Genesis of Renal Cysts Is Associated With Clusterin Expression In Experimental Cystic Disease

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ABSTRACT
Phenol II is a cystogenic chemical that rapidly induces renal cysts, which regress after drug withdrawal. Cyst formation in this model parallels changes in the tubular basement membrane. Clusterin is a potent cohesive factor induced in states of tissue remodeling. The purpose of this study was to determine if renal clusterin was increased in the Phenol II model and to define the time course and distribution of its induction. Male Sprague-Dawley rats were given, by daily gavage, Phenol II (1.2 mg/kg per day) or vehicle (control). The kidneys were harvested after 1, 2, or 4 days of Phenol II treatment or 3 or 7 days after drug withdrawal. An increase in immunoreactive clusterin was seen in the kidneys of Phenol II-treated rats but not in controls. The appearance of clusterin followed a time course similar to that for cyst formation, with expression confined to the epithelial lining and intratubular casts of dilated or cystic tubules. After Phenol II withdrawal, renal cysts regressed and clusterin staining disappeared. The development of cysts was associated with an increase in clusterin mRNA that decreased after drug withdrawal. In conclusion, a marked, yet reversible induction of clusterin occurred in chemically induced polycystic kidney disease. The function of clusterin in this setting remains enigmatic.

Key Words: Phenol II, sulfated glycoprotein-2, testosterone repressed prostate message-2, substratum, adhesion

Multiple factors have been implicated in the pathogenesis of polycystic kidney disease, including alterations in tubular transport function, cell proliferation, and abnormalities in the basement membrane and extracellular matrix (1-3). Models of polycystic kidney disease using cystogenic chemicals have provided an opportunity to study mechanisms and mediators of cyst formation.

Diphenylthiazole causes a reversible form of polycystic kidney disease beginning 2 wk after drug administration (4,5). The hydroxylated metabolite of diphenylthiazole, 2-amino-4-(4-hydroxyphenyl)-5-phenylthiazole (Phenol II), produces an accelerated form of polycystic disease with a greater degree of cystic transformation and much quicker recovery after the drug is stopped (6,7). Thus, this model provides an opportunity to correlate on a daily basis cyst formation with suspected pathogenetic factors. Using such an approach, Carone et al. demonstrated cyst formation after Phenol II administration, which occurred in tandem with basement membrane structural changes, which included thickening and loss of heparin sulfate proteoglycans. Changes in cell proliferation and altered location of the Na, K-ATPase occurred after the appearance of cysts (6,7).

Clusterin is a heterodimeric glycoprotein first isolated from ram rete testes fluid and is so named because of its ability to elicit clustering of Sertoli and red blood cells (8-14). Species and tissue homologs of clusterin have been isolated and/or cloned by a number of groups working in widely divergent areas. This has resulted in a number of different names for clusterin, including complement cytolysis inhibitor or CLI, sulfated glycoprotein-2 or SGP-2, testosterone repressed prostate message-2 or TRPM-2, dimeric acidic glycoprotein or DAG, SP-40,40, gp80, apolipoprotein J, NA1/NA2, and glycoprotein III (10-14). In accordance with recent consensus regarding terminology, this protein will be referred to as clusterin (11). Putative functions for clusterin include cell aggregation, lipid transport, complement inhibition, protein secretion, membrane protection, and apoptosis.

Clusterin is induced during renal and other tissue injuries, as well as in states of tissue remodeling including development and apoptosis (10-14). An increase in clusterin has been demonstrated in renal cysts of the cpk mouse, an autosomal recessive model of polycystic kidney disease, as well as in a variety of human renal cystic disorders (15,16). Despite its immediate and often prominent recruitment in these disorders, the role of clusterin remains elusive.

The purpose of this study was to determine if renal clusterin was increased in the Phenol II model of polycystic kidney disease and to define the time
course and distribution of its induction. We reasoned that greater insight into the role of clusterin could be gained by examining its expression in this well-characterized model, in which the rapid development and regression of cysts occurs in tandem with alterations in the tubular basement membrane.

METHODS

Experimental Model

Renal cystic disease was induced by the administration of Phenol II (6,7). Male Sprague-Dawley rats, 150 to 175 g (specific pathogen free; Charles River Labs, Wilmington, MA), were given, by daily gavage, Phenol II (1.2 mg/g per day) suspended in 1% gum tragacanth. Control rats received an equivalent dose of 1% gum tragacanth.

The kidneys were harvested after 1, 2, or 4 days of Phenol II treatment or 3 and 7 days after drug withdrawal (N = 3 each group). With the rats under ether anesthesia, the kidneys were exposed by a midline incision, and after the vascular pedicle was clamped, the right kidney was removed for RNA extraction. The aorta was catheterized, and the left kidney was perfused with 30 mL of phosphate-buffered saline, followed by Karnovsky formaldehyde-glutaraldehyde fixative for immunohistochemical studies.

Immunohistochemistry

For the detection of clusterin, a rabbit antiserum (gift of Michael Griswold, Washington State University, Pullman, WA) that was originally reported as anti-rat dimeric acidic glycoprotein antibody was used (17). DAG protein, initially isolated from primary cultures of rat Sertoli cells, was later called sulfated glycoprotein-2 (SGP-2) and is identical to clusterin. The specificity of anti-DAG antiserum was demonstrated by immunoprecipitation and western blot analysis with supernatants of cultured Sertoli cells.

Sections from fixed, paraffin-embedded tissue were deparaffinized in Amerecmlear (American Scientific Products, Minneapolis, MN) and rehydrated in graded ethanol and then buffer. Endogenous peroxidase was blocked with 0.8% hydrogen peroxide in absolute methanol. The sections were incubated with 2% sheep serum to block nonspecific binding and then stained by the peroxidase-antiperoxidase procedure as previously described (18,19). Briefly, after overnight incubation at 4°C with the primary antibody (rabbit anti-rat clusterin polyclonal antibody), the sections were incubated with sheep-anti-rabbit antisera (diluted 1:80) (Antibodies Inc., Davis, CA) and with rabbit peroxidase-antiperoxidase (diluted 1:300) (Sternberger Meyer Immunoc deltaXs Inc., Jarretsville, MD). The reaction was demonstrated with 3′,3″-diaminobenzidine tetrahydrochloride (0.25 mg/mL) (Sigma, St. Louis, MO) and 0.03% peroxide; the sections were counterstained with Harris' hematoxylin. Positive controls were represented by sections from tissue known to contain the antigen of interest; negative controls consisted of sections in which normal rabbit serum was substituted for primary antisera. The proportion of cysts staining for clusterin on Day 4 of Phenol II treatment, the time of maximal cyst formation, was determined in 15 randomly chosen fields from each rat kidney.

Northern Hybridization

Total kidney RNA was isolated by guanidinium-isothiocyanate/cesium chloride density centrifugation (20). The RNA pellet was dissolved in sterile diethylpyrocarbonate-treated water, and its concentration was determined by absorbance readings at 260 nm. Samples containing equal amounts of total RNA (10 μg) were glyoxylated and subjected to electrophoresis in a 1% agarose gel containing 0.01 M phosphate buffer (pH 7.6). After electrophoresis, the RNA was blotted onto nitrocellulose membranes (Pall Biosport, East Hills, NY) by the capillary transfer method. The nitrocellulose blots were baked at 80°C for 2 h and stored at ~80°C in polyethylene pouches.

The membranes were prehybridized at 60°C for 4 h in a buffer containing 5× sodium chloride—sodium citrate (SSC), 5× Denhardt's reagent, 50 mM Tris-HCl (pH 7.5), 0.1% sodium pyrophosphate, 0.2% sodium dodecyl sulfate (SDS), 200 μg/mL sonicated, denatured salmon testes DNA, and 100 μg/mL yeast tRNA. The membranes were then hybridized at 42°C with a random oligomer primer-labeled TRPM-2 (clusterin) cDNA probe (see below) for 16 to 18 h in a buffer containing 50% deionized formamide, 5× SSC, 1× Denhardt's reagent, 50 mM Tris-HCl (pH 7.5), 0.1% sodium pyrophosphate, 1% SDS, 100 μg/mL salmon testes DNA, and 100 μg/mL yeast tRNA. The membranes were washed for 45 min in 2× SSC and 0.1% SDS twice at room temperature and once at 60°C; they were then washed in 0.2× SSC and 0.1% SDS at 60°C for 45 min, and autoradiographs (Kodak XAR-5 film; Eastman Kodak, Rochester, NY) were obtained. The cDNA probe was rat TRPM-2 (clusterin) (1650 base pairs) (gift of Martin Tenniswood, University of Ottawa, Ottawa, Ontario, Canada) (21). For preparation of the cDNA probe, we used the method of random oligomer-primer labeling (Promega, Madison, WI) with [32P]-dCTP (6,000 Ci/mmol; NEN Dupont, Wilmington, DE). The specific activity of the probe was 1× 106 to 2× 108 cpm/μg of DNA.

RESULTS

The percentage of nephrons undergoing cystic changes progressively increased during the administration of Phenol II and rapidly decreased after drug withdrawal, as detailed previously (6,7). The appearance of immunoreactive clusterin followed a time course similar to that seen for cyst development. No
Figure 2. Time course of induction of clusterin mRNA after treatment with Phenol II. An autoradiograph of a Northern blot of total kidney RNA (10 μg) hybridized with a clusterin cDNA probe (exposure time, 20 h). A marked increase in clusterin mRNA was seen with Phenol II treatment that decreased after drug withdrawal. Each lane on this Northern blot represents a different time and consists of whole-kidney RNA pooled from three rats.

DISCUSSION

The major finding of this study was the rapid and marked, yet reversible induction of clusterin mRNA and protein that occurred in association with the cystic transformation of renal tubules. Although we cannot exclude a direct effect of Phenol II on clusterin, its expression only in dilated or cystic tubules suggests a direct link between clusterin and cyst formation. We do not think the induction of clusterin is occurring secondary to Phenol II-induced tubular injury, because not all normal or cystic tubules express clusterin. Furthermore, the induction of clusterin is seen in other renal cystic diseases. In the “cpk” mouse, progressive dilation of collecting duct cysts occurs, leading to renal failure in most mice by 3 wk of age. Clusterin mRNA was present in cystic collecting ducts and appeared to increase with progressive disease (15). Clusterin immunostaining has also been detected in the cyst lining in a variety of human renal cystic diseases, including autosomal dominant and recessive polycystic kidney disease, multilocular cyst of the kidney, cystic renal dysplasia, and in neoplastic cysts (16).

The role of clusterin in cyst formation is not known. Clusterin is developmentally regulated in the kidney, being detected in the ureteric bud, induced metanephric, S-shaped bodies, and collecting ducts of fetal and/or newborn mice (22). A decrease in clusterin expression occurs as the kidney matures, being detectable only in some distal tubular epithelial cells in the adult kidney. The reexpression of clusterin in the epithelial lining of collecting duct cysts of the “cpk” mice prompted Harding et al. to suggest that clusterin was a marker of dedifferentiation of these epithelial cells (15).

Clusterin may function to promote cell-substratum and cell-cell interactions that are perturbed in cystic disease. Abnormalities in the tubular basement mem-

renal cysts were present in control kidneys from rats that did not receive Phenol II, and these kidneys did not stain for clusterin (Figure 1a). After Day 1 of Phenol II, some tubules were beginning to dilate and only these dilated tubules stained for clusterin (Figure 1b). By Day 2 of Phenol II treatment, the number of cystic tubules and the degree of dilation increased (Figure 1c). Clusterin staining was seen in the epithelial cells lining cystic tubules as well as in tubular casts. When one cell in a cyst expressed clusterin, all cells lining that cyst were immunoreactive. No glomerular, vascular, or interstitial staining for clusterin was seen. No clusterin staining was observed when these Day 2 kidneys were stained with nonimmune serum (negative control; Figure 1d). The number and size of cystic tubules were further increased on Day 4 of Phenol II treatment (Figure 1e and f). Clusterin was again confined to the epithelial lining of cysts. The percentage of cystic tubules staining for clusterin was 75.7 ± 2.0%. There were no obvious differentiating features between tubules that stained for clusterin and those that did not. No clusterin staining was seen in normal-appearing tubules. A prominent finding was the presence of clusterin in the lumen of many of these cystic tubules. Three days after the administration of Phenol II was stopped, the size of the cysts began to decrease, as did the extent of clusterin staining, although it was still easily detectable in the most dilated tubules (Figure 1g). Seven days after the administration of Phenol II was stopped, the renal cysts had regressed and no clusterin staining was observed (Figure 1h).

An increase in clusterin mRNA was seen on Day 2 of Phenol II and decreased after drug withdrawal (Figure 2). Clusterin mRNA was lower on Day 4 of Phenol II treatment compared with Recovery Day 3, a finding that has been reproducible. No explanation for this decrease is obvious.
brane and extracellular matrix are found in many cystic disorders (2). In the Phenol II model, the baseline membranes are thickened and laminated, demonstrate decreased staining for proteoglycans and increased staining for fibronectin, and have decreased de novo synthesis of proteoglycans (6,7). Alterations in the basement membrane could affect cell-substratum interactions and consequently contribute to phenotypic changes in the lining epithelial cells (23). In addition, the decreased expression of cell adhesion molecules described in some cystic diseases may lead to aberrant cell-cell interactions (24). Clusterin is a potent cohesive factor capable of promoting cell adhesion and aggregation, perhaps accounting for its induction in cystic disease (8,9,25–28).

Other proposed roles for clusterin include complement defense, apoptosis, and membrane protection. Clusterin can inhibit complement-mediated cytolsis by binding to nascent C5b-7 complex, preventing the insertion of the membrane attack complex into cell membranes (29,30). The pathophysiologic relevance of this property of clusterin is unclear, although the depletion of clusterin can exacerbate glomerular immune injury in the isolated perfused kidney (31). The role of clusterin in apoptosis is controversial because many examples exist where the two can be dissociated (11,14,32–34). Recent evidence suggests that apoptosis may be a factor in renal cystic disease because mice deficient in bcl-2, which inhibits apoptosis, develop polycystic kidney disease (35). A role for clusterin in apoptosis is controversial because mice deficient in bcl-2, which inhibits apoptosis, develop polycystic kidney disease (35).

In conclusion, clusterin mRNA and protein are increased in the Phenol II model of polycystic kidney disease in the rat. Immunostainable clusterin is localized to the epithelial lining of dilated or cystic tubules. The time course of clusterin expression parallels cyst formation and previously defined alterations in the basement membrane, all of which rapidly return to baseline after the discontinuation of Phenol II.

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