Nephrol Dial Transplant (2014) 29: i74–i79
doi: 10.1093/ndt/gft389

Sulodexide and glycosaminoglycans in the progression of renal disease

Valentina Masola[1], Gianluigi Zaza[1] and Giovanni Gambaro[2]

[1]Division of Nephrology, Department of Biomedical and Surgical Sciences, University Hospital of Verona, Verona, Italy and [2]Division of Nephrology, Department of Internal Medicine and Medical Specialties, Columbus-Gemelli University Hospital, Catholic University, Rome, Italy

Correspondence and offprint requests to: Giovanni Gambaro; E-mail: giovanni.gambaro@rm.unicatt.it

FULL REVIEW

ABSTRACT

Experimental data in cell cultures and animal models suggest that sulodexide and glycosaminoglycans are potentially effective drugs to treat chronic kidney diseases and prevent progression to renal failure. However, no conclusive evidence support the use of them in human renal disease. In acute and chronic glomerulonephritis, only few studies have been performed. Sulodexide has been more intensely investigated in diabetic nephropathy (DN) where the body of data supports its effectiveness as an antialbuminuric agent in early stages. Unfortunately, there is no study in DN patients on the effect of sulodexide on clinical end points.

Keywords: diabetic nephropathy, FGF-2, glycosaminoglycans, heparanase-1, sulodexide

Patients with primary and secondary chronic glomerular diseases have a considerably high risk to progress to end-stage renal disease (ESRD). There is a dramatic need of new drugs for the treatment of renal diseases, but unfortunately, after the landmark introduction in therapeutics of angiotensin-converting enzyme inhibitors (ACEIs) in 1993 [1] and angiotensin receptor blockers (ARBs) in 2001 [2, 3] for the treatment of overt diabetic nephropathy (DN) and thereafter of other chronic proteinuric renal disorders, although the list of promising drugs periodically changes, our treatment armamentarium has not changed.

Sulodexide and glycosaminoglycans (GAGs) belong to this list.

Experimental evidence shows that sulodexide and GAGs favourably affect proteinuric renal diseases. A number of clinical studies using these agents have produced conflicting results, however.

This paper will review the available evidence on the use of GAGs in renal diseases.

EFFECT OF GAGS IN EXPERIMENTAL RENAL DISEASES AND MECHANISMS OF NEPHROPROTECTION

The majority of experimental studies that investigated the renal effect of GAGs has been carried out in 5/6 nephrectomy models of chronic kidney disease and in DN, while the clinical studies have addressed mainly DN. We will review results from these studies and, when necessary, the few studies on different experimental models and other human renal diseases.

In the subtotal nephrectomy model of chronic renal failure, a number of studies have shown that heparin, sulodexide and other GAGs prevent progression of the renal disease. In DN models, sulodexide and GAGs decrease albuminuria and glomerulosclerosis. The mechanisms of the nephroprotection exerted by GAGs have been the matter of many investigations, since it is quite obvious that its comprehension would lead to the rational design of new drugs capable of preventing and/or curing these renal diseases, but devoid of anticoagulant activity and endowed with a more favourable bioavailability.

Studies by Purkerson *et al.* [4, 5] had shown that both anticoagulant and chemically modified non-anticoagulant heparins are active in preventing the progression of the renal disease at odds with oral anticoagulants engendering the idea that heparin is nephroprotective for mechanisms independent on anticoagulation. The following studies have investigated different hypotheses to explain such a nephroprotection exerted by GAGs. Ichikawa *et al.* [6] ruled out that the slowing down effect of heparin on progression to uraemia in 5/6 nephrectomy models and decrease in proteinuria in patients with DN [7] depends on some interaction between heparin and the renin–angiotensin–aldosteron axes. The hypothesis advanced by Olson [8] that the protective effect exerted by GAGs is due to the mechanical restoration of the negative charge of the glomerular basement membrane (GBM) was

also shown to be unlikely [9]. GAGs inhibit mesangial cell proliferation *in vitro* and in the animal models in which they are nephroprotective, i.e. the subtotally nephrectomized rat, the puromycin and the habu snake venom-induced nephrosis [10–12], the anti-Thy 1.1 mesangioproliferative glomerulonephritis [13, 14] and the spontaneously glomerulosclerostic GH-transgenic mice [15]. Based on some of those observations, Coffey and Karnovsky [12] proposed that exogenous heparin slow the progression of glomerular diseases by replacing lost endogenous heparan sulphate (HS), thus re-establishing the normal proliferation of glomerular cells. However, this should not be the only mechanism since heparins and GAGs prevent and cure the experimental DN [6, 16–18] in which there is no evidence of mesangial cell proliferation [19].

The typically deranged extracellular matrix (ECM) turnover of the DN is repaired by GAGs [20]. Actually, in diabetic animals and *in vitro*, sulodexide and GAGs normalize the sulphation and synthesis of proteoglycans [9, 21], and the diabetes-induced increase in type IV collagen and decrease in perlecan [9, 22]. Interestingly, sulodexide and GAGs prevent the renal ECM expansion, type III and IV collagen deposition [16, 23], all believed to be the result of the overactivation of the transforming growth factor-β (TGF-β) loop by diabetes. Thus, it was suggested that GAGs activity on DN may take place through the modulation of the TGF-β. Evidence that this is the case was obtained. In mesangial cell cultures, the glucose-induced, PKC-dependent synthesis of TGF-β1 was inhibited by GAGs [24]. Similarly, the increase in TGF-β1 gene expression and protein deposition in glomeruli and tubulointerstitium of diabetic rats [24] was prevented by GAGs administration.

The molecular mechanism of such an inhibiting activity of GAG on TGF-β1 has been investigated in mesangial cells cultured in high glucose or stimulated with phorbol 12-myristate 13-acetate (PMA) [24]: in both conditions, GAGs suppressed the stimulated TGF-β1 mRNA levels, protein and bioactivity without interfering with TGF-β1 receptor binding, or intra- or post-receptor signalling.

By transfecting mesangial cells with different TGF-β1 promoter-reporter gene constructs, we showed that the TGF-β1 inhibition induced by GAGs occurs on AP1-containing sites of the promoter without affecting basal TGF-β1 expression [25], a finding consistent with the observation that GAGs administration had no effect on renal TGF-β1 in non-diabetic animals [24].

A more recently investigated target of GAGs to explain nephroprotection is heparanase-1 (HPSE-1), an endo-β(1-4)-D-glucuronidase that cleaves the glycosidic bond within HS. Since renal HPSE-1 is up-regulated in DN, its inhibition was suggested as one of the mechanisms for nephroprotection of GAGs [26]. More recent findings support this idea. As a matter of fact, HPSE-ko mice are resistant to the development of DN in streptozoicin-induced diabetes [27].

Both podocytes and proximal tubular cells express HPSE-1 [28, 29]. At odds with podocytes, high glucose does not influence *HPSE* expression in tubular cells; however, the challenge of proximal tubular cells with albumin (as in proteinuric conditions) and glycated albumin (as in diabetes) induces a dose-dependent increase in *HPSE* expression through the PI3K/AKT signalling pathway [29]. It is such an increased expression of HPSE in the tubular cells which probably explains the decreased HS content in the tubuli of renal biopsies from DN patients [28,30]. Such a decrease is only partly explained by the endoglycosidase activity of HPSE-1; another contributor is the down-regulating effect of HPSE-1 on the expression of HS-proteoglycans, in particular syndecan-1 (SDC1), a cell-membrane associated HS-proteoglycan involved in the fibroblast growth factor (FGF)-2 loop [29] but possibly of others HS-proteoglycans such as betaglycan, one of the TGF-β receptors. This finding puts HPSE-1 in a much intriguing prospective, not just an enzyme degrading glomerular HS and thus deranging glomerular permeability, but also, and perhaps more important, a crucial factor in the cross-talk between tubular cells and renal interstitium mediated by growth factors.

The pivotal role of tubular and interstitial fibrosis in the progression to ESRD of chronic kidney diseases and DN is well known as it is the role of the conversion of tubular epithelial cells into myofibroblasts (EMT) in the pathogenesis of renal fibrosis [31]. HPSE-1 plays an important part in the FGF-2-induced EMT of tubular cells. In fact, it is essential for FGF-2 in two respects: (i) in the activation of the PI3K/AKT pathway which leads to EMT; and (ii) in the generation of an autocrine loop because of the down-regulation of SDC1 and up-regulation of matrix-metalloprotease-9 (MMP9) and HPSE-1 itself [32]. At therapeutic concentrations, sulodexide and parnaparin are capable of inhibiting HPSE-1 and preventing the renal tubular cell EMT induced by FGF-2 [32, 33]. They also stop in tubular cells the increase in HPSE-1 and MMP9 and the associated SDC1 decrease, all triggered by FGF-2, which means that sulodexide and parnaparin switch off the autocrine loop that FGF-2 activates to fuel its signal [32, 33].

Supporting our findings is the observation that sulodexide prevents EMT in another model and tissue, i.e. in the peritoneal membrane of a rat model of peritoneal dialysis [34].

The inhibition by sulodexide of HPSE-1 also leads to the restoration of HS in podocytes grown in high glucose media, and to the restoration of the GBM permeability to albumin *in vitro* [26].

Since enhanced glomerular HPSE-1 has been shown in several renal diseases, the mechanism proposed may also apply to kidney disorders other than DN [30].

Because the rolling of monocytes needs their binding to an endothelial HS-proteoglycan and this is impaired by GAGs [35], in inflammatory glomerulonephritis such as the glomerulosclerosis induced by puromycin, GAGs reduces glomerular macrophage infiltration [36]. Amusingly, in the same experimental model, GAGs also inhibit the TGF-β expression by macrophages [36] which also contribute to preventing the development of glomerulosclerosis.

The already known favourable activity of GAGs on the endothelium, especially in diabetes [37, 38], has recently received interesting confirmation. The endothelial glycocalyx, which is composed by proteoglycans and sialoglycoproteins, contributes to vascular permeability. In Type 2 diabetes, the well-known derangement in the glycocalyx causes abnormal vascular permeability; interestingly, in a small trial, both abnormalities were partially restored by sulodexide in patients [39]. As the endothelial dysfunction contributes to the onset of

microalbuminuria [40], data suggest that in incipient DN, sulodexide favourably affect DN likely because of an endothelial activity [41]. In experimental studies on the streptozotocin diabetic model, sulodexide was also capable of lowering the number of circulating endothelial cells and improving endothelium-dependent relaxation in arteries [42, 43]. We speculate that these activities may also have a favourable impact on the renal haemodynamics of the diabetic kidney which impaired glomerular autoregulation [44] may not be sufficient to protect the glomerulus from a high differential pressure regimen of arteries with impaired relaxation.

Few studies have addressed the effect of GAGs on immunologically mediated glomerulonephritis. Heparin enhanced the removal of antigen from glomeruli in the chronic serum sickness glomerulonephritis and blocked the immune complexes binding to mesangial cells [45]. In the spontaneous glomerulonephritis of lupus-prone mouse, heparin inhibited the binding of nucleosome-containing immune complexes to the GBM, thus slowing the progression of the disease [46].

GAGS IN THE TREATMENT OF NON-DIABETIC GLOMERULAR DISEASES

Only a few studies have been performed on the treatment with GAGs in non-diabetic chronic nephropathies [47].

In a controlled trial on 18 patients with biopsy-confirmed chronic proliferative glomerulitis receiving for a year subcutaneous heparin in monotherapy, glomerular filtration rate improved and in those who were rebiopsied glomerular hyper-cellularity was found to have subsided [48].

The activity of heparin (7000 to 11 000 units, s.c.) and fragmin (60 unit/kg of fragmin) for 4 weeks was evaluated in 5 and 10 subjects, respectively, with proliferative glomerulonephritis [49]. Most patients also received prednisolone and all anti-platelet agents. Urinary proteins decreased, but it is uncertain that GAGs alone were responsible of it.

In an open study on 16 patients with different chronic glomerulonephritides and heavy proteinuria not responding to conventional therapies, sulodexide, i.v. for a week and thereafter orally, induced a significant reduction in proteinuria after 3 and 6 months of treatment [50].

In a polycentric trial on 77 patients with IgA nephropathy randomly allocated in three groups, placebo, oral sulodexide 75 and 150 mg/day for 6 months, the primary end point, i.e. the achievement of at least 50% reduction in proteinuria, did not differ between the three groups, although treatment with sulodexide 150 mg daily significantly reduced it [51].

Randomized controlled trials with larger and homogeneous case populations are necessary to confirm the hypothesis that sulodexide affords renal protection in chronic nephropathy patients with proteinuria.

GAGS IN THE TREATMENT OF DN

Therapies for DN include tight control of glucose metabolism and blood pressure, and of normalization of albuminuria or, if not possible, as low as possible albuminuria levels. However, none is sufficient to arrest progression of DN to ESRD. For instance, in Type 2 diabetic patients with overt nephropathy, ARBs, the most effective agents and first-line treatment of many Clinical Guidelines, consent on average to gain a reprieve from ESRD of ~2 years [2, 3]. Said in a different way, over a 3-year follow-up, ARBs were capable to prevent ESRD in 1 patient out of 15–30, leaving a huge percentage of diabetic patients progressing to ESRD. Furthermore, in a 2-year follow-up trial in Type 2 diabetic patients with incipient nephropathy treated with irbesartan, progression to overt nephropathy was prevented only in 1 out of 10 patients [52].

The search for new drugs for preventing or retarding the progression of DN is therefore essential and the use of GAGs, because of their favourable activity in the experimental model of DN has been suggested.

Following the experimental findings by Gambaro *et al.* [9, 16], Solini *et al.* [53] were the first who investigated GAGs (sulodexide, Vessel®) as anti-albuminuric agents in diabetic patients. Sulodexide is composed of the two GAGs (80% fast-moving heparin and 20% dermatan sulphate) capable to prevent DN in the experimental model [9, 16] and has an oral formulation.

Following this very pilot study, in the period 1994–99, a number of reports with small patient numbers and short duration described favourable results of GAG treatment on proteinuria in DN (see Abaterusso *et al.* [54] for review). Most of these exploratory studies on the anti-proteinuric activity of GAGs were performed on Type 1 and 2 diabetic patients, and employed sulodexide with different schedule and dosages. Other small trials employed different types of LMW heparin, or Danaparoid (Orgaran®), an extractive GAG mainly composed of HS (see Abaterusso *et al.* [54] for review).

Sulodexide and some other GAG formulations were shown to reduce albuminuria in both micro- and macroalbuminuric patients, and in both Type 1 and 2 diabetic patients. Some variability in results, in particular an apparent more effectiveness in Type 1 versus Type 2 diabetic patients, was explained by the inadequate duration of the trial or by insufficient dosage of the agent [54].

In 2002, the Diabetic Nephropathy and Albuminuria Sulodexide (Di.N.A.S.) study was published; it was a multicentre international European trial designed primarily to perform a dose range finding for oral sulodexide [41]. Data were also analysed to investigate the effect of sulodexide on patients concomitantly treated with ACEIs. Three different sulodexide dosages were used, 50, 100 and 200 mg/day, for 4 months followed by a washout period from sulodexide of 4 months. The hypoalbuminuric effect of 200 mg oral sulodexide was particularly evident in microalbuminuric patients with a 40% reduction, and 60% of them had a reduction higher than 50% of the baseline albuminuria. The intermediate 100 mg/day dosage was also effective. More recent studies in Tunisia [55, 56] have shown the hypoalbuminuric effect of sulodexide 50 mg/day too, which keeps on gradually enhancing and reaching a statistically significant reduction in albuminuria while continuing the treatment for over a year.

According to the Di.N.A.S. study, the hypoalbuminuric effect of sulodexide was independent of the type of diabetes or of basal albuminuria and occurred in the absence of any effect on

metabolic control, blood pressure or serum creatinine [41]. Interestingly, the hypoalbuminuric effect of sulodexide was also noted in the subgroup of subjects already treated with ACEI therapy, suggesting that the treatment with an ACEI does not hinder the activity of sulodexide, and that the two agents have different pharmacological targets [41]. That the effect of GAGs on proteinuria is independent on any action on the renin–angiotensin–
aldosteron system in diabetic patients has been confirmed [7].

The Collaborative Study Group (CSG) has investigated in two sufficiently powered trials (SUN-micro and SUN-macro trials) whether sulodexide 200 mg daily was effective in reducing the progression of DN. The rationale behind the trials was that adding sulodexide treatment to ARBs (protocol-preferred) or ACEIs would have led to a further reduction in albuminuria, since sulodexide affects pharmacological targets other than those impacted by these renin–angiotensin blockers. The Phase III SUN-micro trial was performed on microalbuminuric Type 2 diabetic patients treated with maximal dosage of ACEI/ARB for 12 months and did not disclose any significant difference between the sulodexide treated versus the control arm [57]. The parallel confirmatory study, a Phase 4 trial called SUN-macro, i.e. a randomized, double-blind, placebo-controlled study, comparing 200 mg daily of sulodexide and placebo in Type 2 diabetic patients treated with maximal approved therapy with losartan or irbesartan and with overt nephropathy which had the objective to determine the efficacy of sulodexide in reducing the rate of progression of renal disease (doubling of serum creatinine or ESRD) and adverse clinical events was prematurely terminated in its early-mid phase, after that an interim analysis performed because of the negative conclusion of the Phase III SUN-micro trial showed no effect on proteinuria [58].

In the end, the shoulder-to-shoulder comparison between the Di.N.A.S and the SUN-micro trials seemed in favour of the second, because of the much more robustness in terms of (surrogate) end point and number of investigated patients. Thus, it was concluded that sulodexide is unfortunately ineffective on DN [59]. However, some believe that more studies are necessary to rule out the potential role of sulodexide in DN [60, 61].

DI.N.A.S VERSUS SUN-MICRO: DIFFERENCE IN PROTOCOLS MAY HAVE LED TO DISCREPANT RESULTS

The protocol of the SUN-micro trial was substantially different from those of previous clinical studies with sulodexide and the characteristics of the enrolled patients were also considerably different.

The most striking difference is the severity of DN. In fact, in the SUN patients, it was more severe than in previous smaller trials with sulodexide. For instance, in the DINAs [41], most patients were CKD 2 patients, while the CSG trial recruited mainly CKD 3 subjects [57]. Additionally, the 'microalbuminuric' patients of the SUN trial most likely had a more severe albuminuria because of the hypoalbuminuric effect of maximal dosages of ACEI/ARB. This is also recognized by Lewis *et al.* [57].

In view of the more advanced renal disease, the time needed to demonstrate the favourable effect of sulodexide should probably be longer than in previous trials, including the Di.N.A.S (4 months) and possibly longer than the 6 months of the SUN-micro trial. As a matter of fact, Achour *et al.* [55] have shown that even 50 mg sulodexide, which in the Di.N.A.S at 4 months did not decrease albuminuria, is effective, provided the duration of treatment is sufficiently long.

Also very important are the possible differences between the originator sulodexide (Vessel) and Sulonex. The drug used in the CSG trial was evaluated by testing the heparin fraction with standard tests which establish only a rough similarity between the heparin fractions of the two agents. Furthermore, the dermatan sulphate fractions, which makes up to 20% of the active ingredient portion of the two drugs, were not compared at all. Lewis *et al.* [57] themselves do not rule out the possibility that the drug they used [Sulonex, produced by a different company than the original sulodexide (Vessel) used in the previous studies with positive results [41]] was pharmacologically inactive or not absorbed. As for the existing debate on the equivalence of originator drugs with biosimilars, it remains to be demonstrated whether the sulodexide of Sulonex is bioequivalent to the sulodexide of Vessel.

In addition, the finding that GAGs (Sulodexide) are much more effective on true DN than on other glomerular disorders [62] should also be emphasized. Most of the previous studies with sulodexide had also investigated Type 1 DM patients including the Di.N.A.S trial which enrolled 50% of Type 1 diabetics. In Type 1 DM patients, the onset of albuminuria almost invariably underlies the presence of DN. This is not the case in albuminuric Type 2 DM patients who had heterogeneous pathological findings in European reports [63, 64]. It is likely that such heterogeneity is influenced by the ethnicity. Since most of the initial studies on sulodexide were performed in Europeans, we wonder whether the inclusion of patients from so many different ethnic groups enrolled in the multicentre, multinational SUN-micro and SUN-macro may have influenced the results by affecting the prevalence of true DN among the recruited subjects.

Finally, the Di.N.A.S and the SUN trials used different drugs to target the renin–angiotensin system. In the SUN-trials, the protocol-preferred agents were ARBs (irbesartan or losartan) at maximum approved or tolerated doses in lieu of ACEIs. Whereas, among previous studies on sulodexide, only in the Di.N.A.S trial ~50% of patients received contemporaneous treatment with ACEIs for the treatment of hypertension, but in most cases at non-maximal dosages. As observed, in the Di.N.A.S, the *post hoc* analysis disclosed that sulodexide reduced albuminuria even in patients treated with ACEIs. Although ACEIs and ARBs are generally considered to be interchangeable drugs, some differences do exist—ACEIs also affect the bradykinin pathway while ARBs have partial agonist action on PPAR-γ receptors and thus may have effects on glucose and lipid metabolism that are not shared by ACEIs [65]. While these differences may not apply to the present case, one may speculate that lack of effect on albuminuria of the association between Sulodexide-ARB vis-à-vis Sulodexide-ACEI may be caused by one or more antagonistic mechanisms

exerted by ARBs (and not by ACEIs) on the pharmacological targets of sulodexide.

CONCLUSIONS

While experimental data are very interesting and compelling, particularly in non-immunological models, no conclusive evidence support the use of heparin and other GAGs, including sulodexide, in human renal disease. In acute and chronic glomerulonephritis, not enough studies have been performed and, unfortunately, some of them are not of sufficiently good quality. Sulodexide has been much investigated in DN. In the early DN, the body of data suggests that it has an antialbuminuric activity. Unfortunately, there is no sufficiently long study in these patients on the effect of sulodexide on clinical end points. In other clinical conditions, the drug was certainly capable to impact favourably in terms of clinical hard end points. For instance, in post-myocardial infarction patients, it significantly reduced the risk of mortality and of re-infarction [66].

In the more advanced DN, data are in our opinion inconclusive. In these patients, we guess that before concluding that sulodexide is ineffective for prevention of progression of DN, its use in the following situations should ideally be performed: (i) in Type 2 diabetic patients with true DN, (ii) for longer treatment periods than 4–6 months and (iii) evaluating clinical end points rather than the surrogate albuminuria.

ACKNOWLEDGEMENTS

G.G. has been consultant of Alfa Wasserman SpA, Bologna, Italy (the company produces sulodexide) and has received funds for research from the same company.

CONFLICT OF INTEREST STATEMENT

This article has not been published previously in whole or part.

REFERENCES

1. Lewis EJ, Hunsicker LG, Bain RP *et al.* The effect of angiotensin-converting-enzyme inhibition on diabetic nephropathy. The Collaborative Study Group. N Engl J Med 1993; 329: 1456–1462
2. Lewis EJ, Hunsicker LG, Clarke WR *et al.* Renoprotective effect of the angiotensin-receptor antagonist irbesartan in patients with nephropathy due to type 2 diabetes. N Engl J Med 2001; 345: 851–860
3. Brenner BM, Cooper ME, de Zeeuw D *et al.* Effects of losartan on renal and cardiovascular outcomes in patients with type 2 diabetes and nephropathy. N Engl J Med 2001; 345: 861–869
4. Purkerson ML, Tollefsen DM, Klahr S. N-desulfated/acetylated heparin ameliorates the progression of renal disease in rats with subtotal renal ablation. J Clin Invest 1988; 81: 69–74
5. Purkerson ML, Joist JH, Greenberg JM *et al.* Inhibition by anticoagulant drugs of the progressive hypertension and uremia associated with renal infarction in rats. Thromb Res 1982; 26: 227–240
6. Ichikawa I, Yoshida Y, Fogo A *et al.* Effect of heparin on the glomerular structure and function of remnant nephrons. Kidney Int 1988; 34: 638–644
7. Benck U, Haeckel S, Clorius JH *et al.* Proteinuria-lowering effect of heparin therapy in diabetic nephropathy without affecting the renin–angiotensin–aldosterone system. Clin J Am Soc Nephrol 2007; 2: 58–67
8. Olson JL. Role of heparin as a protective agent following reduction of renal mass. Kidney Int 1984; 25: 376–382
9. Gambaro G, Venturini AP, Noonan DM *et al.* Treatment with a glycosaminoglycan formulation ameliorates experimental diabetic nephropathy. Kidney Int 1994; 46: 797–806
10. Pesce C, Purkerson ML, Klahr S *et al.* Early changes in glomerular size and 3H-thymidine labeling index in controls and heparin-treated rats following subtotal nephrectomy. Kidney Int 1991; 43: 369–380
11. Diamond JR, Karnovsky MJ. Nonanticoagulant protective effect of heparin in chronic aminonucleoside nephrosis. Ren Physiol 1986; 9: 366–374
12. Coffey AK, Karnovsky MJ. Heparin inhibits mesangial cell proliferation in habu-venom-induced glomerular injury. Am J Pathol 1985; 120: 248–255
13. Floege J, Eng E, Young BA *et al.* Heparin suppresses mesangial cell proliferation and matrix expansion in experimental mesangioproliferative glomerulonephritis. Kidney Int 1993; 43: 369–380
14. Burg M, Ostendorf T, Mooney A *et al.* Treatment of experimental mesangioproliferative glomerulonephritis with non-anticoagulant heparin: therapeutic efficacy and safety. Lab Invest 1997; 76: 505–516
15. Peten EP, He CJ, Patel A *et al.* Non-anticoagulant heparin suppresses all collagen mRNA and reduces laminin B1 mRNA expression in sclerotic glomeruli from mice transgenic for growth hormone (GH mice). J Am Soc Nephrol 1993; 4: 780
16. Gambaro G, Cavazzana AO, Luzi P *et al.* Glycosaminoglycans prevent morphological renal alterations and albuminuria in diabetic rats. Kidney Int 1992; 42: 285–291
17. Oshima Y, Isogai S, Mogami K *et al.* Protective effect of heparin on renal glomerular anionic sites of streptozotocin-injected rats. Diabetes Res Clin Pract 1994; 25: 83–89
18. Oturai PS, Rasch R, Hasselager E *et al.* Effects of heparin and aminoguanidine on glomerular basement membrane thickening in diabetic rats. APMIS 1996; 104: 259–264
19. Gambaro G, D'Angelo A, Del Prete D *et al.* Mesangial cell proliferation in long-term streptozotocin-induced diabetes mellitus in the rat and the renoprotective activity of heparin. Am J Nephrol 1999; 19: 530–534
20. Gambaro G, van der Woude FJ. Glycosaminoglycans: use in treatment of diabetic nephropathy. J Am Soc Nephrol 2000; 11: 359–368
21. Caenazzo C, Garbisa S, Ceol M *et al.* Heparin modulates proliferation and proteoglycan biosynthesis in murine mesangial cells: molecular clues for its activity in nephropathy. Nephrol Dial Transplant 1995; 10: 175–184
22. Yung S, Chau MK, Zhang Q *et al.* Sulodexide decreases albuminuria and regulates matrix protein accumulation in C57BL/6 mice with streptozotocin-induced type I diabetic nephropathy. PLoS One 2013; 8: e54501
23. Ceol M, Nerlich A, Baggio B *et al.* Increased glomerular alpha 1 (IV) collagen expression and deposition in long-term diabetic rats is prevented by chronic glycosaminoglycan treatment. Lab Invest 1996; 74: 484–495
24. Ceol M, Gambaro G, Sauer U *et al.* Glycosaminoglycan therapy prevents TGF-beta1 overexpression and pathologic changes in renal tissue of long-term diabetic rats. J Am Soc Nephrol 2000; 11: 2324–2336
25. Weigert C, Brodbeck K, Haring HU *et al.* Low-molecular-weight heparin prevents high glucose- and phorbol ester-induced TGF-beta 1 gene activation. Kidney Int 2001; 60: 935–943
26. Lewis EJ, Xu X. Abnormal glomerular permeability characteristics in diabetic nephropathy: implications for the therapeutic use of low-molecular weight heparin. Diabetes Care 2008; 31(Suppl 2): S202–S207
27. Gil N, Goldberg R, Neuman T *et al.* Heparanase is essential for the development of diabetic nephropathy in mice. Diabetes 2012; 61: 208–216
28. van den Hoven MJ, Rops AL, Bakker MA *et al.* Increased expression of heparanase in overt diabetic nephropathy. Kidney Int 2006; 70: 2100–2108
29. Masola V, Gambaro G, Tibaldi E *et al.* Regulation of heparanase by albumin and advanced glycation end products in proximal tubular cells. Biochim Biophys Acta 2011; 1813: 1475–1482

30. van den Hoven MJ, Rops AL, Vlodavsky I *et al.* Heparanase in glomerular diseases. Kidney Int 2007; 72: 543–548
31. Strutz FM. EMT and proteinuria as progression factors. Kidney Int 2009; 75: 475–481
32. Masola V, Gambaro G, Tibaldi E *et al.* Heparanase and syndecan-1 interplay orchestrates fibroblast growth factor-2-induced epithelial-mesenchymal transition in renal tubular cells. J Biol Chem 2012; 287: 1478–1488
33. Masola V, Onisto M, Zaza G *et al.* A new mechanism of action of sulodexide in diabetic nephropathy: inhibits heparanase-1 and prevents FGF-2-induced renal epithelial-mesenchymal transition. J Transl Med 2012; 10: 213
34. Pletinck A, Van Landschoot M, Steppan S *et al.* Oral supplementation with sulodexide inhibits neo-angiogenesis in a rat model of peritoneal perfusion. Nephrol Dial Transplant 2012; 27: 548–556
35. Webb LM, Ehrengruber MU, Clark-Lewis I *et al.* Binding to heparan sulfate or heparin enhances neutrophil responses to interleukin 8. Proc Natl Acad Sci USA 1993; 90: 7158–7162
36. Ceol M, Vianello D, Schleicher E *et al.* Heparin reduces glomerular infiltration and TGF-beta protein expression by macrophages in puromycin glomerulosclerosis. J Nephrol 2003; 16: 210–218
37. Gambaro G, Skrha J, Ceriello A. Glycosaminoglycan therapy for long-term diabetic complications? Diabetologia 1998; 41: 975–979
38. Nader HB, Buonassisi V, Colburn P *et al.* Heparin stimulates the synthesis and modifies the sulfation pattern of heparan sulfate proteoglycan from endothelial cells. J Cell Physiol 1989; 140: 305–310
39. Broekhuizen LN, Lemkes BA, Mooij HL *et al.* Effect of sulodexide on endothelial glycocalyx and vascular permeability in patients with type 2 diabetes mellitus. Diabetologia 2010; 53: 2646–2655
40. Feldt-Rasmussen B. Microalbuminuria, endothelial dysfunction and cardiovascular risk. Diabetes Metab 2000; 26(Suppl 4): 64–66
41. Gambaro G, Kinalska I, Oksa A *et al.* Oral sulodexide reduces albuminuria in microalbuminuric and macroalbuminuric type 1 and type 2 diabetic patients: the Di.N.A.S. randomized trial. J Am Soc Nephrol 2002; 13: 1615–1625
42. Kristova V, Liskova S, Sotnikova R *et al.* Sulodexide improves endothelial dysfunction in streptozotocin-induced diabetes in rats. Physiol Res 2008; 57: 491–494
43. Vasquez J, Mathison Y, Romero-Vecchione E *et al.* Effect of sulodexide on aortic vasodilation capacity and associated morphological changes in rats with streptozotocin-induced diabetes. Invest Clin 2010; 51: 467–477
44. Christensen PK, Hommel EE, Clausen P *et al.* Impaired autoregulation of the glomerular filtration rate in patients with nondiabetic nephropathies. Kidney Int 1999; 56: 1517–1523
45. Drakeley SJ, Furness PN. Heparin inhibits the binding of immune complexes to cultured rat mesangial cells. Nephrol Dial Transplant 1994; 9: 1084–1089
46. van Bruggen MC, Walgreen B, Rijke TP *et al.* Heparin and heparinoids prevent the binding of immune complexes containing nucleosomal antigens to the GBM and delay nephritis in MRL/lpr mice. Kidney Int 1996; 50: 1555–1564
47. Gambaro G, Kong NC. Glycosaminoglycan treatment in glomerulonephritis? An interesting option to investigate. J Nephrol 2010; 23: 244–252
48. Cade JR, DeQuesada AM, Shires DL *et al.* The effect of long term high dose heparin treatment on the course of chronic proliferative glomerulonephritis. Nephron 1971; 8: 67–80
49. Morita Y, Makino H, Ota K *et al.* Beneficial effect of low molecular weight heparin ('fragmin') on proteinuria of proliferative glomerulonephritis. Clin Nephrol 1994; 42: 346–347
50. Rozita M, Md Shahrir MS, Loo CY *et al.* Glycosaminoglycans (sulodexide) for resistant heavy proteinuria of chronic glomerulonephritides. Nephrology 2008; 13(Suppl 1): A13
51. Bang K, Chin HJ, Chae DW *et al.* Anti-proteinuric effect of sulodexide in immunoglobulin a nephropathy. Yonsei Med J 2011; 52: 588–594
52. Parving HH, Lehnert H, Brochner-Mortensen J *et al.* The effect of irbesartan on the development of diabetic nephropathy in patients with type 2 diabetes. N Engl J Med 2001; 345: 870–878
53. Solini A, Carraro A, Barzon I *et al.* Therapy with glycosaminoglycans lowers albumin excretion rate in non-insulin dependent diabetic patients with microalbuminuria. Diab Nutr Metab 1994; 7: 304–307
54. Abaterusso C, Gambaro G. The role of glycosaminoglycans and sulodexide in the treatment of diabetic nephropathy. Treat Endocrinol 2006; 5: 211–222
55. Achour A, Kacem M, Dibej K *et al.* One year course of oral sulodexide in the management of diabetic nephropathy. J Nephrol 2005; 18: 568–574
56. Blouza S, Dakhli S, Abid H *et al.* Efficacy of low-dose oral sulodexide in the management of diabetic nephropathy. J Nephrol 2010; 23: 415–424
57. Lewis EJ, Lewis JB, Greene T *et al.* Sulodexide for kidney protection in type 2 diabetes patients with microalbuminuria: a randomized controlled trial. Am J Kidney Dis 2011; 58: 729–736
58. Packham DK, Wolfe R, Reutens AT *et al.* Sulodexide fails to demonstrate renoprotection in overt type 2 diabetic nephropathy. J Am Soc Nephrol 2012; 23: 123–130
59. House AA, Weir MA. Sulodexide for diabetic nephropathy: another one bites the dust. Am J Kidney Dis 2011; 58: 692–694
60. Coccheri S. Game not over for sulodexide. Am J Kidney Dis 2012; 59: 467
61. Gambaro G. Discounting the efficacy of sulodexide in diabetic nephropathy is premature. Am J Kidney Dis 2012; 60: 169–170
62. Gambaro G, Venturini AP, Chieco AO *et al.* Different heparin dosage are needed o ameliorate the evolution of different experimental nephropathies. Does this mean different heparin targets? Kidney Int Suppl 1995; 47: 689
63. Parving HH, Gall MA, Skott P *et al.* Prevalence and causes of albuminuria in non-insulin-dependent diabetic patients. Kidney Int 1992; 41: 758–762
64. Fioretto P, Mauer M, Brocco E *et al.* Patterns of renal injury in NIDDM patients with microalbuminuria. Diabetologia 1996; 39: 1569–1576
65. Ernsberger P, Koletsky RJ. Metabolic actions of angiotensin receptor antagonists: PPAR-gamma agonist actions or a class effect? Curr Opin Pharmacol 2007; 7: 140–145
66. Condorelli M, Chiariello M, Dagianti A *et al.* IPO-V2: a prospective, multicenter, randomized, comparative clinical investigation of the effects of sulodexide in preventing cardiovascular accidents in the first year after acute myocardial infarction. J Am Coll Cardiol 1994; 23: 27–34

Received for publication: 28.4.2013; Accepted in revised form: 4.8.2013