Pentosan polysulfate sodium prevents kidney morphological changes and albuminuria in rats with Type 1 diabetes

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ABSTRACT

Decreased levels of glycosaminoglycans (GAGs) have been observed in the kidney and other organs, in human and animal models of diabetes. Long-term administration of heparins and other glycosaminoglycans has demonstrated a beneficial effect on morphological and functional kidney abnormalities in diabetic rats. We assessed the effect of pentosan polysulfate sodium (PPS), a semi-synthetic glycosaminoglycan with low anticoagulant activity, on kidney involvement in streptozotocin diabetic rats. Diabetes was induced in male Sprague-Dawley rats by i.v. administration of streptozotocin (STZ). Animals were randomly allocated to three groups: C = control, STZ and STZ + PPS = pretreated with PPS (15mg/kg, s.c.). After three months of follow-up, blood and 24 h-urine samples were obtained, the animals were sacrificed and the kidney microdissected for morphometric analysis. Urinary albumin excretion was markedly increased in untreated diabetic rats (C = 0.26 ± 0.03 vs STZ = 7.75 ± 1.8 mg/24 h) and PPS treatment partially prevented the albumin rise (3.7 ± 0.7 mg/24 h), without affecting the metabolic control HbA1c (C = 3.6 ±1.7; STZ = 8.82 ± 0.47; STZ + PPS = 8.63 ± 0.54). Electron microscope observation revealed typical renal lesions described in experimental diabetes (STZ group). PPS administration prevents the tubular basement membrane thickening and the loss of cytoarchitecture induced by experimental diabetes. Our data demonstrate that long-term administration of PPS has a favourable effect on morphological and functional abnormalities in kidneys of diabetic rats and suggests a potential therapeutic use for this compound.

Key words: Microalbuminuria. Glicosaminoglicanos. Kidney damage. Diabetes

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El pentosán polisulfato de sodio previene las alteraciones morfológicas renales y la albuminuria en ratas con diabetes tipo 1

RESUMEN

Se ha reportado una disminución de los valores de glicosaminoglicanos (GAG) en el riñón y otros órganos en modelos experimentales de diabetes y en humanos. La administración a largo plazo de heparina y otros GAG previene las alteraciones morfológicas y funcionales del riñón en ratas diabéticas. Evaluamos el efecto del pentosán polisulfato de sodio (PPSNa), un mucopolisacárido semisintético similar a los GAG y de baja actividad anticoagulante, sobre la función renal y los cambios estructurales en ratas diabéticas. La diabetes fue inducida a ratas Sprague-Dawley mediante la administración i.v. de estreptozotocina (STZ). Los animales fueron distribuidos al azar en tres grupos (C = control, STZ y STZ + PPSNa = pretratados con 15 mg/kg/día de PPSNa s.c.). Después de 3 meses se tomaron muestras de sangre y de orina de 24 horas; los animales fueron sacrificados y los riñones extraídos mediante microdisección para el análisis morfométrico. Los animales del grupo STZ presentaron un incremento importante de la excreción de albúmina en orina (C = 0,26 ± 0,03 frente a STZ = 7,75 ± 1,8 mg/24 h), que fue parcialmente revertido por el pretratamiento con PPSNa (3,7 ± 0,7 mg/24 h), sin afectar al control metabólico, HbA_{1c} (C = 3,6 ± 1,7; STZ = 8,82 ± 0,47; STZ + PPSNa = 8,63 ± 0,54). En las micrografías electrónicas se observan las lesiones renales típicas descritas en la diabetes experimental (grupo STZ). La administración de PPSNa previene el engrosamiento de la membrana basal tubular y la pérdida de la citoarquitectura inducida por la diabetes. Nuestros resultados demuestran que la administración de PPSNa previene parcialmente el daño renal en este modelo experimental y sugieren un potencial uso terapéutico de este compuesto.

Palabras clave: *Microalbuminuria. Glicosaminoglicanos. Daño renal. Diabetes.*

INTRODUCTION

The efficacy of glycosaminoglycans (GAG) in decreasing urinary albumin excretion in diabetic nephropathy has

been widely demonstrated in various experimental studies and in humans.[1-3] This has stimulated clinical research using these compounds not only in this pathology but also in other microvascular complications of diabetes[4,5] and in different types of glomerulonephritis.[6]

Studies have been conducted with modified molecules in order to obtain preparations with low anticoagulant activity to improve the safety profile. The modified molecules derived from heparin have a similar effectiveness to dermatan sulfate for lowering albumin excretion and inhibiting the expression of TGFb1. This proves that the fraction of heparin is not essential for this activity.[7] Furthermore, studies have been performed with molecules with different degrees of sulfation,[8] with proteoglycans that bind TGFb such as decorin,[9] and GAG with a similar chain to heparan sulfate such as danaparoid sodium.[10] This yielded mixed results. However, the most commonly used GAG in medical practice is sulodexide, a GAG compound of a fast-moving heparin fraction and dermatan sulfate.[11-15]

Pentosan polysulfate sodium (PPS) is a semisynthetic mucopolysaccharide that is structurally similar to GAGs. This has been widely used in the treatment of interstitial cystitis, showing efficacy in controlling symptoms and an appropriate safety profile and tolerance for the range of doses used.[16-18]

PPS shows some similarity with the effects of sulodexide. They both have fibrinolytic effect and activate the lipoprotein lipase and hepatic triglyceride lipase,[19-22] effects that would be beneficial in diabetic patients. PPS anticoagulant activity is very low, about 15 times lower than that of heparin, has a better absorption profile through oral administration than GAG[16] and *in vitro* studies have shown that it inhibits cell proliferation mediated by the heparin-binding growth factors.[23]

Pharmacokinetic studies on rats, through the administration of radiolabeled PPS, show that it concentrates mainly in the kidneys and urinary tract.[24] This suggests that it may have a possible functional role in these structures.

The possibility that PPS prevents or decreases albumin excretion in diabetic nephropathy has not been evaluated. Therefore, our aim is to evaluate whether PPS decreases urinary albumin excretion in rats that have been made diabetic through the administration of streptozotocin (STZ) and whether it can prevent the morphological changes in the kidney caused by diabetes.

MATERIAL AND METHODS

Animals

The laboratory animals were male albino Sprague-Dawley rats, between 6 and 7 weeks of age, weighing between 220g and 250g, originating from the laboratory animal facility of the Escuela José María Vargas, School of Medicine, UCV, Caracas, Venezuela. These rats were kept with alternating periods of light and dark, and they were allowed free access to water and food (standard feed for laboratory rats, containing approximately 20% protein).

The rats were randomly assigned to two groups: a control group and a group that was induced with diabetes. All experiments were performed following the best practices for handling laboratory animals[25] and were approved by the Bioethics Committee of the Escuela de Medicina (School of Medicine) José María Vargas.

Induction of Experimental Diabetes

Diabetes was induced through the administration of a single injection of STZ (Sigma Chemical Co., St. Louis, MO), at a dose of 60mg/kg of weight, in the caudal vein. The induction of diabetes was confirmed by measuring blood glucose levels using an enzymatic method (Glucose HK Reagent, Bayer) at 2 and 7 days after STZ administration. A control group was kept in which diabetes was not induced. This group was only administered a saline solution in the caudal vein. After 7 days, the control and diabetes-induced animals were randomly assigned to the following treatment groups:

Control: saline solution or vehicle.

STZ-Control: STZ.

STZ-PPS: STZ + 15mg/kg/day of PPS administered subcutaneously for 3 months.

PPS was administered subcutaneously 5 days a week (Monday to Friday). The animals did not receive insulin during the experiment. During the monitoring period, the animals' weight, blood pressure and capillary glucose levels were measured every month. At the end of the treatment period, blood samples from the caudal vein in the tail were taken to determine glycaemia and HbA_{1c}. The animals were placed in metabolic cages to collect urine for 24 hours in order to determine albuminuria. The animals were then put down and their kidneys were extracted by microdissection for morphometric analysis.

Glycaemia was measured in the blood samples through an enzymatic assay using a commercial kit (Glucose HK Reagent, Bayer). HbA_{1c} and urinary albumin excretion were measured using commercial kits (DCA 2000®, Bayer).

Measuring Blood Pressure

Blood pressure readings were conducted on conscious rats employing a non-invasive method using a digital

plethysmograph of the tail (LE 5000, LETICA Scientific Instrument, Barcelona, Spain).

The rats were subjected to heat (42ºC) for 15 minutes in an oven (Memmert, 854 Schawabach, Germany) to vasodilate the peripheral vessels.[26] Subsequently, they were immobilised in a restraint and the blood pressure measuring device was attached to their tail. This device was connected to a pulse transducer that recorded this parameter. The readings reported are the average of three successive readings.

Ultrastructural and Morphometric Analysis

Kidney cortex samples, 2mm in diameter, were extracted. The samples were fixed in 3% glutaraldehyde and 1% OsO4 in Milloning's phosphate buffer (pH = 7.4, 320 mOsmol). They were then dehydrated in increasing concentrations of ethanol and embedded in Epon. After being embedded, the samples were sectioned in a Porter-Blum MT2-B ultramicrotome, contrasted with uranyl acetate and lead citrate and examined under a JEM-1011 transmission electron miscroscope with an accelerating voltage of 80 kV.

The digital records of the images were analysed with a morphometry software (Image-Tool version 3.0), which measured the basement membrane thickness (n=16/each treatment).

Statistical Analysis

The data were expressed as the mean ± SEM and were plotted and analysed using the GraphPad Prism version 4.1 and STATISTICS version 7 software. The comparison between the arithmetic means of the groups was performed using analysis of variance (ANOVA). Those values with $P<.05$ were considered statistically significant.

RESULTS

Table 1 shows the values for weight, glycaemia and urine volume for the groups studied. The groups which were induced with diabetes showed a significant increase in glycaemia and urine volume excreted in 24 hours, as well as a lower weight compared with the control group after 3 months of treatment.

Induction of diabetes with STZ produced a significant increase in urinary excretion of albumin as compared to the control group (C=0.27 ± 0.03; STZ=7.8mg/24 h ± 1.8). Treatment with PPS for 3 months partially prevented this increase (STZ+PPS=3.7mg/24h ± 0.70), decreasing albumin excretion by 52.5% compared to the untreated diabetic group (Figure 1).

HbA_{1c} values in the treatment groups were: C=3.6 ± 1.7; STZ=8.8 ± 0.47 and STZ+PPS=8.65% ± 1.23. This clearly shows that treatment with PPS does not alter the increase in the HbA_{1c} values which are characteristic of this diabetes model. Additionally, there were no significant differences between values of mean arterial pressure between the different treatment groups (C=122.8 ± 5.1; STZ=134.6 ±12 and STZ+PPS=110mm Hg ± 5.7).

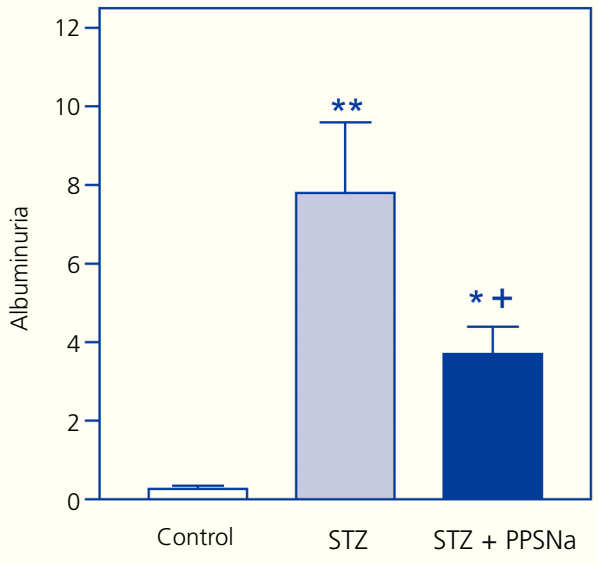
The electron micrographs (Figure 2) show the normal appearance of kidney tubules in the control rats (A) with adequate interdigitation and basement membrane of normal thickness. Note that the capillary has a uniform and thin endothelium. In contrast, significant tubular damage was observed in the STZ (B) group with loss of cytoarchitecture as evidenced by the disappearance of interdigitations and thickening of the basement membrane. Treatment with PPS (C) partially prevented tubular damage since more interdigitations were observed than with STZ but they were arranged irregularly and did not correspond with the number of mitochondria.

Morphometric analysis of the thickness of the tubular basement membranes for the various treatment groups (Figure 3) produced the following values: C=0.077μm ± 0.003; STZ=0.266μm ± 0.021 and STZ+PPS=0.082μm ± 0.04. The thickness of the basement membrane increased significantly in the STZ group compared to the control group, while in the group of rats with diabetes who received treatment with PPS the thickening of the basement membrane was much lower. There was no significant difference with the control group but there was a statistically significant difference with the STZ group.

Table 1. Parameters evaluated at 3 months

	Control	STZ	STZ + PPS
Body weight (g)	383.1 ± 3.7	283 ± 7.1[a]	299.7 ± 3.2[a]
Glycaemia (mg/dl)	95 ± 7.6	632 ± 27[a]	556.8 ± 88[a]
Urinary Vol. (ml/24 h)	17.4 ± 0.4	109.3 ± 12.9[a]	105.2 ± 25[a]

Data correspond to the mean ± SEM of 6-10 rats. [a]p <.01 compared to control



Data correspond to the mean ± SEM of 6-10 rats.
*P <.05 **P <.01 versus control and P <.01 comparing the STZ
+PPS group versus the STZ group

Figure 1. Urinary albumin excretion in 24 hrs in control rats, diabetic (STZ) rats y diabetic rats treated with pentosan polysulfate sodium (STZ + PPS).

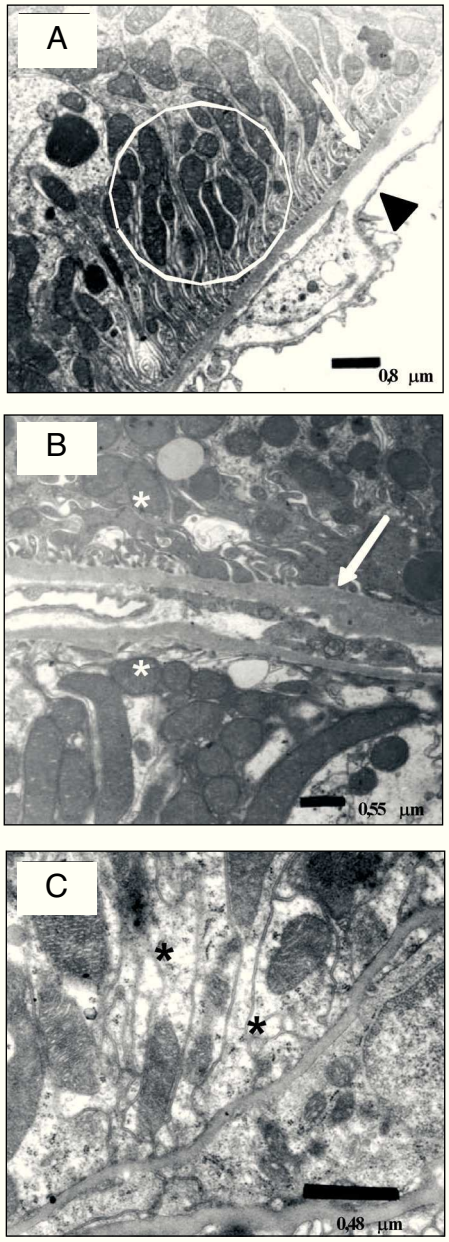


Figure 2. (A) Control: Regular arrangement of interdigitations and mitochondria (circle), basement membrane of normal thickness (arrow) and uniform capillary endothelium (triangle). Magnification x12 000. (B) STZ: loss of interdigitations, mitochondria arranged randomly (asterisks) and thickened basement membrane (arrow). Magnification x18 000. (C) STZ + PPS: preserved interdigitations but irregular areas devoid of mitochondria (asterisks). Magnification x21 000.

DISCUSSION

GAG, particularly heparan sulfate, are synthesised in endothelial and mesangial cells and, after a process of sulfation in the Golgi apparatus, are incorporated into the extracellular matrix in the glomerulus and the great arteries, where they help maintain the structural integrity of the basement membrane and the vascular wall.[27]

A general reduction of the negative charges of the extracellular matrix and the plasma membranes associated with a reduced content of heparan sulfate or with changes in its degree of sulfation has been reported in diabetes.[28-31] This alteration in the charge of the basement membrane would result in a loss of charge selectivity and facilitate increased elimination of proteins in the urine.[30,32]

In this way, experimental models of diabetes in rats and mice have found a decrease in proteoglycan synthesis in the glomerulus and decreased content of basement membrane heparan sulfate proteoglycans.[33,34] Furthermore, it has been reported that GAG content was reduced in the kidneys and in the intima of the aortas obtained from autopsies done on diabetic patients.[35,36] This suggests that alterations in the metabolism of heparan sulfate are not restricted to the kidney and may be involved in the pathogenesis of other complications of diabetes.

It has been shown that the changes in the metabolism of GAG, produced in an experimental diabetes model in rats, may be modified if they are administered exogenously, with the consequent restoration of normal kidney function.[1,2]

Our results are consistent with those found in medical literature since the kidney alterations brought on by diabetes are partially prevented by administrating PPS for 3 months following administration of STZ in rats.

The subcutaneous administration of PPS for 3 months prevented the increase in renal excretion of albumin in this experimental model. This coincides with what has been previously reported in medical literature,[1,2] and corresponds to the effect of decreased renal excretion of albumin demonstrated in patients with diabetes mellitus through the administration of other GAGs.[12,14,15,37-39] The administration of sulodexide is the most studied GAG.

Similarly, treatment with PPS protects the kidneys from the structural changes caused by diabetes. This prevents the basement membrane from thickening and the loss of cytoarchitecture. This coincides with the results of other authors who have shown that the administration of heparin and other GAGs prevents diabetic nephropathy in rats. It also maintains the normal thickness of the basement membrane and the anionic charge density, and simultaneously delays the onset of microalbuminuria.[1-3]

A correlation has been found between albuminuria and heparan sulfate content of the basement membrane of the glomerulus in patients with diabetic nephropathy.[40] A significant reduction in microalbuminuria or macroalbuminuria in diabetic patients who have been treated with GAG has also been observed.[11,37-39]

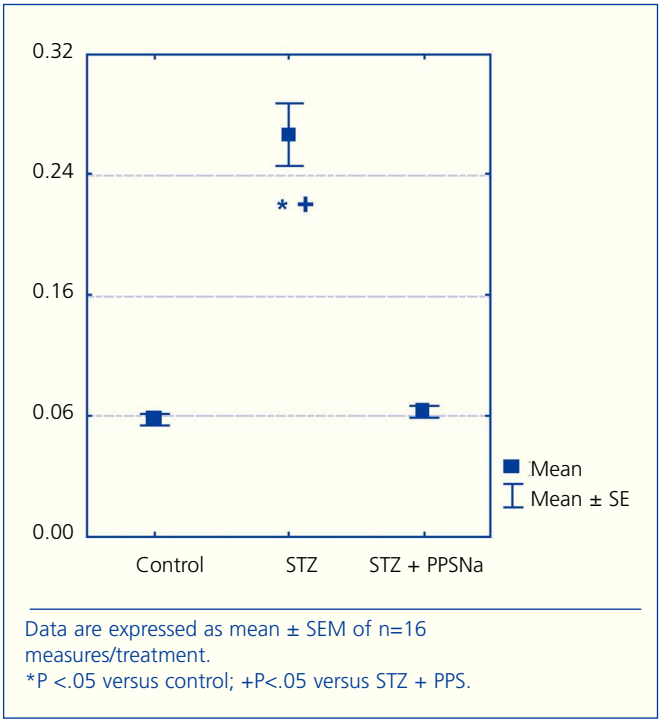


Figure 3. Diameter of basement membrane.

These findings indicate that GAG may play an important role in the pathophysiology of diabetic nephropathy, and that an abnormal metabolism of GAG may be the cause of this disease.[1,41]

The mechanism by which GAG exert this protective effect in diabetic nephropathy is not fully understood. Initially it was proposed that its effect was limited to restoring glomerular permselectivity by replacing negative charges, thus reducing urinary albumin excretion and restoring renal function.[3,32,42] It is now known however that GAG can modulate protein synthesis in the extracellular matrix, an effect that may contribute to its therapeutic usefulness.

The administration of low molecular weight heparin and dermatan sulfate prevents the thickening of the basement membrane, the reduction of anionic charges and the onset of albuminuria in rats with STZ-induced diabetes.[1] Additionally, it has been shown that the administration of heparin reduces the overexpression of collagen, possibly blocking the TGF-b1-mediated pathway that is activated in diabetic nephropathy.[2,7] Recently, Lewis and Xu (2008) demonstrated that GAG inhibit the heparanase enzyme, which is stimulated by hyperglycaemia, thereby preventing the breakdown of heparan sulfate.[31] Through these combined effects, GAG are able to prevent structural and functional alterations, mainly mesangial, that occur in diabetic nephropathy.

In nephrectomised rats, PPS has been shown to be capable of preventing atrophy of epithelial cells and decreasing the inflammatory infiltrate in the interstitium.[43] It also inhibits the extracellular matrix from proliferating, reducing type I and IV collagen in mesangial cell cultures[44] and in vascular smooth muscle cells obtained from patients in whom the implant failed to obtain vascular access for haemodialysis.[45] PPS also reduces cyclosporine-induced nephropathy in rats subjected to salt depletion. This is clearly seen by the decrease in the number of affected arterioles and tubulo-interstitial lesions.[46] These findings, together with our results, clearly show the nephroprotective role of PPS.

Our findings may be explained by the similarity between the effects of PPS and sulodexide; namely, the fibrinolytic effect and activation of the lipoprotein lipase and the hepatic triglyceride lipase,[19-22] as well as the structural similarity with GAG. The anti-inflammatory action of PPS also plays a role in its efficacy for treating interstitial cystitis,[47,48] and may be involved in its nephroprotective effect. Thus, in models of nephrectomised rats, the nephroprotective effect of PPS, as evidenced by

decreased sclerosis and tubular dilation, was associated with a reduction in the infiltration of lymphocytes and macrophages, in a similar way to the effects reported for losartan.[43,49]

This experimental model of diabetic nephropathy shows that PPS partially prevents kidney damage. The physiological and ultrastructural findings were consistent. This suggests that there is a potential therapeutic use for this compound.

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