed previously in three of 10 patients; these individuals were on stable \(\beta\) blocker treatment for at least 8 wk, and none of them was on ACE inhibitor treatment. Mean systolic office cuff BP was 131 ± 5.59 mmHg. Mean diastolic office cuff BP was 82 ± 6.24 mmHg. All participants gave written informed consent for the study before enrollment.

**Protocol**

The protocol was approved by the local medical ethics committee. We performed a prospective, controlled, nonrandomized trial with the three study populations and a total study duration of 11 wk. The trial is illustrated in Figure 1. In the treatment period, we used enoxaparin (Clexane; Aventis Pharma, Berlin, Germany) as low molecular weight heparin (LMWH) in a dosage of 4000 IU/d subcutaneously. Blood samples were drawn 5 and 10 d after beginning of the treatment period to exclude LMWH-induced hyperkalemia and low platelet count and to check compliance to therapy by determination of anti-factor Xa activity. Normal salt diet was defined as liberal sodium intake of 200 mmol/d. Salt restriction was defined as low salt intake of 50 mmol/d. All participants were instructed by an experienced dietician. Compliance to the diet was checked twice 5 and 3 d before each study day by 24-h urine collections to ensure inclusion only when dietary compliance was good.

On study days, participants were advised to drink approximately 700 ml of liquids before and 400 ml during the examination. At 8 a.m., they emptied their bladders and then rested in a supine position in a quiet room. After completion of the baseline clearance period of 50 min, AngII (Clinalfa AG, Switzerland) was administered intravenously in a dosage of 8 \(\mu\)g/kg per min for 60 min. This dosage is known to cause a moderate systemic and renal hemodynamic pressure response, as described previously (23). This was followed by a 20-min recovery period. Mean arterial pressure (MAP) and heart rate (HR) were recorded during examination by an automatic recording device (Dinamap; Critikon, Tampa, FL) at 5-min intervals.

**Measurements of Renal Hemodynamics**

Using a single-compartment dual-tracer infusion clearance and the tracers \(^{99m}\)Tc-diethylentriamine penta-acetic acid (DTPA) and \(^{131}\)I-hippurate, we measured both GFR and effective renal plasma flow (ERPF) simultaneously in the supine position using the method of Pedersen et al. (24). The clearance examination of participants began 40 min after simultaneous intravenous injection of 4.4 MBq \(^{99m}\)Tc-DTPA and 4.4 MBq \(^{131}\)I-hippurate.

A superficial vein of the right arm was used for a continuous infusion clearance. Both tracers were monitored by two scintillation probes placed over the right and the left shoulder of each individual. This site is selected because of the large vessels in the probe’s field of view. Each detector monitored one of the two radioisotopes by means of energy discrimination. The signals that were monitored by the probes activated an infusion pump system via feedback control. Two pumps were used; one contained \(^{99m}\)Tc-DTPA, and the other contained \(^{131}\)I-hippurate.

**Figure 1.** Study protocol. All participants were studied on four occasions: Twice before and twice after an 8-wk treatment period on low-dosage, low molecular weight heparin (LMWH) and on normal salt diet and on salt restriction, respectively. Participants were studied after week 1 (A; normal salt, before heparin), after week 2 (B; low salt, before heparin), after week 10 (C; normal salt, after heparin), and after week 11 (D; low salt, after heparin). Scr, screening.
rate. A separate step motor drove the pumps, whereby steady-state conditions were reached. The data from the first 10 min of the clearance examination were discarded because this time was needed to equilibrate the feedback control system. After completion of the 50-min baseline clearance period, 10 ml of blood was drawn from the cubital vein of the contralateral arm to obtain a plasma sample. A probe from each pump that contained the respective isotope served as standard. A microcomputer registered the motor step rates, documented the serum activity level of each isotope in the probe’s field of view, and calculated the clearance after the activity of the standard and the serum sample had been registered using the equation:

\[ CI = \frac{N \times A_s}{A_{pl}} \]

where CI is the clearance (ml/min), N is the number of motor steps per minute, \( A_s \) is the activity pumped per motor step (Bq), and \( A_{pl} \) is specific activity of plasma (Bq/ml).

The clearance was calculated for 2-min time intervals. These separate GFR and ERPF values then were used to compute a mean clearance for any appropriate time interval studied, such as baseline (50 min), AngII infusion (60 min), and recovery (20 min).

Filtration fraction was calculated as ratio of GFR and ERPF. Renal vascular resistance was calculated as the ratio of MAP and ERPF multiplied with (1 – hematocrit) \( \times 1000 \).

Single-compartment infusion clearance measurements are highly reproducible. Phantom studies showed that clearance values varied <5% in consecutive measurements. Head-on comparison between classical clearance that was based on urine collection and infusion clearance in the same individual had shown that the measured clearance values differ on average by 8.4% and no more than 11% in individuals with a GFR >50 ml/min (25). This was reconfirmed in 1998: Clearance values (GFR) that were based on urine collection (without catheterization relying on spontaneous voiding) and single-compartment infusion clearance were compared in five individuals for 75 min. Clearance that was based on urine collection was 105 ± 6.51 ml/min, and infusion clearance 96.2 ± 10.2 ml/min, the former being slightly but consistently higher by an average of 10% (2 to 20%) (26).

**Laboratory Procedures**

Venous blood samples were taken from a catheter that was inserted into the left antecubital vein at the end of the baseline and AngII periods, respectively. Blood samples were collected on ice and centrifuged immediately at 4°C, and the samples were stored at −20°C before assay.

Active renin was measured by an immunoradiometric assay (27). Aldosterone and AngII were determined by RIA (28,29).

Proteinuria was determined from 24-h collections with the pyrogallol red-molybdate method (30). The intra-assay coefficient of variation of this method is <3.3%, and the interassay coefficient of variation is <3%.

Each value of urine protein was validated with concurrent creatinine as baseline (50 min), AngII infusion (60 min), and recovery (20 min). Statistical Analyses

Data are means ± SD, unless stated otherwise. Within-group differences were analyzed with paired t test. Between-group differences were analyzed with one-way ANOVA and Tukey multiple comparisons. For nonparametric data, Wilcoxon test for paired differences was used. Statistical significance was defined as \( P < 0.05 \). Power analysis that compared ERPF before and after AngII infusion was performed with \( \alpha = 0.05 \) and \( 1 - \beta = 90\% \) to define the sample size of each group to detect a 20% difference in the AngII-induced change of ERPF.

**Results**

Baseline characteristics of the participants are listed in Table 1. According to power analysis, a sample size of 10 patients in each group was required to meet the criteria mentioned previously.

**Hormonal Parameters**

Aldosterone levels on baseline were not significantly different between patients with diabetes, patients with GN, and healthy control subjects. AngII induced a significant rise in aldosterone levels on every occasion except in patients who had diabetes and were on low salt and heparin (measurement D). As expected, aldosterone levels were significantly higher on low salt (measurements B/D) compared with normal salt intake (measurements A/C) on every occasion in all groups. This reflects RAAS activation on salt restriction. It is interesting that heparin therapy never lowered aldosterone levels. Figure 2 depicts aldosterone levels in patients with diabetes.

Baseline active renin values were significantly higher in patients with diabetes and patients with GN compared with healthy control subjects, reflecting RAAS activation in renal disease. In all groups, active renin was significantly attenuated by AngII. As expected, active renin values were significantly higher on low salt intake compared with a normal salt diet (data not shown).

AngII levels increased on average by a factor of 950 during AngII infusion. There were no significant differences in AngII levels between any of the groups (data not shown).

**Systemic Hemodynamics**

AngII infusion was followed by a marked systemic pressor response with significant increases in MAP in all groups (for patients with diabetes, see Figure 3A). In patients with diabetes, salt restriction resulted in a BP reduction, which reached statistical significance only after heparin therapy (from 97.0 ± 5.41 to 85.5 ± 4.1 mmHg). Notably, in patients with DN, the pressor response was more pronounced (29.5 ± 9.86 versus 24.3 ± 10.5 mmHg in patients without diabetes and 24.5 ± 9.51 mmHg in control subjects), but this trend failed to reach statistical significance. However, in four patients with diabetes, dosage reductions of AngII were necessary in seven of 16 measurements to avoid increases in BP above 200/100 mmHg. In contrast, patients with GN and healthy control subjects did not require any dosage reductions. This finding suggests that patients with DN are more susceptible to the hemodynamic actions of AngII. Heparin treatment did not lower baseline BP values on any occasion.

AngII infusion induced a moderate increase in HR, reaching statistical significance in all groups (in patients with diabetes 76.6 ± 4.47 versus 70.0 ± 2.7 bpm, in patients with GN 70.8 ±
4.74 versus 66.4 ± 4.25 bpm, in control subjects 67.5 ± 2.70 versus 63.3 ± 2.34 bpm, AngII versus baseline; *P* < 0.05, respectively). Figure 3B shows HR in patients with diabetes.

### Renal Hemodynamics

Mean GFR at baseline was 83.8 ± 20.5 ml/min per 1.73 m² in patients with diabetes, 80.4 ± 21.3 ml/min per 1.73 m² in patients with GN, and 107 ± 26.9 ml/min per 1.73 m² in healthy control subjects, indicating mild impairment of renal function in renal patients with and without diabetes and normal renal function in healthy control subjects. As expected, GFR remained unchanged after AngII infusion in all groups and all measurements (for patients with diabetes, see Figure 4A).

Mean ERPF at baseline was 521 ± 110 ml/min per 1.73 m² in patients with diabetes, 564 ± 140 ml/min per 1.73 m² in patients with GN, and 583 ± 152 ml/min per 1.73 m² in control subjects. AngII caused an average 35% reduction of the ERPF in all groups (for patients with diabetes, see Figure 4B). AngII infusion induced hyperfiltration (for patients with diabetes, see Figure 4C) and a marked increase in renal vascular resistance (data not shown), as expected and described previously (31).

Heparin therapy did not alter the AngII-induced changes in renal and systemic hemodynamics during normal salt intake or during salt restriction. All groups showed identical renal hemodynamic responses to AngII before and after heparin therapy, as demonstrated for ERPF in Figure 4D.

### Proteinuria

Heparin therapy caused a significant decrease in proteinuria only in patients with DN: On normal salt intake from 961 ± 830 to 561 ± 493 mg/g creatinine per 24 h and even after salt restriction from 720 ± 709 to values of 494 ± 555 mg/g creatinine per 24 h (*P* = 0.01 by Wilcoxon test; Figure 5A). There was a trend to a decreased proteinuria on low salt compared with normal salt intake, but this trend failed to reach statistical significance.

Figure 5B denotes the individual decrease of each patient

### Table 1. Baseline characteristics

<table>
<thead>
<tr>
<th>Parameters</th>
<th>DN</th>
<th>GN</th>
<th>C</th>
<th><em>P</em> (ANOVA)</th>
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<tr>
<td>No. of patients</td>
<td>10</td>
<td>10</td>
<td>10</td>
<td></td>
</tr>
<tr>
<td>Age (yr)</td>
<td>55.2 ± 5.55^b</td>
<td>45.6 ± 11.7</td>
<td>49.8 ± 10.3</td>
<td><em>P</em> &lt; 0.05 (DN versus GN)</td>
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<td>Gender distribution (M/F)</td>
<td>7/3</td>
<td>6/4</td>
<td>6/4</td>
<td></td>
</tr>
<tr>
<td>Disease distribution</td>
<td>—</td>
<td>7 IgA-GN, 3 membranous GN</td>
<td>—</td>
<td></td>
</tr>
<tr>
<td>Body mass index (kg/m²)</td>
<td>30.1 ± 5.61^c</td>
<td>28.1 ± 6.11</td>
<td>25.5 ± 3.75</td>
<td><em>P</em> = 0.05 (DN versus C)</td>
</tr>
<tr>
<td>Duration of diabetes (yr)</td>
<td>15.1 ± 4.65</td>
<td>(21 to 41)</td>
<td>(19 to 39)</td>
<td></td>
</tr>
<tr>
<td>Age of onset of diabetes (yr)</td>
<td>38.5 ± 9.40</td>
<td>(19 to 52)</td>
<td>—</td>
<td></td>
</tr>
<tr>
<td>HbA1c (mmol/L)</td>
<td>7.34 ± 1.07</td>
<td>—</td>
<td>—</td>
<td></td>
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<tr>
<td>BP (mmHg)</td>
<td>135 ± 15.1/77 ± 6.80</td>
<td>126 ± 16.8/77 ± 7.29</td>
<td>131 ± 5.59/82 ± 6.24</td>
<td>NS</td>
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<tr>
<td>Serum sodium (mmol/L)</td>
<td>139 ± 6.47</td>
<td>(125 to 146)</td>
<td>142 ± 2.66</td>
<td>139 ± 3.17</td>
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<td>Serum potassium (mmol/L)</td>
<td>4.36 ± 0.31</td>
<td>(3.77 to 4.75)</td>
<td>4.47 ± 0.45</td>
<td>4.57 ± 0.61</td>
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<td>Serum creatinine (mg/dl)</td>
<td>1.42 ± 0.68^d</td>
<td>(0.81 to 2.78)</td>
<td>1.33 ± 0.55^d</td>
<td>(0.73 to 2.39)</td>
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<td>Proteinuria (mg/24 h)</td>
<td>800 ± 508^d</td>
<td>(194 to 1816)</td>
<td>1958 ± 1951^d</td>
<td>(259 to 6651)</td>
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</table>

^aData are means ± SD (range). C, healthy controls; DN, diabetic nephropathy; GN, nondiabetic renal disease (IgA or membranous glomerulonephritis); HbA1c, glycosylated hemoglobin.

^bSignificant differences between DN and GN.

^cSignificant differences between DN and C.

^dSignificant differences between DN/GN and C.
with DN on a normal salt diet. Note that patients with the most severe proteinuria experienced the greatest reduction in protein excretion. In striking contrast, proteinuria remained unchanged in patients with GN: On normal salt 1632 versus 1640 mg/g creatinine per 24 h and on low salt 1752 versus 2059 mg/g creatinine per 24 h, before versus after heparin, respectively.

**Discussion**

This study shows that treatment with enoxaparin effectively lowers proteinuria in DN. The antiproteinuric effect of enoxaparin persists even after lowering proteinuria by salt restriction, which is known to improve the beneficial effect of ACE inhibitor treatment (32). The treatment with enoxaparin did not lower proteinuria in the other nephropathies tested. Because patients with the most severe proteinuria had the greatest reduction in protein excretion, we postulate that patients with diabetes and overt proteinuria will experience the greatest benefit from heparin therapy. There was no evidence that enoxaparin treatment modulated proteinuria by interfering with the RAAS; aldosterone levels remained unchanged as did systemic and renal hemodynamic reactivity after AngII infusion. Enoxaparin did not lower BP values on any occasion, although BP control is an important determinant of proteinuria in treatment of DN (33). This finding is in contrast to animal models of hypertension, in which unfractionated heparin lowered BP in spontaneously hypertensive rats and Goldblatt hypertensive rats (34).

Our results confirm and extend previous findings, demonstrating an antiproteinuric effect of heparins and glycosaminoglycans (GAG) in DN (for review, see reference [19]). For enoxaparin in particular, a small, placebo-controlled crossover trial in patients with type 1 diabetes suggested that this LMWH might have antiproteinuric properties. However, the demonstrated trend to a reduced proteinuria failed to reach statistical significance as a result of the very small group of six patients, who had therapy for only 4 wk (17). Furthermore, two clinical studies in which sulodexide was used in microalbuminuric type 2 diabetes showed that this GAG formulation of fast-moving heparin and dermatansulfate that offers oral bioavailability also decreases proteinuria (35,36). It should be emphasized that a great pharmacologic gap separates heparins from GAG, because these polysaccharides have different degrees of sulfation, molecular weights, and diverse biologic activities. Nonetheless, our results suggest that these diverse compounds with heterogeneous structure have a common antiproteinuric effect in DN.

The results in DN differ from those reported by Rostoker et al. (37), who examined patients with primary GN. They found no decrease in proteinuria even in patients with nephrotic-range disease when they were treated with enoxaparin. These contrasting results in patients with GN support our hypothesis that the proteinuria-lowering effect of enoxaparin might be specific for DN (37). However, there is no evidence from clinical studies yet that heparins or GAG preserve renal function in any entity of glomerular disease. How can the results of this study be explained in the context of what is known about the interactions between proteoglycans and the RAAS?

First, there is substantial evidence that heparins may reduce aldosterone levels (20). However, the effect of heparins on aldosterone secretion may depend on their molecular weight or sulfation pattern. Most studies that described hyperkalemia
and decreased aldosterone levels after heparin treatment used unfractionated heparins. Second, heparins and GAG modulate calcium fluxes in smooth muscle cells and mesangial cells after AngII stimulation in vitro (21,22). These effects seem to depend on the sulfation pattern of the heparins used and on the sulfation pattern of the GAG side chains in the HSPG on the surface of the investigated cells (22). Our in vivo data do not support the hypothesis that the antiproteinuric effect of enoxaparin in DN is mediated through modulation of AngII signaling in intrarenal smooth muscle cells or mesangial cells. Third, AngII modulates HSPG production in cultured human podocytes (13). To what extent a preservation of HSPG synthesis after ACE inhibition or ATRI blockade contributes to their antiproteinuric effects in DN remains to be elucidated. ACE inhibition has been shown to preserve staining of the GBM for HSPG core protein and GAG side chains in the adriamycin model in the rat (35).

Figure 3. Systemic hemodynamics. Mean arterial pressure (MAP; A) and heart rate (HR; B) in patients with diabetes and with measurement A (before heparin on normal-salt diet), C (after heparin on normal-salt diet), B (before heparin on low-salt diet), and D (after heparin on low-salt diet). Baseline values (□) and after AngII infusion (■). *P < 0.0001 (MAP) and P < 0.05 (HR) after AngII versus baseline by two-sided paired t test. #P < 0.05 after salt restriction versus normal-salt diet by ANOVA.
However, it has been shown convincingly by several groups (36,37) that nephrin expression is reduced in experimental and human nephropathy and that AngII antagonist treatment or ACE inhibition can prevent these changes. We are not aware of any studies on the effect of heparins on nephrin expression in podocytes. If not by interaction with the RAAS, then how do heparins reduce proteinuria in DN?

The exact mode of action of heparins and sulodexide in DN is not clear at present. As reviewed by Striker et al. (38), in vitro studies demonstrate that HSPG and GAG modulate extracellular matrix composition (e.g., by increasing fibronectin and thrombospondin synthesis and by regulating the assembly of laminin and collagen IV via specific receptors). HSPG and GAG influence mesangial cell turnover by acting on both production and effects of several growth factors, such as TGF-β1 and basic fibroblast growth factor (38), but they also affect vascular endothelial growth factor and have, depending on dosage, antiproliferative rather than proliferative effects (19).

An interesting hypothesis on the mechanism of the proteinuria-lowering effect of heparins emerged recently: Xu et al. (39) demonstrated in an in vitro study that sulodexide inhibits heparanase-1 activity, an endoglycosidase that specifically degrades HSPG. Furthermore, heparanase-1 seems to be overexpressed in patients with DN, but, in contrasting, not in patients with nondiabetic nephropathies (40). These findings provide an intriguing hypothesis to explain, why—in our study—heparins have antiproteinuric effects in DN but not in patients with GN.

In evaluating the results of our study, we point to specific limitations of our protocol: The patients were not studied in a randomly assigned sequence for reasons of feasibility. However, we do not believe that a random order could have influenced our results. Furthermore, a crossover design with washout periods of long duration was not considered necessary to exclude carryover effects of ACE inhibition on proteinuria. Relevant interference of ACE inhibition with the effects of enoxaparin was believed to be minimized by the long-term ACE inhibitor pretreatment for 6 mo in all patients with diabetes except one. Second, the method used—which is applicable in human volunteers—to investigate the influence of heparin therapy on the systemic and renal hemodynamic effects of AngII cannot rule out that a modulation of

Figure 4. Renal hemodynamics. GFR (A), effective renal plasma flow (ERPF; B), and filtration fraction (FF; C) in patients with diabetes and with measurement A (before heparin on normal-salt diet), C (after heparin on normal-salt diet), B (before heparin on low-salt diet), and D (after heparin on low-salt diet). Baseline values (□) and after AngII infusion (■). *P < 0.01 after AngII versus baseline by two-sided paired t test. (D) Percentage decrease of ERPF in patients with diabetes (DN), patients with glomerulonephritis (GN), and healthy controls (C) after AngII infusion versus baseline with measurement A (before heparin on normal-salt diet), C (after heparin on normal-salt diet), B (before heparin on low-salt diet, and D (after heparin on low-salt diet).
the local RAAS occurred. Third, we were not able to obtain data on proteinuria after AngII infusion. However, we believe that this limitation is of minor importance, because it was demonstrated convincingly by Gansevoort et al. (41) that the antiproteinuric effects of RAAS blockade occur long after the acute renal hemodynamic alterations.

We believe that the mechanisms of heparin action in renal disease are of substantial clinical relevance. The beneficial effect of sulodexide suggested by two preliminary studies (42,43) was confirmed recently by a larger randomized trial. In the Di.N.A.S. trial, an oral sulodexide dosage dependently reduced albumuria in 223 patients with micro- and macroalbuminuria and DN that was caused by type 1 as well as type 2 diabetes. The antiproteinuric effect of sulodexide was long lasting and seemingly additive to the ACE inhibitory effect (44).

Conclusion

Our results show that treatment with the LMWH enoxaparin effectively lowers proteinuria in patients with DN, who were on ACE inhibition therapy. The efficacy of this treatment cannot be explained by the effects of enoxaparin on aldosterone levels. Our study on the effects of heparin on AngII-induced renal hemodynamic changes does not support the notion that the known heparin-induced modulation of AngII signaling, in mesangial or smooth muscle cells, can explain its antiproteinuric effect. These findings suggest that heparin treatment may have a beneficial effect in DN independent from and in addition to ACE inhibition or ATR1 blockade.

Acknowledgments

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Figure 5. (A) Proteinuria in patients with diabetes validated with concurrent creatinine before heparin (■) and after heparin (□). A, normal-salt diet before heparin; C, normal-salt diet after heparin; B, low-salt diet before heparin; D, low-salt diet after heparin. *P = 0.01 after heparin versus before heparin by Wilcoxon test for paired differences. (B) Individual values of proteinuria with concurrent creatinine in 10 patients who had diabetes and were on a normal-salt diet and had measurements A (before heparin) and C (after heparin).
Disclosures
None.

References
It is well known that blockade of the renin-angiotensin system lowers protein excretion in diabetic nephropathy. Benck et al. show that heparin can also lower proteinuria without affecting the renin-angiotensin system. In this month’s issue of JASN, Sasser et al. (pp. 143–154) show that endothelin receptor blockade can also reduce diabetic renal injury via an anti-inflammatory mechanism that does not involve the renin-angiotensin system, which gives us the possibility that other therapeutic strategies and pathways can reduce proteinuria.