Original article

Congenital murine polycystic kidney disease

II. Pathogenesis of tubular cyst formation

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Abstract. In the current study, the pathogenesis of proximal tubular cyst formation was studied in an animal model of polycystic kidney disease, the CPK mouse. The specific roles of (a) sodium-potassium adenosine triphosphatase (Na-K ATPase) activity, determined by an enzyme-linked kinetic microassay, (b) proximal tubular epithelial hyperplasia, determined by calculation of mitotic indices, and (c) altered proximal tubular basal lamina formation, determined by immunohistological localization of basal lamina glycoproteins, were investigated at progressive developmental stages of CPK proximal tubular cyst formation. Increases in renal Na-K ATPase were present at the earliest fetal stages of proximal tubular cyst formation, and subsequently paralleled the course of proximal tubular cyst progression. Proximal tubular epithelial hyperplasia, although not present at the earliest stages of cyst formation, was a consistent feature of progressive proximal tubular cystic enlargement. Abnormalities in basal lamina glycoprotein expression were not present at any stage of proximal tubular cyst development. We conclude that increased Na-K ATPase and tubular epithelial hyperplasia are significant features of proximal tubular cyst formation in the CPK mouse.

Key words: Cystic kidney disease — Animal models — Sodium-potassium ATPase — CPK mouse

Introduction

Genetically determined renal cystic diseases produce significant morbidity and mortality in both pediatric and adult patients [1, 2]. The processes of renal cyst formation and progressive enlargement are variable in human disease states and may evolve over protracted time periods [3]. Therefore, studies of renal cystogenesis have focused on the epidemiology of genetically determined human cystic disease [4], the anatomy of diseased human renal cystic tissue [5], or the investigation of renal cyst formation in a variety of experimental animal models [6]. Such studies have identified at least three factors of potential pathophysiological significance in tubular cyst formation and enlargement: (1) tubular epithelial hyperplasia [5, 7–9]; (2) abnormal epithelial cell basal lamina structure and function, presumably leading to increased tubular wall compliance [10–12]; and (3) altered tubular epithelial cell metabolism and transport, leading to intratubular fluid accumulation [13–15]. The relative importance of each of these factors and their complex interaction in genetically determined renal cystic diseases remain unknown [2, 6].

A series of recent studies has focused on a new animal model for genetically determined polycystic kidney disease, the cpk/cpk mutant of the murine C57BL/6J strain (CPK mouse) [16–18]. In this mutant strain, a lethal form of polycystic kidney disease is transmitted in an autosomal recessive fashion [16, 17]. In a previous study, we chronicled the origin and progression of cyst formation in different nephron segments of the CPK mouse by utilizing the techniques of nephron microdissection in addition to standard light and
transmission electron microscopy [18]. These studies demonstrated that the earliest morphological alterations in fetal CPK kidneys were localized to the distal portions of developing proximal tubules, and suggested that alterations of transtubular transport in abnormally shortened proximal tubular segments, in concert with epithelial hyperplasia and cytoskeletal alterations, were factors in cyst formation and progressive enlargement.

The current studies were therefore designed to explore the interrelationships between: (a) the major determinant of transtubular transport energetics, sodium-potassium adenosine triphosphatase activity (Na-K ATPase); (b) tubular cell hyperplasia; and (c) abnormal tubular basal lamina formation during proximal tubular cyst formation in the CPK mouse. Na-K ATPase was measured in CPK kidneys at progressive stages of proximal tubular cyst formation and compared with indices of proximal tubular cell hyperplasia as well as immunohistologically determined proximal tubular basal lamina glycoprotein production. Such studies suggest a significant role for increased Na-K ATPase and tubular epithelial hyperplasia in proximal tubular cystogenesis in the CPK mouse.

**Materials and methods**

**Experimental animals.** Offspring of matings of homozygous C57BL/6J mice served as controls in the current experiments, while affected CPK animals were obtained from matings of breeder pairs known to be heterozygotes for the CPK trait [16–18]. Based on previous anatomical studies [18], renal tissue was obtained for Na-K ATPase determination, tubular cell hyperplasia determination, and immunohistology at the following developmental stages: 17-day gestation fetal, newborn (= 21-day gestation), and postnatal days 5, 12, and 21.

**ATPase determination.** Na-K ATPase was determined in control and CPK tissue by our previously described modification of the linked pyruvate kinase-lactate dehydrogenase kinetic spectrophotometric method [13, 14, 19]. Kidneys were washed in 0.2 mol/l sucrose – 0.02 mol/l Tris-HCl buffer at 4°C and then homogenized in the same solution with a Kontes tissue microgrinder (Kontes Glass Co., Vineland, NJ, USA). Tissue homogenates were stored frozen at – 80°C until assayed. At the time of assay, after thawing and rehomogenization, samples of tissue homogenate were added to duplicate cuvettes containing the reaction mixture and two additional cuvettes containing ouabain, 2.5 mmol/l (final concentration), in addition to the reaction mixture. The reaction mixture contained (final concentration) 100 mmol/l NaCl, 10 mmol/l KCl, 2.5 mmol/l MgCl₂, 1 mmol/l Tris-ATP, 1 mmol/l tri(cyclohexylammonium)phosphoenoxypruvate, 30 mmol/l imidazole- HCl buffer (pH 7.3), 0.15 mmol/l NADH, 50 μg/ml lactate dehydrogenase, and 30 μg/ml pyruvate kinase. After initial stabilization, the oxidation of NADH was monitored at 340 nm in a Perkin-Elmer Lambda 3 spectrophotometer (Perkin-Elmer Corp., Norwalk, CT, USA). The temperature of the reaction was maintained at 37°C ± 0.1°C by a thermostatically controlled cell holder. ATPase was calculated from the rate of change of optical density by use of the L3CP kinetic software program of the Perkin-Elmer 3600 Data Station, and expressed as micromoles per minute per microgram of tissue DNA as assayed by the fluorometric method of Nordling and Aho [20]. Specific Na-K ATPase was determined as ouabain-sensitive ATPase and computed as the difference between the reaction rate with and without the addition of ouabain, 2.5 mmol/l (final concentration). As noted previously, this concentration of ouabain is 10 times the concentration required for maximal inhibition of ATPase in murine fetal or adult kidney homogenates [13].

Enzyme activity was expressed as the mean value of ten homogenates of control or six homogenates of CPK tissue at each time period studied. Each tissue homogenate represented a total of three to eight individual kidneys. In addition, Na-K ATPase was determined in kidneys of phenotypically normal littermates of affected CPK animals at each of the five noted developmental stages. For these studies, enzyme activity was expressed as the mean value of two to four tissue homogenates representing one to two individual kidneys.

**Immunohistochemistry and determination of epithelial hyperplasia.** In the current study, immunohistological techniques were applied to both control and cystic CPK kidneys to clearly localize control and cystic proximal tubular segments for epithelial hyperplasia determination, and to define patterns of proximal tubular basal lamina glycoprotein antigenic expression. The primary antibody used for identifying proximal tubules was affinity-purified antibody to the brush border enzyme, gamma-glutamyl transpeptidase (γ-GTP). Anti-γ-GTP was kindly provided by Dr. N. P. Curthoys (Department of Microbiology, Biochemistry, and Molecular Biology, University of Pittsburgh School of Medicine) and was prepared by his previously described methods [21]. Previous studies have demonstrated that γ-GTP is distributed along the entire proximal tubule of the murine nephron (S₃ > S₂ > S₁) [22, 23], and that anti-γ-GTP identifies normal as well as cystic developing murine proximal tubules both in vivo and in vitro [14, 24]. Primary antibodies used for localization of basement membrane glycoproteins included affinity-purified antibody to antelactin (kindly provided by Dr. A. E. Chung, Department of Biological Sciences, University of Pittsburgh, and prepared by his previously described methods [25]) and affinity-purified anti-laminin and anti-fibrinectin (obtained commercially from Bethesda Research Laboratories, Gaithersburg, Md., USA). All other antisera and sera were DAKO immunochromatics, obtained through Accurate Chemical and Scientific Corporation, (Westbury, NY, USA).

The specific immunostaining procedure utilized was our previously described post-embedding technique specifically developed for immunolocalization of basement membrane and brush border antigens in plastic sections of fetal murine tissue [14, 24]. Control and CPK tissue were fixed in 2% formaldehyde-2.5% glutaraldehyde in phosphate buffer, pH 7.4, for 2 h at 4°C. Explants were then washed, dehydrated through graded acetone, and infiltrated and embedded with Immuno Bed Plastic embedding medium (Polysciences, Inc., Warminster, Pa., USA). Three-micron sections were cut on an ultramicrotome and mounted on glass slides. After trypsinization at 37°C for 30 min in a solution containing 0.4% trypsin and 0.2% calcium chloride in phosphate-buffered saline solution, sections were incubated with primary antibodies or pre-immune rabbit serum for 48 h at 4°C. Primary antibody was then reapplied to sections for 30 min at 32°C. Tissue was washed and incubated with swine anti-rabbit IgG (1:20) for 2 h, and then in peroxidase-antiperoxidase complex 1:100 for 1 h. Tissues were stained in diaminobenzidine, 0.05% with 0.1% hy-