The glycosaminoglycan content of renal basement membranes in the congenital nephrotic syndrome of the Finnish type


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Abstract. A decrease in the concentration of heparan sulphate proteoglycan (HSPG) in the glomerular basement membrane (GBM) is supposed to cause the increased GBM permeability in the congenital nephrotic syndrome (CNS). Therefore, we analysed the glycosaminoglycan (GAG) content and composition of the GBM and tubular basement membrane (TBM) from 3 patients with CNS of the Finnish type (FCNS) and 16 control infants. The GAG content, determined by spectrophotometric assay after papain digestion, was not significantly different in FCNS patients compared with controls. In addition, the GAG composition was comparable in the two groups, with heparan sulphate (HS) constituting at least 75% of the total GAG content. The urinary GAG content (expressed as mg GAG/mmol creatinine) was age dependent, but similar in both groups. Indirect immunofluorescence studies on kidney tissue from normal human infants, using monoclonal or polyclonal antibodies against the core protein of human GBM HSPG, showed linear staining of almost all renal basement membranes. A monoclonal antibody directed against the HS chain of HSPG showed strong GBM and a weak TBM staining. Kidney tissue from three patients with FCNS displayed no discernible differences in the distribution or quality of staining with the same antibodies. These biochemical and immunohistochemical results are in contrast to the decrease in anionic sites (by polyethylenimine staining) and the replacement of GBM HS by chondroitin sulphate, observed by others in CNS of the diffuse mesangial sclerosis type.

Key words: Congenital nephrotic syndrome - Finnish type - Glomerular basement membrane - Heparan sulphate - Heparan sulphate proteoglycan

Introduction

Congenital nephrotic syndrome (CNS) is usually a fatal disorder in which heavy proteinuria and the nephrotic syndrome are present from birth or early infancy. The main subgroups are the Finnish type (FCNS) and diffuse mesangial sclerosis [1]. FCNS is an autosomal recessive renal disease which is characterized, even in utero, by elevated levels of α-fetoprotein both in the maternal circulation and in the amniotic fluid [2, 3]. After birth, patients with FCNS have massive proteinuria resistant to treatment, and renal transplantation is the only therapeutic option [2].

The precise pathogenesis of FCNS is unknown. It has been assumed that the proteinuria is due to faulty structure of the glomerular filtration barrier, probably in the glomerular basement membrane (GBM) itself [4]. Electron-microscopic studies of kidneys from fetuses and infants with FCNS have generally revealed a normal GBM [5]. However, on morphometric analysis a thinner GBM lamina densa has been demonstrated in FCNS compared with age-matched controls [6]. A number of biochemical studies of the collagen composition of the GBM have failed to show conclusive evidence of any basic abnormalities in FCNS [7]. In addition, immunohistochemical studies for laminin, type IV collagen, fibronectin, brush border antigens, Tamm Horsefall protein and binding of wheat germ agglutinin have not revealed changes in FCNS compared with age-matched controls [8], except for an accumulation of laminin, fibronectin and type IV collagen in the mesangial matrix [9]. This mesangial change is, however, regarded as a secondary phenomenon.

Alterations in the charge as well as the size barrier have been suggested as a cause of the proteinuria. Changes in the charge barrier have been observed in aminonucleoside nephrosis [10–13], IgA nephropathy and membranous nephropathy [14–18]. Vernier et al. [14] reported a decrease in heparan sulphate(HS)-rich anionic sites in the GBM in five patients with CNS, revealed by a decreased staining with polyethylenimine. However, none of the patients in this study had CNS of the Finnish type. In a recent study Vermylein et al. [17] found no change in the total gly-
cosaminoglycan (GAG) content of the GBM, but a total replacement of HS with chondroitin sulphate in one patient with diffuse mesangial sclerosis. The urinary excretion of HS was significantly increased in two patients with FCNS (expressed both in relation to creatinine and to chondroitin sulphate) compared with normal children, but it was normal or only slightly increased in two patients with diffuse mesangial sclerosis and in nine with acquired nephrotic syndrome [17].

To ascertain whether changes in proteoglycans are involved in the pathogenesis of FCNS, we analysed the GAG content and composition of the GBM and tubular basement membrane (TBM) in 3 children with FCNS and in 16 children of comparable age who died from unrelated disorders. The availability of specific polyclonal and monoclonal antibodies against human GBM HS proteoglycan (HSPG) [19–21] enabled us to investigate more extensively the changes in HS content in FCNS. We also determined the urinary GAG excretion of patients with FCNS and of age-matched controls.

Materials and methods

Materials

Kidney tissue. Biochemical and immunohistochemical investigations were performed on kidneys from 3 infants with FCNS (between 4 and 12 months) and 16 controls ranging from premature neonates to children up to 4 years old. All the patients with FCNS had typical clinical features [2], with proteinuria in excess of 5.0 g/24 h. In all patients glomerular changes consisted of mesangial proliferation. Kidneys were obtained at nephrectomy and the diagnosis was confirmed by routine pathological investigation. Control kidneys were obtained at autopsy within 20 h of death. During life, there was no clinical evidence of any renal disease in any of these subjects.

Urine. Complete 24-h urine samples were collected (and preserved with sodium azide) from 39 healthy children with normal renal function ranging from premature infants to children up to 4 years old, and from 14 patients with FCNS. During the collection period the urine was kept at 4°C and frozen at −70°C within a few hours.

Other materials. All other chemicals have been described in detail elsewhere [19], or were of analytical grade.

Methods

Isolation of basement membranes. Isolation of glomeruli and tubules from renal cortex was carried out following established procedures [22] which included the use of ethylenediaminetetra-acetic acid (EDTA), a temperature below 6°C, mincing of the cortical tissue through a stainless steel screen and sieving. To obtain sufficiently pure glomerular preparations, repeated sieving of glomerular fractions was necessary. Purity control was performed by light microscopy, which showed that glomerular preparations had a purity of at least 90% and tubular preparations of at least 95%.

Basement membranes were isolated from glomeruli and tubules by the detergent procedure [22, 23] which required lysis in water, extraction with 1 M sodium chloride (NaCl), extraction with 4% sodium deoxycholate (for 5 h at room temperature) followed by digestion with ribonuclease and deoxyribonuclease (for 2 h at room temperature 5 mg each enzyme/100 ml 50 mM Tris-HCl/7.5 mM MgCl₂, pH 7.3). The final basement membrane preparations were lyophilized and dried above phosphorus pentoxide.