Glycosaminoglycans: Use in Treatment of Diabetic Nephropathy

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Because diabetic nephropathy occurs in 20 to 40% of patients with diabetes mellitus, it has become one of the most important causes of end-stage renal disease. The latest incidence figures vary from 16% of patients starting with dialysis in The Netherlands (1) to 42% in the United States (2). Diabetic nephropathy has a large impact in terms of associated morbidity and mortality for the individual patient and in terms of costs for health care (3). The duration and efficacy of treating hyperglycemia (4), as well as BP regulation (5,6) and genetic factors (7–10), are probably all important in the pathogenesis of diabetic nephropathy in insulin-dependent diabetes mellitus (IDDM) and non-insulin-dependent diabetes mellitus (NIDDM). Several retrospective and prospective studies have demonstrated that micro- and macroalbuminuria predict cardiovascular morbidity and mortality in diabetes mellitus (reviewed in reference (11)). Although Rossing et al. recently reported that the prognosis for patients with overt diabetic nephropathy has improved, 15% of IDDM patients with normoalbuminuria, 25% with microalbuminuria, and 44% with overt nephropathy died during their 10-yr follow-up study (12). How albuminuria and proteinuria are linked with macroangiopathy is poorly understood, and many hypotheses have been formulated and tested. The Steno hypothesis, first advanced by Deckert et al. in 1988, held that a genetic defect in the regulation of heparan sulfate (HS) production by endothelial, myo-medial, and mesangial cells determines the susceptibility of diabetic patients to develop proteinuria and angiopathy with its associated cardiovascular risk (13). The sulfation pattern of the glycosaminoglycan (GAG) side chains of HS proteoglycans (HSPG) plays a pivotal role in this hypothesis. The central idea is that diabetic patients susceptible to nephropathy and macroangiopathy have a genetic trait leading to a lower activity of the enzymes responsible for GAG sulfation under high glucose conditions. The resulting undersulfated GAG chains would then play a crucial role in the pathogenesis of proteinuria (due to the loss of anionic charges in the glomerular basement membrane [GBM]) and its morphologic substrate, diabetic nephropathy, as well as in the pathogenesis of diabetic micro- and macroangiopathy. Although genes have been cloned for N-deacetylase/N-sulfotransferase, 3-O-sulfotransferase, and 6-O-sulfotransferase enzymes (reviewed in reference (14)), it is still unclear whether different allotypes of these enzymes with a different susceptibility to high glucose concentrations really exist. Although the Steno hypothesis has not been formally proven, it has stimulated in vitro studies and therapeutic trials in experimental animal models and patients.

Pathogenesis of Diabetic Nephropathy and the Possible Role of Abnormal GAG Metabolism

Possible GAG metabolism abnormalities in diabetic nephropathy were originally investigated for the following reasons: (1) albuminuria and proteinuria appear in diabetic nephropathy; (2) this phenomenon suggests abnormal GBM permeability; and (3) GAG, in particular HS, were thought to be important determinants of GBM permeability. Several groups reported a decreased 35S sulfate incorporation in the GBM of diabetic glomeruli (15,16). In mice and rats with diabetes, reduced synthesis of glomerular proteoglycans and basement membrane HSPG was found (17–19). However, the findings of the numerous sulfate incorporation experiments are not without controversy, and a marked increase in radiolabeled sulfate incorporation in proteoglycans in diabetic tissues has also been reported (reviewed in reference (20)). Studies using biochemical techniques to measure GAG content of kidneys obtained at autopsy demonstrated that GBM of patients with diabetic nephropathy contained less GAG than kidneys of nondiabetic control subjects (21,22). Similar changes in HS content in the intima of the aortas of patients with diabetes mellitus have been observed (23), suggesting that the abnormalities in HS metabolism are not necessarily restricted to the kidney. This would explain the association between cardiovascular mortality and diabetic nephropathy. Biochemical techniques, as well as immunohistology and histochemical staining procedures combined with electron microscopy morphometric studies have been used to examine glomerular HSPG and GAG content in diabetic nephropathy. With the last technique, Vernier et al. (24) described a reverse correlation between GBM HSPG expression and mesangial expansion in diabetic nephropathy. In the past decade, new monoclonal antibodies...
against GAG chains and core proteins of various HSPG have become available (25). JM-403, a very interesting monoclonal antibody that reacts with HS-GAG chains, mainly stains the GBM in normal kidneys, but largely fails to stain tubular basement membranes. The epitope recognized by JM-403 contains one or more N-unsubstituted glucosamine and D-glucuronic acid units, and is located in a region of the HS chain composed of mixed N-sulfated and N-acetylated disaccharide units (26). Using this antibody, we could show that a decreased GBM staining intensity (Figure 1) correlated with proteinuria, expressed as a function of creatinine clearance in patients with diabetic nephropathy (27). We also could show that staining of skin basement membrane was significantly reduced in patients with diabetic nephropathy compared to patients with longstanding diabetes without nephropathy (28). It should be noted that the exact meaning of a decreased staining by this antibody under pathologic conditions in relation to total GAG content or sulfation is not yet known. The first major basal membrane

Figure 1. (A) Control section stained with a monoclonal antibody directed against heparan sulfate (HS) (JM-403). A linear staining pattern of high intensity is observed in the glomerular basement membrane. Magnification, ×250. (B) Kidney section of a patient with diabetic nephropathy showing mesangial expansion without nodular lesions stained with the same anti-HS antibody. Compared with Panel A, the intensity of staining of HS chains is clearly diminished. From reference 27. Reproduced with permission from Springer-Verlag, Heidelberg, Germany.

HSPG to be identified was perlecan (20); however, immunohistologic staining with antibodies against perlecan HSPG core proteins did not reveal any significant changes in diabetic nephropathy (25,27). In 1999, three HSPG core proteins have been identified in the GBM: perlecan, agrin (29), and collagen XVIII (30). Interestingly, domain-specific monoclonal antibodies revealed a differential expression of agrin in glomerular and tubular basement membranes, suggesting alternative splicing and/or posttranslational processing of the molecule within the kidney (31). Agrin is present only in the kidney and the nervous system, and studies on renal agrin metabolism in diabetes are currently in progress.

The effects of the diabetic milieu on glomerular HSPG synthesis have been studied in vitro by different groups, using glomerular cell cultures (reviewed in reference (32). When human glomerular visceral epithelial and mesangial cells were cultured under normal (5 mM) and high (25 mM) glucose conditions, and then stained with the same monoclonal antibody (JM-403) that had been used on the tissue sections of diabetic kidneys, a decreased extracellular matrix staining after culture in 25 mM glucose was observed (33). The rise of metabolic labeling also disclosed an altered proteoglycan production under high glucose conditions, with predominantly a decrease in HS, compared with dermatan or chondroitin sulfate proteoglycan. N-sulfation analysis of HSPG produced under high-glucose conditions revealed less di- and tetrasaccharides, compared with larger oligosaccharides, indicating an altered sulfation pattern.

Renal function deterioration and proteinuria in diabetic patients are correlated with mesangial expansion as the main morphologic parameter (34). Because angiotensin-converting enzyme (ACE) inhibitors are known to slow the progression of diabetic nephropathy (5) and because we were interested in the role of HSPG in diabetic nephropathy, we studied the effect of angiotensin II (AngII) on mesangial HSPG production. Because AngII is also known to stimulate extracellular matrix production through transforming growth factor-β (TGF-β) production in rat mesangial cells (35), we also studied the role of TGF-β in this regard (36). Metabolic labeling studies revealed that AngII induced a decrease in HSPG synthesis, with a decrease in N-sulfation of the GAG side chains. Enzyme-linked immunosorbent assay measurements using JM-403 confirmed that AngII decreased HS production. AngII increased TGF-β production in a dose-dependent manner. Specific mRNA for perlecan HSPG decreased, while mRNA for TGF-β increased after incubation with AngII. Blockade of the subtype 1 AngII receptor (ATR1) reversed the effects of AngII on both HSPG and TGF-β production. Coincubation of the mesangial cells with neutralizing antibodies against TGF-β did not prevent the AngII-induced reduction of HS. These results indicate that the decrease in HS synthesis induced by AngII is not mediated by an increase in TGF-β, but, on the contrary, the increase in TGF-β partially counteracts the inhibition of HS production by AngII.

Because defects in HS-GAG synthesis are so striking in in vitro models of diabetic nephropathy (induced either by high glucose concentrations or AngII), and because decreased renal
and extrarenal JM-403 staining seems to be a rather specific marker for nephropathy in patients with diabetes, treatment of diabetic nephropathy with HS-GAG-like substances can be viewed as an experiment to test the Steno hypothesis.

Heparin and GAG Renoprotection in Experimental Diabetic Nephropathy

Indeed, numerous reports showed that heparin and more generally GAG prevent and cure experimental diabetic nephropathy (37–41). However, it soon became clear that the activity of these drugs could not be explained, according to the Steno hypothesis, only in terms of recovery of the diabetes-induced abnormalities in HSPG metabolism, and restoration of anionic-HS charges in glomerular and other basement membranes. Several hypotheses to explain the renoprotective effect of heparin and GAG in experimental diabetes were formulated: downregulation of proteases, modulation of extracellular mesangial matrix synthesis, and restoration of GBM anionic charges (reviewed in references (42) and (43)).

Most in vitro and in vivo data support the idea that the degradation of extracellular matrix is impaired in diabetic nephropathy (44) and that heparin and GAG may correct the balance between collagen synthesis and degradation by acting on synthesis rather than on degradation (45). Heparin and GAG improve glomerular permeability to proteins, as deduced by evaluating the fractional clearances of neutral and anionic dextrans (37). This has been indirectly confirmed by a number of investigators reporting a reduction in the albumin excretion rate after heparin treatment in diabetic animals. This effect is probably related to the activity of heparin on extracellular matrix and GBM protein synthesis, rather than adhering to the GBM and thereby correcting the charge deficiency. The activity of heparin on the sulfation and synthesis of proteoglycans (37,46) and on the collagen IV/perlecan mRNA ratio might be relevant to maintaining the authentic architecture of the membrane with normal permeability characteristics (37). However, although a recent study confirmed some of the glomerular morphologic effects, it reported increased albuminuria after heparin therapy (47). We suggest that this contrasting result might be due to the high heparin dosage and the type of commercial heparin used in this study (48). Indeed, at such high dosages, heparin appears in the glomerular ultrafiltrate; it then may interfere with the charge-dependent proximal tubule reabsorption of albumin due to its high degree of sulfation, thereby increasing albuminuria. Furthermore, it should be emphasized that when referring to heparin, it is generally overlooked that heparin is a heterogeneous group of polysaccharides, with varying degrees of sulfation, molecular weights, and biologic activities. Commercial heparins are similar regarding their anticoagulant activity, but not with respect to many other activities. For example, let us consider anti-proliferative activity, which is probably heparin’s best-investigated non-anticoagulation-related activity. Although heparins from different suppliers have the same anticoagulant activity, they show a broad range of anti-mitogenicity, and some are even mitogenic (49). Furthermore, depending on its dosage, the same heparin preparation may be both anti-proliferative and proliferative (49). Similarly, in studies on the effect of heparin and GAG in experimental diabetic nephropathy, despite differences in drug formulation and dosage, some effects were constant, especially the effects on the ultrastructure of the glomerulus, whereas others, such as those on albuminuria, were contradictory. Most likely these differences were due to the quality and the quantity of heparins used in the different protocols.

It is also possible that the renoprotective effect of heparin and GAG in diabetic nephropathy depends on the modulation of the overactivated TGF-β cascade. Although TGF-β was shown to increase HSPG synthesis (36), a potentially favorable effect according to the Steno hypothesis, as a whole it has a more prominent and pivotal role in the pathogenesis of glomerulosclerosis. Thus, TGF-β inhibition might have a useful impact in the evolution of diabetic nephropathy. Indeed, GAG therapy in long-term diabetic rats prevents characteristic manifestations of diabetic nephropathy that are possibly related to TGF-β activity, mesangial matrix expansion, and deposition of periodic acid-Schiff-positive material, collagen III, and α1(IV) collagen (39). Heparin/GAG can dissociate TGF-β from α2-macroglobulin in serum, a protein possibly involved in the clearance of TGF-β (50). Accordingly, heparin/GAG should increase the concentration of active TGF-β, thus augmenting mesangial matrix production and worsening diabetic nephropathy rather than ameliorating it as found in a number of studies. However, in theory, heparin/GAG can also have at least two inhibitory targets in the TGF-β cascade. Heparin/GAG may interfere with TGF-β1 signaling or inhibit increased TGF-β1 formation. We have data indicating that GAG do not interfere with TGF-β1 receptor binding or with intra- or postreceptor signaling via binding to active TGF-β1 and thus by blocking binding to TGF-β receptor I and/or II, or by interfering with intracellular processes, e.g., with type II receptor serine-threonine kinase (51). On the contrary, GAG act on increased de novo formation of TGF-β1. In cultured mesangial cells, we found that heparin/GAG suppress high glucose-induced TGF-β1 mRNA, protein, and bioactivity, similar to treatment with TGF-β1 antisense oligonucleotides (52). Because the effects of high glucose concentrations on mesangial matrix production have been attributed to protein kinase C (PKC) activation (53,54), it is worth noting that renoprotective GAG (modified heparins and dermatan sulfate) also inhibit the PKC-dependent induction of TGF-β1 mRNA obtained with phorbol myristate acetate. These data confirm that GAG inhibit TGF-β function by prevention of TGF-β1 mRNA overexpression, and argue against inhibition of TGF-β activation, e.g., by inhibiting tissue plasminogen activator formation and thus subsequently inhibiting 1-TGF-β activation to mature TGF-β (55). The hypothesis that heparin/GAG can modulate TGF-β1 gene expression was specifically addressed in an in vivo study by evaluating TGF-β1 mRNA expression with in situ hybridization (56). Indeed, heparin/GAG treatment inhibited the disease-induced increase in TGF-β1 gene expression in glomerular and tubular cells. Together, these findings support the view that heparin/GAG are directly involved in the inhibition of TGF-β1.
overexpression, most likely, as ongoing experiments indicate, by inhibiting stimulated TGF-β1 promoter activity without reducing basal activity.

Interestingly, in unstimulated mesangial cells, the addition of renoprotective GAG has no effect on basal TGF-β1 mRNA levels. This observation mirrors the fact that heparin/GAG treatment has no effect on TGF-β1 mRNA levels in nondiabetic animals. Together, the data indicate that GAG treatment prevents overexpression of TGF-β1 mRNA in mesangial cells induced by different stimuli, but does not affect basal levels.

In theory, the chronic blockage of all TGF-β1 activity may be deleterious because of the risk of autoimmune-like diseases (57) and malignant cell transformation (58). The fact that GAG inhibit TGF-β1 overexpression leaving its basal expression unaffected seems to argue against such risks. Indeed, in vivo long-term (8 to 12 mo) studies showed that GAG treatment prevents diabetes-induced overexpression of TGF-β1 with no occurrence of autoimmune-like disease, excess mortality, or cancer (37,38,41).

Heparin and GAG Renoprotection in Human Diabetic Nephropathy

Previous experimental findings and the observation that increased TGF-β1 expression exists in humans with diabetic nephropathy (59) suggest that the results obtained in animal studies may also be relevant to human disease. Recent reports have indeed described favorable results of GAG treatment on proteinuria in diabetic nephropathy (Table 1). Treatment with a low molecular weight heparin reduced albuminuria in both micro- and macroalbuminuric IDDM patients (60,61). Danaparoid, a mixture of sulfated GAG consisting mainly of HS, also could lower proteinuria in a small, double-blind cross-over study in IDDM patients with albumin excretion rates exceeding 300 mg/24 h (62). A minimum of 6 wk was necessary for the antiproteinuric effect to become manifest with no modifications in BP and creatinine clearance. Sulodexide, a formulation composed of the two GAG (80% fast-moving heparin and 20% dermatan sulfate) that were active in preventing diabetic nephropathy in the experimental model (38), was reported to reduce albuminuria in IDDM and NIDDM patients (63–67), and this effect lasted several weeks after its withdrawal (64,67). Although sulodexide treatment in IDDM seems to be consistently effective in reducing microalbuminuria, this hyposalbminuric effect is observed in only 30 to 50% of NIDDM patients. It is known that microalbuminuria in NIDDM patients is not always caused by the typical diabetic nephropathy because different, nondiabetic histologic changes were reported in two-thirds of the patients (68). Because the antiproteinuric effects of heparin differ among the various nephropathies (e.g., experimental diabetic nephropathy is much more sensitive to heparin than puromycin nephrosis) (69), we suggest that in some NIDDM patients sulodexide and other heparins/GAG (70) do not reduce albuminuria due to a dosage that is low relative to the underlying renal lesion. The relatively low dosages could also be related to differences in the type of the administered heparin. In this regard, it is interesting to note that tinzaparin, which gave negative results in microalbuminuric NIDDM patients (70), has an average molecular weight of 6500 Daltons versus 4500 for enoxaparin, and moreover has an anti-factor Xa to anti-factor IIa activity ratio of 1.5:2.5 versus 3.3:5.3. Thus, in molar terms, NIDDM patients in the study by Nielsen et al. (70) received 30% less low molecular weight heparin than in the study by Tamsma et al. (61). Furthermore, differences in molecular weight and anti-X and anti-II activity may be indirect hallmarks of different renoprotective potency of the heparin formulation used. Although promising, the studies published thus far were too short-term to clarify whether GAG treatment in diabetic patients is capable of curing diabetic nephropathy, instead of simply influencing one of its surrogate end points, albuminuria.

Risks of the Chronic Use of GAG

A major point of concern in the treatment of diabetic patients with GAG and heparin has been the risk of bleeding, particularly in subjects with retinal neangiogenesis, due to the fragility of new vessels. Although the dosages used were relatively low, sufficient only to inhibit factor X, we recently demonstrated that therapy with the GAG danaparoid was safe (71). Even more interesting, we observed a reduction in retinal hard exudates. This was surprising because it is known that a spontaneous reduction in such abnormalities is not likely to occur within a few months. How can these findings be explained?

Overexpressed vascular endothelial growth factor (VEGF) plays a pivotal role in diabetic retinopathy, both the proliferative form and maculopathy (72,73). Interestingly, under physiologic conditions, some VEGF isoforms are sequestered and modulated by extracellular matrix-sulfated GAG. Alterations in the sulfation of GAG in diabetes could thus lead to an altered disposal of VEGF (74), and exogenous GAG putatively can reverse this phenomenon (37). Thus, the effect of danaparoid on hard exudates might well be related to the modulation of sequestered VEGF in the retina.

Anticoagulation may also constitute a critical problem in long-term treatment with heparin-related drugs. The problem of anticoagulation with heparin and heparin-derived GAG is twofold. Indeed, it depends on both the chemical structure of the molecule and the route of administration. The chemical structure is certainly very important; anticoagulant activity depends on the degree and pattern of GAG sulfation, so that chemical desulfation of GAG might be essential for safe chronic use. On the other hand, anticoagulant activity also depends on the type of molecular repeat, i.e., heparin is anticoagulant, whereas chondroitin and dermatan sulfate are not anticoagulant. As a matter of fact, in our long-term experiments we did not observe any hemorrhagic deaths in either desulfated heparin-treated or dermatan sulfate-treated animals (37,38). The second aspect of the problem is that to achieve therapeutic anticoagulation, heparin must be administered intravenously; in this way, its serum concentrations are high enough to inhibit the coagulation cascade. When heparin is administered through other routes, its pharmacokinetics are completely different, and anticoagulant levels cannot be
Table 1. Summary of the phase 2 studies with heparins/GAG in diabetic patients with albuminuria

<table>
<thead>
<tr>
<th>Study</th>
<th>Design</th>
<th>Type of Diabetes Mellitus</th>
<th>AER (mg/24 h)</th>
<th>No. of Patients</th>
<th>Duration of Treatment</th>
<th>Dosages Administered</th>
<th>Results on AER</th>
</tr>
</thead>
<tbody>
<tr>
<td>Solini et al., 1994 (63)</td>
<td>Open</td>
<td>NIDDM 30 to 300</td>
<td>6</td>
<td>2 mo</td>
<td>Sulodexide, 100 mg/d; oral</td>
<td>Reduction in 4 of 6 patients</td>
<td></td>
</tr>
<tr>
<td>Myrup et al., 1995 (60)</td>
<td>Randomized, open, placebo-controlled, 2 drugs (LMW heparin and standard heparin)</td>
<td>IDDM 30 to 300</td>
<td>11 LMWH</td>
<td>3 mo</td>
<td>LMW heparin, 2000 IU anti-Xa; s.c.</td>
<td>Reduction (P = 0.004)</td>
<td></td>
</tr>
<tr>
<td>Tamsma et al., 1996 (61)</td>
<td>Randomized, double-blind, placebo-controlled cross-over</td>
<td>IDDM &gt; 300</td>
<td>6</td>
<td>1 mo</td>
<td>Enoxiparin, 4000 IU anti-Xa/d; s.c.</td>
<td>Reduction (P &lt; 0.05)</td>
<td></td>
</tr>
<tr>
<td>Velussi et al., 1996 (79)</td>
<td>Randomized, placebo-controlled cross-over</td>
<td>NIDDM 30 to 300</td>
<td>24</td>
<td>6 mo</td>
<td>Sulodexide, 100 mg/d (approximately 9000 IU anti-Xa); oral</td>
<td>Reduction (P &lt; 0.01)</td>
<td></td>
</tr>
<tr>
<td>Dedov et al., 1997 (66)</td>
<td>Randomized, open, placebo-controlled</td>
<td>IDDM 30 to 300 and &gt;300</td>
<td>18</td>
<td>3 wk</td>
<td>Sulodexide, 60 mg/d (approximately 5400 IU anti-Xa); i.m.</td>
<td>Reduction (P &lt; 0.01)</td>
<td></td>
</tr>
<tr>
<td>van der Pijl et al., 1997 (62)</td>
<td>Randomized, double-blind, placebo-controlled cross-over</td>
<td>IDDM &gt; 300</td>
<td>9</td>
<td>6 wk</td>
<td>Danaparoid, 750 IU/d (approximately 100 mg/d); s.c.</td>
<td>Reduction (P &lt; 0.03)</td>
<td></td>
</tr>
<tr>
<td>Poplawskas et al., 1997 (65)</td>
<td>Open</td>
<td>IDDM 30 to 300 and &gt;300</td>
<td>7</td>
<td>1 mo</td>
<td>Sulodexide, 60 mg/d; i.m. (for 10 d) and 50 mg/d; oral (for 3 wk)</td>
<td>Reduction (P &lt; 0.001)</td>
<td></td>
</tr>
<tr>
<td>Skrha et al., 1997 (64)</td>
<td>Multicentric, open</td>
<td>IDDM 30 to 300 and &gt;300</td>
<td>26 IIDD and NIDDM (29 macro AER, 24 micro AER)</td>
<td>3 wk</td>
<td>Sulodexide, 60 mg/d; i.m.</td>
<td>No reduction in 28%</td>
<td></td>
</tr>
<tr>
<td>Solini et al., 1997 (78)</td>
<td>Randomized, double-blind, placebo-controlled cross-over</td>
<td>NIDDM 30 to 300</td>
<td>12</td>
<td>4 mo</td>
<td>Sulodexide, 100 mg/d; oral</td>
<td>Reduction (P &lt; 0.033)</td>
<td></td>
</tr>
<tr>
<td>Szelenowska et al., 1997 (67)</td>
<td>Open</td>
<td>IDDM 30 to 300</td>
<td>15</td>
<td>3 wk</td>
<td>Sulodexide, 60 mg/d; i.m.</td>
<td>Reduction (P &lt; 0.0007)</td>
<td></td>
</tr>
<tr>
<td>Nielsen et al., 1999 (70)</td>
<td>Randomized, double-blind, placebo-controlled</td>
<td>NIDDM &lt;10, &lt;100, &gt;100</td>
<td>22 tinzaparin</td>
<td>3 wk</td>
<td>Tinzaparin, 50 IU anti-Xa/kg per d; s.c.</td>
<td>No reduction</td>
<td></td>
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GAG, glycosaminoglycan; AER, albumin excretion rate; NIDDM, non-insulin-dependent diabetes mellitus; IDDM, insulin-dependent diabetes mellitus; LMWH, low molecular weight heparin; SH, standard heparin; i.m., intramuscularly; s.c., subcutaneously.
reached; thus, to expand the use of these drugs as well as increase patient compliance, it would be relevant to study the pharmacokinetics and pharmacodynamics of heparin and GAG administered through different routes. A few reports have demonstrated that heparin and dermatan sulfate have oral bioavailability. After oral administration of heparin to rats, Larsen et al. (74) found fragments as large as octasaccharides with anti-factor Xa activity in the plasma. Furthermore, heparin was shown to be rapidly absorbed at the gastric level, in quantities sufficient to prevent venous thrombosis in rats, but with no “clinically” significant anticoagulant effect due to its rapid sequestration by the endothelium (75,76). Dermatan sulfate is also absorbed in active form at the gastrointestinal level with a 3 to 9% oral bioavailability (77). These investigations address the possibility of oral bioavailability of GAG in terms of the anticoagulant and antithrombotic activity, but two clinical studies in microalbuminuric diabetic patients treated with sulodexide (78,79) show that after oral administration GAG maintain their effects not only on fibrinolytic parameters, but, interestingly, also on albuminuria.

Osteopenia is frequently reported as a possible adverse effect of long-term heparin administration, but few studies have evaluated this issue. Interestingly, however, and contrary to the basic assumption, it was reported that a 12-wk course of heparin therapy in rats with chronic renal failure did not influence bone metabolism (80).

The risk of antibody induction is very low with GAG therapy because low molecular weight GAG are poor antigens. Therefore, the use of GAG structure-based anti-TGF-β agents certainly carries a lower risk of inducing antibody formation, compared with protein-based anti-TGF-β agents (e.g., antibodies or decorin) (57). Heparin-induced thrombocytopenia is a well-recognized complication of heparin therapy that is frequently associated with severe thrombotic events due to the formation of immune complexes between IgG, heparin, and platelet factor 4 (PF4). Although about 5% of patients receiving standard unfractionated heparin may develop this complication, its prevalence is significantly lower in patients treated with low molecular weight heparin and heparin-related GAG (81) probably because of the lower affinity for PF4. Therefore, these agents have been used to replace standard heparin to avoid thrombocytopenia and thrombosis (82,83). The use of other GAG, such as chondroitin or dermatan sulfate, remains an interesting possibility because thrombocytopenia is much less likely to occur; however, there is no experience with these compounds in patients.

**Why Are ACE Inhibitor and Heparin/GAG Treatments Both Effective?**

ACE inhibitors decrease progression of diabetic nephropathy and reduce proteinuria by mechanisms that only in part can be attributed to effective control of systemic and intraglomerular hypertension and might be related to an increased activity of the renin angiotensin aldosterone system (RAAS), local synthesis of AngII, and the nonhemodynamic effects of the RAAS components. Indeed, AngII may work as a growth factor and a profibrogenic peptide. It induces hypertrophy and/or proliferation of glomerular and tubular cells, stimulates the synthesis of collagen and fibronectin, inhibits the synthesis of perlecan, and finally reduces extracellular matrix turnover (84). Although clinical and experimental findings support the existence of an interaction between diabetes and RAAS, how this interaction occurs is unclear. A number of observations demonstrate that AngII-mediated signal transduction may be diminished rather than augmented as expected in the presence of high glucose (85–88). However, the finding that AngII stimulates glucose uptake and transcription of the glucose transporter GLUT-1 in a number of different cells (89–91) raises the intriguing possibility that a primary increased activity of the RAAS at the kidney level, for instance due to a particular genetic background, might lead to a much higher intracellular glucose concentration in nephropathic diabetic patients than in diabetic subjects with normal renal RAAS activity (92). The consequence of such a phenomenon is self-evident, because a number of cellular abnormalities believed to lead to diabetic nephropathy (i.e., extracellular matrix protein synthesis, TGF-β expression) are related to the intracellular rather than to the extracellular glucose concentration.

Furthermore, additive synergistic mechanisms between the effects of RAAS stimulation and high glucose levels might also exist. Both effects trigger similar or parallel signal transduction pathways in cultured renal cells. PKC activation in renal cells is typical of diabetes and high glucose concentrations (53,54) and is important in the high glucose-mediated expression of fibronectin, collagen IV, and TGF-β in renal cells (84). AngII activates PKC in mesangial and tubular cells through ATR1 as well (93). It is therefore possible that hyperglycemia and AngII might exert additive effects on PKC activation and subsequent target phosphorylation. Indeed, the additive effects of these conditions on the hypertrophy of proximal tubular cells was demonstrated (94).

*In vitro* studies on cultured vascular smooth muscle, mesangial cells, and proximal tubular cells have shown that AngII induces hypertrophic, fibrogenic, and mitogenic responses reminiscent of those induced by growth factors including TGF-β and platelet-derived growth factor. AngII is a potent inducer of TGF-β, and some of its reported effects are directly mediated by expression of this growth factor (35,95–98). TGF-β induction by AngII is also a PKC-dependent phenomenon (99) similar to induction by glucose. The body of available data has led to the theory that TGF-β might constitute the missing link between glomerular hyperfiltration, *i.e.*, a purely hemodynamic and RAAS-dependent event, and glomerulosclerosis (100). This theory can be extended to diabetic nephropathy; TGF-β could be the missing link between RAAS and glomerulosclerosis, with PKC playing a pivotal role between AngII and high glucose (92). However, it is unknown whether intracellular high glucose and AngII share the same intracellular signaling cascade (*i.e.*, the same or different PKC isoforms) in TGF-β gene induction, although it was demonstrated in glomerular epithelial cells that at least some effects are distinct, thus suggesting different pathways (101). Moreover, there is no perfect overlap between the biologic effects of...
AngII and TGF-β on mesangial cells in vitro, as clearly shown by differential effects on perlecan production (36).

The proposed model of interaction between high glucose, AngII, and TGF-β, therefore, is somewhat simplistic, but its relevance to understanding is not merely a matter of speculation. Rather, it may offer the possibility of discerning additional pharmacologic targets for the treatment of diabetic nephropathy. As clearly shown by the cited exceptions to this model (36,101), full intervention on the different events triggered by high glucose, AngII, and TGF-β in diabetes mellitus most likely requires drugs that work on different pathogenetic pathways. Therefore, the search for ancillary therapies for diabetic nephropathy to add to metabolic control and RAAS modulation seems to be warranted. In this context, it seems interesting that heparin/GAG work on different targets than ACE and ATR1 inhibitors; as a consequence, the combination of ACE inhibitors and GAG could synergistically inhibit the TGF-β axes in the kidney, thereby increasing the possibility of preventing glomerulosclerosis.

Conclusion

An article on the controversy regarding the therapeutic use of heparin appeared in 1984 in the American Journal of Kidney Diseases (102), after more than 10 yr of sporadic experimentation and clinical use. The consensus at that time was that anticoagulants were of little value in the treatment of renal disease. Indeed, after the first experiments with heparin, the emphasis in the early 1970s was on anticoagulation for the treatment of a few, very acute, and dramatic glomerular diseases. So, it seemed logical to substitute intravenous heparin with oral anticoagulants.

The Steno hypothesis postulated a crucial role for heparin-like structures in the pathogenesis of diabetic nephropathy. We now may reasonably assume that there are some specific structures in the GAG chains of heparin molecules, which are useful for renoprotection and do not necessarily have a relationship with the anticoagulant activity of heparin.

We conclude that experimental and human data on the effects of GAG treatment on morphology and proteinuria in diabetic nephropathy, collected over the past 7 yr, have disclosed a new therapeutic avenue to improve the treatment of a patient category with a dismal prognosis. The possibility of synthesizing newer, less toxic compounds, together with the favorable “side effects” that these compounds may have on retinal and other vessels (103), could make this kind of treatment even more attractive. Additional studies will be needed to confirm and extend the data, and most of all clarify whether in humans not only proteinuria, but also renal function and morphologic abnormalities can be improved.

Acknowledgments

Dr. Gambaro thanks Dr. E. Schleicher and Dr. C. Weigert of the University of Tübingen, Germany, and Dr. M. Ceol, University of Padova, Italy, for the invaluable contribution in the development of the TGF-β-GAG paradigm.

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