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THE EFFECTS OF THE THERAPY WITH NATURAL GLYCOSAMINOGLYCANS (SULODEXIDE) ON PROTEINURIA IN DIFFERENT TYPES OF GLOMERULONEPHRITIS

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Summary. *Alterations of the glycosaminoglycans (GAG) from the glomerular basement membrane have been demonstrated in glomerulonephritis (GN) with heavy proteinuria. Experimental data suggest that GAG could be used in the treatment of GN for their antiproteinuric and antiproliferative effects. The aim of this study was to assess the evolution of proteinuria during the therapy with a natural GAG (Sulodexide), which comprises a heparin-like substance and dermatan-sulphate. The study was carried out in 20 patients with different types of GN (males-12; females-8; mean age- 38.95±12.49 y). 18 p underwent kidney biopsy processed in light microscopy. In 9 p histochemical stainings were performed (toluidine blue, Alcian blue) in order to reveal GAG deposits and immunohistochemical stainings (Cytokeratin, Desmin, Vimentin, PCNA, Actin) to evaluate interstitial involvement in renal fibrosis. Sulodexide was administered i.v., 1 ampoule daily, for 30 days. The following parameters were assessed at the beginning and at the end of the therapy: hemoglobin, leucocytes, fibrinogen, thrombocytes, prothrombin index, BUN, serum creatinine, creatinine clearance, hematuria. Proteinuria was evaluated initially and on day 8, 16, 30 of therapy and after 10 days from the discontinuation of therapy. The short-course i.v., therapy with Sulodexide reduced proteinuria in 85% of the patients. The initial mean value of proteinuria in these patients decreased significantly at the end of the therapy ($p<0.01$). Proteinuria decreased in all patients with mesangio-capillary (MCGN), whereas the therapeutic result was far more modest in patients with membranous- and mesangio-proliferative GN. The decline of proteinuria is more relevant in GAG(+) patients, with important proximal tubular necrosis and moderate to severe myofibroblast infiltrates in comparison with GAG(-) patients, with mild interstitial involvement. The therapy had no significant side effects. The short-course therapy with Sulodexide decreases significantly proteinuria in patients with MCGN and in p with interstitial GAG deposits, without any significant side effects.*

Key words: *Glomerulonephritis, proteinuria, glycosaminoglycans, interstitial fibrosis, Sulodexide, renal function*

Introduction

Over the last years, basic and clinical research has brought new insights into the pathogenic sequence of glomerulonephritis (GN). Relevant details with regard to the electronegative filter of the glomerular basement membrane (GBM) and to the role of certain glycosaminoglycans (GAG-heparan-sulphate) in the layout of this filter have been recently forwarded (1). Furthermore, alterations of the electronegative filter, (of GAG from the GBM respectively), have been demonstrated in glomerulonephritis with heavy proteinuria. At the same time, it has been demonstrated that high level proteinuria contributes to the evolution of this disease towards chronic renal failure (CRF)

These data have pointed to the idea that the decline of proteinuria might slow the progression rate of GN towards CRF. and that proteinuria could be reduced by recovering the GAG layer of the GBM by means of exogenous intake of GAG,. Studies which assess the evolution of proteinuria during the therapy with heparin have been initiated accordingly (2).

Sulodexide (Vessel Due F from ALFA Wassermann S.p.A Bologna, Italy) is a natural extract from bowel mucosa, which contains a heparin-like substance (80%) and dermatan sulphate (20%). This drug is being used in vascular diseases at a high risk of thrombosis because it inhibits several coagulation factors, improves rheological parameters and also activates lipoproteinlipase, thus controlling hyperlipidaemias (3). Sulodexide also seems to reduce proteinuria in diabetic nephropathy (4,5).

In our study we have used Sulodexide as an exogenous source of GAG and we have investigated the effects of this intake on proteinuria and the glomerular function in patients with different types of glomerulonephritis.

Methods

This prospective study was carried out on a group of 20 patients with different types of glomerulonephritis (12 males, 8 females, mean age 38.95±12.49 years), admitted to the Dpt. of Nephrology, Timisoara, during 1996-1998.

All patients were assessed from a clinical and biological point of view and 18 patients out of this group also underwent a percutaneous echoguided renal biopsy.

Sulodexide was administered i.v., 1 ampoule daily, for 30 days. The following parameters were assessed at the beginning and at the end of the therapy with Sulodexide: hemoglobin, leucocytes, fibrinogen, thrombocytes, prothrombin index, serum ureea, serum creatinine, creatinine clearance and hematuria.

Proteinuria was evaluated initially and on day 8, 16, 30 of therapy as well as 10 days after the therapy was discontinued (samples 1,2,3,4,5,respectively).

In 9 patients histochemical stainings were performed (toluidine blue and alcian blue) in order to reveal GAG deposits (heparan- sulphate and chondroitin- sulphate). Immunohistochemical stainings were also used (DAKO kits), as follows:

- Cytokeratin MNF116 for the assessment of the distal and collecting tubules
- Desmin D33 for proximal convoluted tubules
- Actin A4 for myofibroblasts
- Vimentin Vim6 for mesangial cells
- PCNA PC 10 (proliferating cell nuclear antigen) to reveal the proliferation of mesangial and tubular cells and of myofibroblasts.

On these histological sections we studied the presence of: proximal and distal tubular necrosis; GAG deposits; interstitial fibrosis; proliferation of tubular, mesangial and interstitial cells; myofibroblasts.

The immunohistochemistry data were evaluated by means of the following histological index:

- *Interstitial infiltrate with myofibroblasts*:
  - mild < 2 myofibroblasts in the peritubular area
  - moderate 2-5 peritubular myofibroblasts
  - severe > 5 peritubular myofibroblasts / numerous periglomerular myofibroblasts.
- *Proximal tubular necrosis*
  - absent
  - present
- *Number of mesangial cells per glomerulus* – standard score
- *Proliferation evaluated by PCNA*
  - mild - < 5% stained nuclei
  - moderate - 5-15% stained nuclei
  - severe - >15% stained nuclei
- *Distal tubular necrosis*
  - mild - <2 cellular necrosis/ tubule
  - moderate to severe - >2 cellular necrosis/ tubule.

Results

Kidney biopsies performed in 18 patients revealed:

- mesangio- capillary GN (MCGN) - 8 cases
- mesangial proliferative GN (MPGN) - 4 cases
- membranous GN (MGN) - 3 cases
- focal glomerular sclerosis and hyalinosis (FGSH) - 1 patient
- acute diffuse GN (ADGN) - 1 case
- renal amyloidosis (RA) - 1 case

Associated diseases were liver chirrosis - 1 patient and rheumatoid arthritis, 1 patient.

From the clinical and biological standpoint, the patients presented the following parameters at the beginning of the study:

- nephrotic range proteinuria - 9 cases (3.5-5.4 g/24h)
- non-nephrotic proteinuria - 11 patients (0.3-2.5 g/24h)
- azotemia - 11 patients (serum creatinine - 1.7-4.8 mg%)
- hypertension - controlled under treatment in all cases.

Angiotensin converting enzyme inhibitors, steroids and non-steroidal antiinflammatory drugs were not administered during the study.

Table 1 displays the average values, the standard deviation and the results of the Student t-test assessed for the above-mentioned parameters.

In our group, proteinuria decreased between the samples 1 and 5 in 16 out of 20 patients (80% of the cases), while between the samples 2 and 5, proteinuria decreased in 15 out of 20 patients (75%).

Average values of proteinuria and standard deviation evaluated in 5 samples of proteinuria collected from the investigated patients and their statistic significance are presented in Table 2.

The response to the treatment with Vessel Due F was variable and showed the following pattern:

- in patients with MCGN (8 cases) – proteinuria decreased in all cases
- in patients with MGN (3 cases) – proteinuria de-

Table 1. Basal and post-treatment laboratory parameters

Variable	Initial	Final	Significance
Hb (g/dl)	11.3 ± 2.25	10.88 ± 2.05	0.42
Leucocytes (/mm²)	6205 ± 1784	6380 ± 2091	0.43
Fibrinogen (mg/dl)	452.94 ± 109.42	421.10 ± 158.45	0.23
Thrombocytes (x10³/mm³)	188.94 ± 63.14	198.5 ± 46.25	0.29
Prothrombin index (%)	87.72 ± 22.43	85.09 ± 23.96	0.36
Serum urea (mg/dl)	66.9 ± 46.21	69.5 ± 48.72	0.43
Serum creatinine (mg/dl)	2.4 ± 1.42	2.35 ± 2.60	0.47
Creatinine clearance (ml/min)	52.4 ± 37.01	54.59 ± 36.21	0.42
Hematuria (NR/ml/min)	17126 ± 45397	10175 ± 26911	0.27

Table 2. Basal and follow-up levels of proteinuria

Sample	Proteinuria 1	Proteinuria 2	Proteinuria 3	Proteinuria 4	Proteinuria 5
Value (g/24h)	2.9±1.29	2.95±1.30	2.77±1.52	2.3±1.33	2.05±1.26
Significance					
vs Proteinuria 1		0.45	0.38	0.15	**0.02**
vs Proteinuria 2			0.34	0.09	**0.01**

creased in 1 patient

- in patients with MPGN (4 cases) – proteinuria decreased in 2 cases
- in patients with ADGN, amyloidosis, FGSH, as well as in patients who did not undergo renal biopsies-proteinuria decreased in all cases.

Statistical analysis and correlations between these results and the types of GN were not possible due to the small number of the investigated cases. Individual analysis however showed in each case a wavy pattern evolution of proteinuria, consistent with the general trend of the whole group.

GAG deposits (heparan- sulphate and chondroitin sulphate) were evidenced in 4 patients out of the group of 9 patients who had the renal biopsies processed by histochemical and immunohistochemical stainings. These GAG deposits were located in the interstitium and /or the peritubular areas, in the periglomerular area and in the capillary loops. In the other 5 cases of this group, GAG deposits were absent. The histological diagnosis of these 9 patients was as follows:

- GAG+ patients: -MCGN- 3 cases
  -MPGN- 1 case
- GAG- patients: -MCGN- 2 cases
  -MPGN- 1 case
  -MGN- 1 case
  -ADGN- 1 case

The immunohistochemical stainings performed in this group of 9 patients revealed: - proximal tubular necrosis in 75% of the GAG+ patients and in 20% of the GAG- patients; - moderate to severe distal tubular necrosis in 25% of the GAG+ patients and in 40% of the GAG- patients; - moderate or severe myofibroblast infiltrate in 75% of the GAG+ patients and in 60% of the GAG- patients.

Proteinuria and serum creatinine in GAG+ and GAG- patients had the following evolution during therapy with Sulodexide (Table 3).

Table 3. Proteinuria and creatinine levels during therapy

	GAG(+)	GAG(–)
Proteinuria initial g/24h	3.42 ± 1.4	2.66 ± 1.2
Proteinuria final g/24h	2.47 ± 1.3	2.38 ± 0.87
Creatinine initial mg/dl	2.47 ± 1.2	1.50 ± 0.9
Creatinine final mg/dl	2.27 ± 1.1	1.47 ± 0.8

The short-term therapy with Sulodexide provided inconsistent response in the investigated patients. Proteinuria decreased in all patients with MCGN, whereas the therapeutic result was far more modest in patients with MGN or MPGN. Because of the small number of patients we evaluated, statistical analysis and correlations between the pathological features of GN and the anti-proteinuric effect of Sulodexide were not possible. The results presented in Figures 3 and 4 depict the individual tendency of the therapeutical response to Sulodexide, in relationship with the type of GN (Fig. 1,2,3,4).

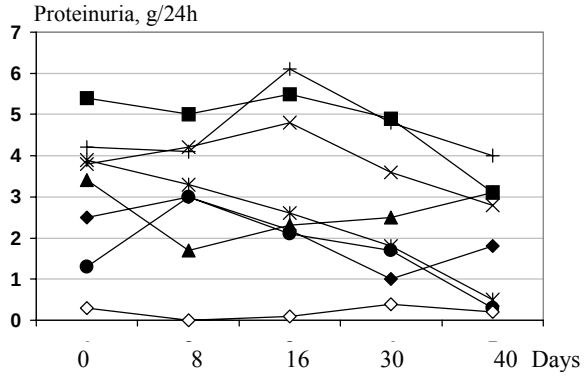

Fig 1. Proteinuria in the MCGN patients under sulodexide treatment

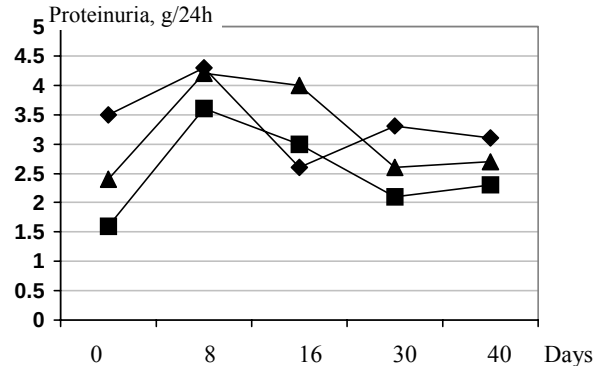

Fig 2. Proteinuria in MGN patients treated with sulodexide

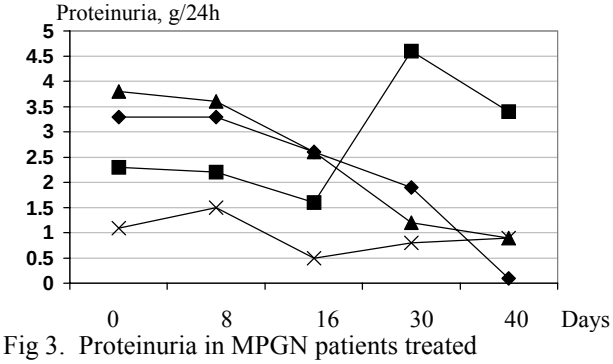

Fig 3. Proteinuria in MPGN patients treated with sulodexide

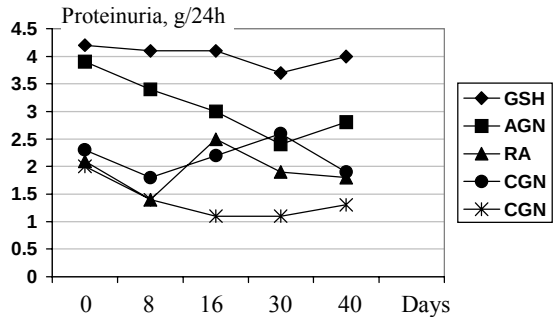

Fig 4. Proteinuria of patients with glomerular fibrosis and hyalinosis (GSH), acute glomerulonephritis (AGN), renal amiloidosis (RA), and chronic glomerulonephritis (CGN) without kidney biopsy treated with sulodexide

Discussion

Over the last decade a large number of studies have advanced the role of GAG, especially of heparan- sulphate, in the structure of the electronegative filter of the GBM (1). These authors have evidenced a straightforward relationship between the concentration of GAG in the GBM and the level of proteinuria in numerous glomerulonephritis (lupus nephritis, IgA nephropathy, MGN, MCGN, minimal change GN, diabetic nephropathy). At the same time they issued the hypothesis that the administration of heparin- sulphate might reestablish the concentration of GAG in the anionic filter and, which could thus lead to the decline of proteinuria in patients with different types of glomerulonephritis.

In 1994-1997 several favourable results with GAG in diabetic nephropathy, in both insulindependent and non- insulindependent diabetes mellitus were published (4,6). Sulodexide, a natural compound of GAG, containing an important amount of heparian-like substances, was used in the treatment of proteinuria and microalbuminuria in diabetic nephropathy by Ionescu-Targoviste et al, with encouraging results in 1997 (7).

In this paper we have evaluated the effects of Sulodexide on proteinuria in patients with different types of glomerulonephritis. In our group, the short-term i.v. therapy with Sulodexide (one month) reduced proteinuria in 85% of the investigated patients. The initial average value of proteinuria in these patients (2.90±1.29 g/24h) decreased significantly ($p<0.01$) after the 30-day therapy with Sulodexide (2.05±1.26 g/24h).

More recently, attention has focused on renal effects of heparin. Caenazzo et al. (2), Gambaro et al. (5) have demonstrated the antiproliferative effect of heparin on mesangial cells, as well as its effects on the metabolism of GAG, fact which involves the correction of abnormalities of the matrix/ GBM ratio. Caenazzo et al. have also suggested that heparin might modulate the synthesis of matrix proteins and could intervene in the availability of growth factors, in the migration of fibroblasts and in the processes of fibrosis. These actions of heparin can be ascribed to its properties to modify the metabolism of decorin- a dermatan- sulphate GAG.

The therapy with Sulodexide in the one-month scheme was not accompanied by any notable side effects. The mean values of hemoglobin, leukocytes, fibrinogen and the prothrombin index did not change significantly during the treatment and no significant changes in azotemia and the creatinine clearance were recorded.

In 9 patients histochemical and immunohistochemical stainings were performed on the kidney biopsy specimens. In this group, GAG deposits (heparan- sulphate and chondroitin sulphate) were revealed with toluidine-blue and Alcian-blue stainings, with the following localization: -interstitium, -peritubular, -periglomerular and in the capillary walls in 44.44% of the patients (4 cases). These results divided our group of patients into two subgroups i.e.: GAG (+) and GAG (-) patients. The immunohistochemical stainings underlined the fact that, generally, in a relatively high percentage of cases the GAG(+) patients presented proximal tubular necrosis (75%) and moderate to severe myofibroblast infiltrates, when compared to the GAG(-) patients. No significant differences between the two subgroups were noticed with regard to distal tubular necrosis or the PCNA staining (Fig. 5).

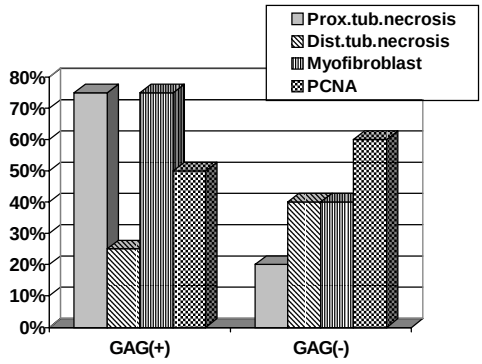

Fig 5. Kidney damage in GAG(+) and GAG(-) patients

Although we are dealing with small number of patients, the clinical and biological picture of glomerulonephritis seems to be more severe in GAG(+) patients. The mean value of proteinuria in GAG(+) patients (3.24±1.4 g/24h) is higher than that one in GAG(-) patients (2.66±1.2 g/24h). In addition, the mean value of serum creatinine is higher in GAG(+) patients (2.47± 1.2 mg%) in comparison with GAG(-) patients (1.5± 0.9 mg%).

The role of GAG in the processes of interstitial renal fibrosis has been suggested by Davies et al (8). It is worth mentioning that in two previous studies we demonstrated that interstitial GAG deposits, as well as the location of GAG in the peritubular areas, correlated directly with severe forms of glomerulonephritis and with a significant process of interstitial fibrosis (9,10). It is debatable whether or not the administration of a natural GAG derivative would influence both, the alterations of the anionic filter and these processes of fibrosis.

The treatment with Sulodexide, performed after the

above mentioned protocol in 9 patients evaluated by histochemical and immunohistochemical stainings, revealed that the decline of the mean values of proteinuria is more important in GAG(+) patients (from 3.42± 1.4 g/24h to 2.47±1.3 g/24h) in comparison with GAG(-) patients (from 2.66±1.2 g/24h to 2.38±0.17 g/24h). It also is worth pointing out the fact that this therapy did not significantly alter the serum creatinine in this group. The small number of patients prevents us from applying an adequate statistical analysis of the results. Therefore, reiterated renal biopsies should be performed after a long-term therapy with Sulodexide, in order to demonstrate the efficacy of this treatment on renal interstitial fibrosis.

In conclusion, we might state that GAG(+) patients presented more severe forms of glomerulonephritis. From the histological point of view, it seems that the incidence of proximal tubular necrosis and the moderate to severe myofibroblast infiltrate was much higher in this category of patients.

Based on the foregoing discussion, we suggest that the short-term therapy with Sulodexide decreases proteinuria significantly in patients with different types of GN. This effect seems more relevant in patients with MCGN and in patients with interstitial GAG deposits.

Further studies are required in order to draw pertinent conclusions with regard to the relationship between exogenous GAG therapies and their influence on proteinuria and renal fibrosis.

References

1. Van Den Born J, Berden J.H.M. Glomerular heparan sulpphate in renal filtratiion and glomerular disease with emphasis on diabetic nephropathy, in: Bilo H.J.C., Viberti G.C. Diabetic nephropathy. De Weezenlanden series no.2, Zwolle, 1994, 83
2. Caenazzo C, Garbisa S, Ceol M, Baggio B, Borsatti A, Marchi E, Gambaro G. Heparin modulates proliferation and proteoglycan biosynthesis in murine mesangial cells: a molecular clue for its activity in nephropathy, Nephrol Dial Transplant 1995, 10:175
3. Gaddi A, Galetti C, Illuminati B, Nascetti S. Meta-analysis of some results of clinical trials on sulodexide therapy in peripheral occlusive arterial disease J Int Med Res 1996; 24:389-406
4. Dedov I, Shestakova M, Vorontzov A, Palazzini E. A randomized, controlled study of sulodexide therapy for the treatment of diabetic nephropathy Nephrol. Dial.. Transplant.. 1997, 12:2295-2300
5. Gambaro G, Venturini A.P, Noonan D.M, Fries W, Re G, Garbisa S, Milanesi C, Pesarini A, Borsatti A, Marchi E, Baggio B Treatment with a glycosaminoglycan formulation ameliorates experimental diabetic nephropathy, Kidney Int 1994, 46:797
6. Solini A, Carraro A, Barzon I, Crepaldi G. Therapy with glycosaminoglycans lowers albumin excretion rate in non insulin dependent diabetic patients with macroalbuminuria, Diab Nutr Metab, 1994, 7:304
7. Ionescu-Tirgoviste C, Dumitrescu C, Hancu N, Serban V, Mota M, Veresiu D, Lichiardopol R, Farcasiu E, Ghise G, Graur M. Tratamentul cu sulodexid in nefropatia diabetica. Studiu clinic efectuat in Romania, Viata Medicala 1997
8. Davies M, Kastner S, Thomas GJ. Proteoglycans: their possible role in renal fibrosis Kidney Int 1996, suppl 54, S55
9. Petrica L, Schiller Ad, Raica M, Velciov S, Gluhovschi Gh, Trandafirescu V, Bozdog Gh. Aspects of mesangio- capillary chronic glomerulonephritis due to glycosaminoglycans, The XXXVth Congress of ERA-EDTA, Rimini, Italy, 1998
10. Schiller Ad, Raica M, Petrica L, Gluhovschi Gh, Trandafirescu V. Glycosaminoglycan (GAG) renal deposits and interstitial fibrosis in chronic primary GN, The XXXVth Congress of ERA-EDTA, Rimini, Italy, 1998

UTICAJ TERAPIJE PRIRODNIM GLIKOZAMINOGLIKANIMA (SULODEKSID) NA PROTEINURIJU U RAZLIČITIM OBLICIMA GLOMERULONEFRITISA

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Kratak sadržaj: *U glomerulonefritisu sa masivnom proteinurijom nađene su promene glikozaminoglikana (GAG) glomerulske bazalne membrane. Eksperimentalni nalazi sugerišu da se antiproteinuričkі i antiproliferativni efekti GAG mogu koristiti u terapiji. Cilj ovog ispitivanja bio je da se ispita kretanje proteinurije u toku terapije sa GAG (Sulodeksid), koji sadrže heparinu-slične substance i dermatan sulfat. Kratkotrajna i.v. terapija Sulodeksidom dovela je do smanjenja proteinurije u 85% bolesnika. Na kraju lečenja došlo je do statistički značajnog smanjenja proteinurije ($p<0,01$). Smanjenje proteinurije nađeno je u svih bolesnika sa MCCG, dok je rezultat bio skroman u bolesnika sa membranoznim i mezangioproliferativnim GN. Sniženje proteinurije bilo je izraženije u GAG(+) bolesnika. Nije bilo značajnih nuzgrednih efekata terapije.*
U zaključku, kratkotrajna terapija sa Sulodeksidom dovela je do značajnog sniženja proteinurije u bolesnika sa MCGN i bolesnika sa depozitima GAG.

Ključne reči: *Glomerulonefritis, proteinurija, glikozaminoglikani, intersticijska fibroza, Sulodeksid, bubrežna funkcija*