Breaking Down the Barrier: Evidence against a Role for Heparan Sulfate in Glomerular Permselectivity

Scott J. Harvey and Jeffrey H. Miner
Renal Division, Washington University School of Medicine, St. Louis, Missouri

Charge barrier dysfunction has long been touted as an underlying cause of human glomerular disease (10–12). This may be brought about by decreased expression or undersulfation of GBM-HSPG (13,14). Segmental or global loss of GBM-HS has been reported in human membranous nephritis, lupus nephritis, minimal change disease, and diabetic nephropathy (13,15), as well as in rat models of adriamycin and Heymann nephritis (16,17). The intensity of GBM labeling inversely correlates with severity of disease, which supports the theory that reductions in GBM-HS contribute directly to a loss of barrier function. However, in a recent study, GBM-HS was reported to be normal in diabetic humans and rats with microalbuminuria, and it was concluded that its loss may be a secondary event occurring at advanced stages of this disease (18).

In this issue of JASN, Wijnhoven and colleagues provide evidence that challenges the notion that HS is an important constituent of the glomerular filtration barrier (19). Here they report on a short-term study of renal function in rats following intravenous administration of heparinase to degrade glomerular HS. As a control, rats were injected with neuraminidase, which cleaves neuraminic acid from glomerular sialoproteins such as podocalyxin and causes proteinuria. Careful analysis of the specificity and efficiency of deglycosylation was performed using lectin histochemistry and immunostaining with a panel of well-defined HS antibodies. It is this approach, combined with the simple determination of albumin excretion (as opposed to the use of a surrogate tracer), that distinguishes this study. Injection of heparinase was followed quickly by urinary excretion of HS. After 24 h there was complete loss of glomerular HS as assessed by immunostaining, which indicated there was no regeneration of HS over the course of the experiment. Staining for the core protein agrin, the major GBM-HSPG, was not disrupted. Labeling with the cationic probe cuprolicin blue, which detects proteoglycans with their anionic GAG side chains as a filamentous network in the GBM, was virtually abolished after heparinase injection, yet glomerular and tubular architecture was otherwise normal. The key finding of this study is that the glomerular filtration barrier of heparinase-treated rats was functionally intact, despite the loss of HS and a disruption of GBM charge. The authors conclude that the loss of GBM-HS does not lead to increased permeability to protein. Their findings are in accordance with recent work using isolated perfused kidneys that have pointed to other GAG (chondroitin sulfate and hyaluronic acid) present in the endothelial...
glycocalyx as determinants of glomerular permselectivity (20,21). The nature of the study by Wijnhoven et al. leaves open questions about the possibility of compensation by tubular resorption and whether there might be long-term consequences arising from the loss of glomerular HS. Nevertheless, their important finding highlights the fact that it is time to revisit (and likely revise) the concept of the glomerular charge barrier.

The study by Wijnhoven et al. complements recent work by others that have turned to knockout mouse models to address these questions by targeting HS-bearing core proteins. Three genetically distinct GBM-HSPG are recognized: perlecan, collagen XVIII, and agrin. Mice that lack the HS attachment sites on perlecan have a normal glomerular ultrastructure and no evidence of renal disease, but show increased susceptibility to protein-overload proteinuria (22,23). Collagen XVIII mutants have mild mesangial expansion and only slightly elevated serum creatinine levels (24). Our own work shows that podocyte-specific agrin-knockout mice lack GBM-HS and have a significant GBM charge defect, but kidney function is normal (25).

Others have taken an alternative approach by targeting synthesis of the GAG chains themselves, thereby disrupting all forms of HS linked to both GBM and cell-associated proteoglycans (e.g., syndecans and glypicans). Transgenic overexpression of heparanase leads to changes in podocyte ultrastructure and a slight increase in urinary protein excretion (26), whereas disruption of HS biosynthesis specifically in podocytes was very recently reported to cause profound structural and functional defects (27).

Finally, the findings of Wijnhoven et al. should be of interest to clinical nephrologists, as unraveling this aspect of glomerular biology will hopefully advance our understanding of the cause and treatment of kidney disease. Increased heparanase activity has been implicated in the pathogenesis of experimental Heymann nephritis, and its inhibition in this model prevents the loss of glomerular HS and reduces proteinuria (28,29). Heparanase has also been associated with the pathogenesis of human diabetic nephropathy (30,31). With this report, Wijnhoven and colleagues raise important questions about the significance of this finding in the setting of kidney disease. Moreover, they bear upon the possible mechanism of action of a promising heparinoid compound (32), which is currently in advanced clinical trials for the treatment of overt diabetic nephropathy.

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Disclosures.

None.

References


See the related article, “In Vivo Degradation of Heparan Sulfates in the Glomerular Basement Membrane Does Not Result in Proteinuria,” on pages 823–832.