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SHORT COMMUNICATION

Maturation of urinary proteoglycan excretion

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Urinary proteoglycan excretion was studied using two newly established methods in subjects aged between 1 and 22 years. Analysis of glycan moieties showed an age-dependent decrease from 9.1 ± 5.5 (SD) g/mol creatinine ($n = 5$) at the age 1–6 years to 1.9 ± 1.3 ($n = 5$, $P < 0.01$) in those aged 16–22 years. Marked qualitative changes in the proteoglycan electrophoretic pattern occurred during the first and second years of life. Two major proteoglycan bands with a molecular weight of 50 kDa decreased in intensity so that the pattern resembled the adult configuration after 6 years of age. The latter consisted of a major band with a molecular weight of 80–100 kDa, the bands corresponding to a molecular weight of 50 kDa and lighter bands of molecular weight around 32 kDa. These changes may be related to functional maturation of the kidney as a whole and to an increase in the number of mature nephrons.

Keywords: blotting, electrophoresis, glycosaminoglycans, maturation, proteoglycans, urine

Introduction

Glomerular and tubular basement membrane undergoes marked biochemical and morphological changes during development and maturation [1, 2]. Specific peptides are added to the glomerular membrane during ontogenesis [3]. For example, it is currently held that the negative surface charge is achieved and maintained by heparan sulphate proteoglycans [4].

During the foetal period, proteoglycans are first deposited in granular form in the putative membrane [5], with a later organization into layers. In general, the proteoglycan content increases from the beginning of metanephros development to the stage of a functional kidney. While the glomerular membrane contains mainly heparan sulphate, urine and perhaps also the tubular portion of the nephron contain more chondroitin 4/6 sulphate [6] in their proteoglycans. This agrees with the finding that chrondroitin sulphate proteoglycan synthesis is increased during branching morphogenesis of the foetal kidney [7].

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If there exists relative specificity of the proteoglycan content of nephron segments and the glomerulus, urinary proteoglycan analysis could have great diagnostic importance. There are indications that this might be the case. Isolated cells from polycystic kidneys seem to sulphate proteoglycan to a lesser extent than controls [8]. Even in acquired kidney diseases such as nephrolithiasis [9, 10] and proteinuria [11], proteoglycans are involved.

In this study, developmental changes in the urinary excretion of proteoglycans during early life and adolescence are described. The analytical method used is applicable to random urine samples so that no hospitalization is necessary.

Subjects and methods

Twenty healthy subjects, aged 1–22 years, provided random 10 ml urine samples. The subjects comprised 12 boys and eight girls divided into four age categories (1–6 years, $n = 5$; 7–12 years, $n = 6$; 14–15 years, $n = 4$; and 16–22 years,

$n = 5$). The creatinine concentration was determined in an aliquot of urine by the alkaline picric acid method and spectrophotometric analysis was used to determine the urinary glycosaminoglycan (GAG) content [12].

Proteoglycans were precipitated from the rest of the sample by cetyl pyridium chloride [13]. Samples containing 0.2 μg of protein were electrophoresed on 12% polyacrylamide gel slabs [11], blotted on a nitrocellulose membrane and stained for the glycan moieties as previously described [14].

Statistical evaluation was performed using two-way analysis of variance.

Results

The urinary GAG excretion decreased according to age. Typical adult GAG concentrations were found after the age of 6 (Table 1).

Two prominent proteoglycan bands were found in the electrophoretic pattern of 1- and 2-year-old children, with an approximate molecular weight of 50 kDa (Figure 1). Their intensity decreased in samples from older children so that the pattern corresponded to that of adults at an age of 6 and later. The adult pattern consisted of a major band with a molecular weight of 80–100 kDa and lighter bands with molecular weights around 32 kDa together with the aforementioned band at 50 kDa (Figure 1).

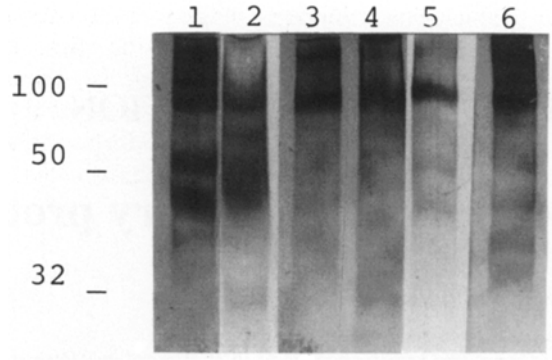

Figure 1. Electrophoresis of urinary proteoglycans. The origin is at the top and the scale on the left indicates molecular weights in kilodaltons. 1, sample from a 1-year-old; 2, sample from a 2-year-old; 3, sample from a 3-year-old; 4, sample from a 5-year-old; 5, sample from a 6-year-old; 6, sample from a 12-year-old. The major band present in all samples has a molecular weight of approximately 100 kDa. Note the prominent bands in lanes 1 and 2 at a molecular weight of 50 kDa.

Discussion

The major GAG species in the urine is chondroitin 4/6 sulphate [13, 15], but other GAG chains may also exist in the urine. Whereas no specific physiologic function can be assigned to the changes in specific bands in the electrophoretic pattern, it can be assumed that they reflect the functional maturation of the kidney, which is known to be roughly completed in the same time scale.

It seems that most of the proteoglycan bands are of renal or of urinary tract origin [11]. They may be secreted or shed by the tubular epithelium [16]. This secretion seems to be directional, as chemical disruption of microtubular function in secreting cells alters secretory process. The microtubules probably organize secretion of special adhesion macromolecules to ensure the formation of epithelial monolayers [17].

If this interpretation is correct then the two prominent bands found in the patterns of 1- and 2-year-olds may be fragments of adhesion molecules, which reflect the biochemical maturation of the epithelium. Experimental evidence indicates that, in case of regeneration, molecular events comparable to morphogenesis can be found (for review, see Ref. 17). At a later age, therefore, the appearance of proteoglycan bands could be an indication of repair of tubular damage.

Table 1. Age-dependent changes in urinary proteoglycan excretion

	Age (years)			
	1–6 ($n = 5$)	7–12 ($n = 6$)	14–15 ($n = 4$)	16–22 ($n = 5$)
Proteoglycans (g/mol creatinine)	9.1 ± 5.5*	4.7 ± 3.0	1.2 ± 0.5	1.9 ± 1.3

Each figure represents the mean ± SD.
*Differs from all other values at $P < 0.01$ (two-way variance analysis).

In conclusion, human urinary proteoglycans undergo marked changes during the first and second year of life. These maturational changes may shed light on the regeneration of renal tissue after sublethal damage, and the products of these changes may provide powerful diagnostic aids in manifest kidney disease.

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