Preconditioning of the distal tubular epithelium of the human kidney precedes nephrocalcinosis

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Preconditioning of the distal tubular epithelium of the human kidney precedes nephrocalcinosis.

Background. Preterm neonates and renal transplant patients frequently develop nephrocalcinosis. Experimental studies revealed that crystal retention in the distal nephron, a process that may lead to nephrocalcinosis, is limited to proliferating/regenerating tubular cells expressing hyaluronan and osteopontin at their luminal surface. Fetal and transplant kidneys contain proliferating and/or regenerating cells since nephrogenesis is not completed until 36 weeks of gestation, while ischemia and nephrotoxic immunosuppressants may lead to injury and repair in renal transplants. This prompted us to investigate the expression of hyaluronan and osteopontin and to correlate this to the appearance of tubular calcifications both in fetal/preterm and transplanted kidneys.

Methods. Sections of fetal/preterm kidneys and protocol biopsies of transplanted kidneys (12 and 24 weeks posttransplantation from the same patients) were stained for osteopontin, hyaluronan, and calcifications (von Kossa).

Results. Hyaluronan and osteopontin were expressed at the luminal surface of the epithelial cells lining the distal tubules of all fetal kidneys at birth and in all kidney graft protocol biopsies 12 and 24 weeks posttransplantation. In 7 out of 18 surviving (at least 4 days) preterm neonates crystal retention developed. In renal allografts a striking increase (from 2/10 to 6/10) in tubular crystal retention between 12 and 24 weeks posttransplantation was observed. In addition, crystals were selectively retained in distal renal tubules containing cells with hyaluronan and osteopontin at their luminal surface.

Conclusion. The results of this study show that luminal expression of hyaluronan and osteopontin preceded renal distal tubular retention of crystals in preterm neonates and renal transplant patients. We propose that the presence of this crystal binding phenotype may play a general role in renal calcification processes.

Key words: nephrocalcinosis, osteopontin, hyaluronan.

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Nephrocalcinosis, recently defined [1] as increased calcium content of the kidney, is observed in specific patient populations, including renal transplant patients and preterm neonates. Renal calcifications in preterm neonates, detected by roentgenograms or ultrasonography, were first described in 1982 [2]. Varying incidences (17% to 64%) have been reported since then [3]. Nephrocalcinosis in preterm neonates is the cause of later complications such as urinary tract obstruction and infection resulting in decreased renal function on the longer term [4, 5]. Increases in urinary stone salts saturation because of the calcium and vitaminD–rich diet, low urinary citrate concentration, and long-term furosemide treatment are all factors that have been previously considered to explain the enhanced crystal formation in tubules of preterms [3, 6, 7]. Recently, intratubular microcalcifications have been described in 13% of transplanted kidneys 26 weeks posttransplantation [8] and in 42% 1 year posttransplantation [9]. Importantly, these intratubular calcifications have been associated with poor long-term graft survival [10]. As in other individuals, a protein-rich diet may result in high urinary calcium and oxalate concentrations and hence intratubular crystallization [6, 11].

The exact mechanisms leading to nephrocalcinosis in these two patient groups, however, are unclear. The localization of renal calcifications in preterm neonates is unknown, while in transplanted kidneys nephrocalcinosis clearly presents as intratubular microcalcifications [9, 10]. Recent investigation has shown involvement of hyaluronan, osteopontin, CD44, nucleolin-related-protein, annexin II, and Tamm-Horsfal-protein in the processes of tubular crystal formation and/or retention [12–16]. An important step in the development of nephrocalcinosis is the retention/trapping of passing crystals to the luminal surface of cells in the distal nephron. Our own studies, both in vitro and in vivo, have demonstrated that calcium crystals do not adhere to an intact epithelium but solely to a proliferating/regenerating epithelium with dedifferentiated cells expressing hyaluronan, osteopontin, and their receptor CD44 at the luminal surface [12, 16]. Luminal hyaluronan and osteopontin expression is absent/sparse in normal renal epithelium but extensively up-regulated following renal damage and the subsequent proliferation/regeneration [12, 17–22]. During nephrogenesis, hyaluronan may play a role during branching morphogenesis [23] and osteopontin has been proposed to be involved in tubulogenesis [24]. In proliferating renal tubular cell cultures, hyaluronan is identified as a major crystal-binding molecule since hyaluronidase treatment diminished crystal adhesion [16, 25]. Although controversial, osteopontin is also considered to be a crystal-binding molecule, it is found associated with hyaluronan and their mutual receptor CD44 on a crystal-binding epithelium, and therefore may play a role in crystal retention [12, 16, 26, 27].

In an attempt to extend our experimental results showing the important role of the epithelial phenotype in crystal retention, in a human clinical setting, we investigated two patient populations presenting with a high incidence of nephrocalcinosis (i.e., preterm born infants and renal transplant patients) [3, 9]. Kidneys of both populations contain proliferating/regenerating nephrons since nephrogenesis is not completed until 36 weeks of gestation and posttransplant kidneys are damaged and regenerate because of ischemia, nephrotoxic drugs (e.g., cyclosporine) and chronic graft rejection.

METHODS

Patients

Renal tissue from 52 human fetuses with gestational age 15 to 40 weeks was available (archive Antwerp University Hospital, Professor Dr. Van Marck). These preterm neonates died soon after birth (<1 day) and expression of hyaluronan and osteopontin during nephrogenesis could be studied, in the absence of any influence from diet and fluid intake. Consequently, these kidneys did not contain crystals. Therefore, tubular crystal localization and colocalization with luminal osteopontin and hyaluronan was studied in 18 preterm neonates who lived for at least 4 days (Dr. R. De Krijger, Department of Pathology, Erasmus Medical Center, Rotterdam, The Netherlands) and hence received a diet known to promote urinary crystallization for at least 4 days. Two kidney biopsies, 12 and 24 weeks posttransplantation, were available from 10 transplant-patients (Transplant Center, Medical School Hannover, Hannover, Germany). The protocol biopsy program at the Hannover Medical School is part of the routine medical care following transplantation and had been approved by the local ethical committee. Patients were informed well about the program before transplantation. Written consent was obtained and participation in the program was not a requirement for transplantation. The use of archive material for research is permitted by local ethical committees on the understanding of anonymity.

Hyaluronan and osteopontin staining

For hyaluronan staining, sections were incubated 20 minutes with 0.1% bovine serum albumin (BSA), 4 hours with biotinylated hyaluronan-binding-protein (Seikagaku, Tokyo, Japan) (1/10000), 1 hour with avidin-biotinylated-peroxidase-complex (Vector Laboratories, Burlingame, CA, USA) and finally with peroxidase substrate [diaminobenzidine (DAB)]. Nuclei were methyl green counterstained.

Osteopontin was stained with LF123, a polyclonal rabbit-antihuman osteopontin antibody (provided by Dr. Fisher, National Institutes of Health, Bethesda, MD, USA). Sections were incubated 20 minutes with normal goat serum, overnight with LF123, and subsequently 30 minutes with biotinylated goat-antirabbit antibody (Vector Laboratories). Avidin-biotinylated-peroxidase-complex (Vector Laboratories) and peroxidase substrate [DAB or aminoethyl carbazole (AEC)] were used for detection. Nuclei were methyl green counterstained.

von Kossa staining

Calcium deposits were visualized by von Kossa staining. Sections were incubated 45 minutes in 5% silver nitrate, 3 minutes with 1% pyrogallic acid, and 1 minute fixed in 5% sodium thiosulfate. Sections were hematoxylin and eosin counterstained.

Tubular crystals

Renal tissue from preterm neonates that lived at least 4 days and from von Kossa–positive transplant patients was used. Three sequential sections, stained for von Kossa, hyaluronan and osteopontin were used to evaluate the expression pattern of hyaluronan and osteopontin in tubules with von Kossa–positive deposits.

RESULTS

Hyaluronan and osteopontin

Developing kidneys. Hyaluronan expression was present in the interstitium of fetal kidneys during the whole period of nephrogenesis studied (week 15 to 40). However, the amount of interstitial hyaluronon diminished along with the decrease in interstitial volume resulting from tubular growth. Besides the interstitium, hyaluronan was found at the luminal surface of the tubules. This tubular localization was observed, both in cortex and medulla, from week 15 to 40. During later nephrogenesis (from around week 27), luminal hyaluronan was especially evident in newly formed, immature tubules of the medulla and outer layer of the cortex.

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