Ultrastructural Tubular Basement Membrane Lesions in Adult Polycystic Kidney Disease*

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ABSTRACT

The pathogenesis of adult polycystic kidney disease (PCKD) remains an enigma. In an attempt to find a defect that might explain the cyst formation, an ultrastructural study was performed on seven fresh bilateral nephrectomies of seven patients suffering from adult PCKD. Marked electron microscopic changes of the tubular basement membranes were detected, including thickening, splitting, fraying, and multilayering of the basement membranes. By contrast, glomerular basement membranes lacked these alterations. The kidneys from two control groups (five donor kidneys harvested for transplantation; 10 patients who suffered from end stage renal disease) showed none of the lesions detected in the polycystic kidneys. The lesions of the tubular basement membrane, the principal support of tubular wall, may be the primary phenotypic expression and cause of the inherited defect.

Introduction

Adult polycystic kidney disease (PCKD) is the most common human cystic renal disease. According to Win-gaarden, it occurs in approximately one of every 1,250 births, one in every 500 to 800 autopsies, and it ranks third to glomerulonephritis and pyelonephritis as a cause of end-stage renal failure. Since Lejars’ original description in 1888 of PCKD, many large surveys of the disease have been reported. Despite the vast literature, however, the cause of the alterations remain an enigma.

The heritable nature of the disease has been recorded by Dalguard who showed that adult PCKD is an autosomal dominant trait in contrast to the autosomal recessive inheritance of the infantile form. Studies of the genetics of PCKD, however, have failed to uncover a spe-
specific biochemical, developmental or morphological reason for the lesions. Microdissection studies revealed that cysts originate from nearly all segments of the nephron, with the Bowman's space, Henle's loop, and corticomedullary collecting tubules being the preferential sites. Studies by Lambert illustrated the anatomical as well as the functional continuity of the cystic nephrons. These findings were subsequently confirmed by Bricker and Patton and Gardner. Microscopically, the cysts are lined by cuboidal epithelium indistinguishable from the lining of other cystic renal diseases.

Ultrastructural features of adult PCKD might provide clues to the pathogenesis, but the literature on this subject is sparse. Studies of renal biopsy tissue obtained from asymptomatic complete sibships at risk for adult PCKD but with normal excretory urograms revealed ultrastructural glomerular splitting of lamina densa which, according to Milutinovic, may be the earliest recognizable histologic change in the carriers of the abnormal gene for PCKD. Similar results were obtained by Cuppage, but the relevance of these observations to the pathogenesis of PCKD remains unclear.

In an attempt to find a defect that might yield information regarding the pathogenesis of the cyst formation, an ultrastructural study was performed on renal specimens from autopsies and biopsies of patients suffering from PCKD. The lesions of the tubular basement membrane (TBM) described herein may provide a clue to the primary defect of PCKD.

Materials and Methods

Population

Renal tissue from seven unrelated patients was studied by light, immuno-fluorescence and electron microscopy. The patients ranged in age from 49 to 59 years; five were females and two were males. All specimens were obtained fresh from bilateral nephrectomies performed for massive renal enlargement in patients who had end-stage renal failure. Two control groups were as follows: (a) five unused donor kidneys, harvested for transplantation; (b) 10 cases of end-stage kidney, five from chronic glomerulonephritis, and five from a tubulointerstitial process (four males, six females).

Light Microscopy

Samples of kidney obtained from nephrectomy specimens were fixed in 10 percent formalin for at least 12 hours, processed in an automatic tissue processor* and embedded in paraffin. Sections were cut at three micrometers on a rotary microtome and stained with hematoxylin and eosin to evaluate renal morphology. Special histochemical stains included periodic acid-Schiff and Jones' silver stain to assess glomerular, tubular, and vascular basement membranes, and Masson's trichrome stain to evaluate fibrosis.

Immunofluorescence Microscopy

Fresh unfixed specimens of renal tissue were snap frozen in isopentane precooled on dry ice. The tissue was cut at three micrometers on a cryostat and sections were placed on glass sides. Each of eight slides was overlayed with one of the following fluorescein conjugated goat antihuman sera: IgG, IgA, IgM, IgE, C3, Clq, albumin, and fibrinogen. Slides were incubated with antiserum for 30 minutes, washed twice in phosphate buffered saline, and examined using an ultraviolet microscope.† The intensity of

* Technicon.
† Leitz Ortholux.
the fluorescence was scored subjectively by two observers and graded from 0 to 4+.

**Electron Microscopy**

Fresh renal tissue was fixed in 2.5 percent glutaraldehyde overnight, placed in osmium-tetroxide (30 min), dehydrated with increasing percentages of alcohol, and embedded in Spurr® resin. The thick sections (1.2 micron range), cut on an ultramicrotome using a glass knife‡ were then stained with toluidin blue and examined using a light microscope to identify the region to be studied. This region was then trimmed and thin sectioned (600 to 100Å) using a diamond knife. The sections were floated in a water bath, retrieved on Formvar® coated copper grids and stained with five percent uranyl acetate in 50 percent ethanol lead citrate. The sections were then examined using an electron microscope.§

**Results**

**Gross Morphology**

Characteristic bilateral cystic enlargement was observed in each of the bilateral nephrectomy specimens (figure 1).

**Light Microscopy**

All seven cases of PCKD showed the classic light microscopic morphology. Bilateral cysts were found in the cortex and medulla of both kidneys, markedly distorting the renal parenchyma. Cysts were variable in size and lined by flattened to cuboidal epithelium. Some cysts showed papillary and polypoid hyperplastic epithelium. The cysts contained eosinophilic coagulum, cholesterol clefts, granular debris, blood and blood pigments. Most of the noncystic tubules were dilated, and some were filled with colloidal material, macrophages and eosinophilic material. Glomerular, tubular, and cystic basement membranes were thick. The interstitium between the cysts showed multiple foci of chronic inflammation and fibrosis, but lipid was not apparent. Arteriopneuro-sclerosis and arterioluteosclerosis were conspicuous findings, and there was marked medial thickening and luminal narrowing. Glomeruli ranged from normal to ischemically contracted to totally sclerosed. Immunofluorescent studies were negative except for focal deposits of albumin and fibrinogen.

**Electron Microscopy (figures 2 to 5)**

Many cysts were lined by cuboidal epithelium. Most tubules showed conspicuous thickening and fraying of the basement membranes. Irregular rings of electron-dense and electron-lucent zones were evident, creating an "onion-skin" appearance. Tubules not appreciably dilated had thinner basement membranes than those exhibiting marked cystic change. The most prominent splitting, fraying, and thickening surrounded large cysts lined by low cuboidal epithelium. In fact, the changes became most striking in the larger and more cystic lesions and were not as apparent in tubules that showed no dilatation. Most arteries and arterioles showed medial hyperplasia, but membranes were unremarkable. Likewise, glomerular basement membranes lacked the fraying and splitting seen in the tubular basement membranes but did show signs consistent with ischemia, notably homogeneous, segmental thickening and wrinkling. Mesangial zones demonstrated

‡ LKB.
§ Zeiss EM-10.
foci of increased matrix. Rare foot processes were effaced, and Bowman's capsules occasionally showed similar fraying and lamellar alterations.

The kidneys from donors obtained for transplantation exhibited normal tubular basement membranes, whereas the kidneys from patients who suffered from
end-stage renal disease showed thick, homogeneous basement membranes without the extensive multilayering seen in the polycystic group.

Discussion

In spite of the fact that a century has passed since the adult PCKD was first described, and although the heritable nature of the disease has been recognized for decades as an important factor in the pathogenesis, the cause of cyst formation is still unknown. By electron microscopy, splitting and multilayering of the tubular basement membrane was detected in kidneys of patients suffering from adult PCKD. These findings were absent from the control group. Similar but milder lesions were observed by Milutinovic and associates in studies of asymptomatic unrelated sibships at risk for PCKD, leading them to conclude that these ultrastructural findings may be the earliest recognizable histologic abnormalities.

Several hypotheses are advanced to explain the pathogenesis of cyst formation in adult PCKD. The study of human material has been supplemented by observations in several experimental models of cystic renal disease, both genetically transmitted and chemically induced. Because of certain
pathological and functional similarities of chemically-induced cysts to the human cystic disease,\(^9\) it has been proposed that adult PCKD in humans might be induced by some toxic hereditary metabolite concentrated in the kidneys. However, cysts in experimental animals arise mostly from the distal portion of the nephron where maximal concentration of the circulating toxic filtrate is present,\(^4,14\) whereas cystic lesions in adult PCKD in humans are found randomly distributed throughout the nephron.\(^2\) Moreover, if the circulating toxic metabolite is involved in human cyst formation, cystic disease should recur in transplanted kidney of patients with adult PCKD, which, according to Gardner,\(^11\) does not occur.

Recent ultrastructural studies of adult PCKD showed splits in glomerular basement membrane.\(^7,18,19\) Alport's syndrome, an autosomal dominant inherited glomerulopathy,\(^5,23\) is typified by irregularly thickened glomerular basement membrane in which the lamina densa is split and frayed. The ultrastructural findings of Alport's syndrome bear some resemblance to those described herein.

![Figure 4. A portion of a basement membrane surrounding a cyst, seen at high magnification, shows thin, continuous lines split and separated by electron-lucent regions. Also seen are rarified and grey zones. This basement membrane is markedly thick and distorted. No normal architecture was detected (uranyl acetate and lead citrate; \(\times 43,000\)).](image-url)
for PCKD; however, unlike Milutinovic,\textsuperscript{18} splits in the glomerular basement membrane were not detected in our series of PCKD.

The electron microscopic findings of our study show marked abnormalities of the tubular basement membrane. Similar lesions of the tubular basement membrane were observed in the DPT (2-amino-4, 5-diphenyl-thiazole HCl) induced rat model of polycystic kidney and regressed when the toxin was discontinued. In human adult PCKD, the defects in the tubular basement membrane, which is the principal support of tubular wall,\textsuperscript{4,25} may be due to defective synthesis of one or more components of basement membrane as a result of a defective gene which controls synthesis of the membrane. It is possible that the primary pathology resides in the tubular basement membrane of the nephron and may, in fact, be the basic phenotypic lesion responsible for cyst formation.

References

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