Altered primate glomerular development due to in utero urinary tract obstruction

DOUGLAS G. MATSELL, AMY MOK, and ALICE F. TARANTAL

Department of Pediatrics, Anatomy and Cell Biology, and Child Health Research Institute, University of Western Ontario, London, Ontario, Canada, and California Regional Primate Research Center, Department of Pediatrics, University of California, Davis, California, USA

Altered primate glomerular development due to in utero urinary tract obstruction.

Background. In utero urinary tract obstruction is an important cause of newborn and childhood renal failure. Ureteric obstruction during active nephrogenesis results in cystic renal dysplasia; the earlier and longer the obstruction the more severe the histopathological changes of dysplasia. We have reported on a non-human primate model of non-surgical in utero fetal ureteric obstruction that accurately reflects the human equivalent of obstructive renal dysplasia. A striking feature of this model is the effect of obstruction on normal glomerular development and podocyte survival.

Methods. To study the effect of urinary obstruction on glomerular development, kidneys were studied from fetuses undergoing unilateral ureteric obstruction by ultrasound guided injection of alginate beads as early as 75 days gestation (term gestation = 165 ± 10 days). These kidneys displayed all the features of human obstructive cystic dysplasia, had reduced weights, and significant deficiencies in terminal ureteric duct branching.

Results. A combination of histochemistry, histomorphometry, and immunocytochemistry was used to demonstrate deficient cortical ureteric duct development and branching, reduced glomerular number, and altered glomerular basement membrane formation with in utero urinary tract obstruction.

Conclusions. These data suggest that urinary tract obstruction during active nephrogenesis results in a defect in ureteric duct branching morphogenesis, and altered vascularization of the glomerulus with consequent podocyte dropout and decreased glomerular number. These abnormalities reflect human renal dysplasia, which is associated with compromised postnatal renal function and, thus, should be predictive of postnatal outcome.

Differentiation and maturation of the mammalian kidney involves the reciprocal induction of the undifferentiated metanephric blastema and the branching ureteric duct. Recent excellent reviews of this topic have been published [1, 2]. Induction of the metanephric stem cells initiates the epithelial cell fates of the mature kidney, however, the processes by which the various epithelia of the nephron (glomerular epithelia, tubular epithelia, and collecting duct epithelia) are defined, how these epithelia functionally mature, how they differentiate, and the stimuli necessary for their survival are largely unknown. In addition, there is a lack of knowledge about the functional impact of interrupting these events.

Published models of fetal obstructive nephropathy have identified a number of important factors that may contribute to compromised renal function, including expansion of the interstitial mesenchymal space and interstitial fibrosis, tubular atrophy, and tubular cell apoptosis [3, 4]. Although apoptosis is an important cellular event in the normal development of many tissues and organs including the kidney, unregulated apoptosis during critical stages of development may result in irreversible histopathologic changes and altered organ function. Although tubular cell apoptosis is apparent in most models of in utero urinary obstruction, we have identified significant podocyte cell death in our fetal monkey model as early as the S-shaped nephron stage of development, with obstruction as short as 10 days, and in developing vascularized glomeruli that histologically otherwise appear normal [5]. Podocyte apoptosis and abnormal glomerular development have also been noted in fetal sheep models with short periods of obstruction and at relatively later stages of gestation [6–8]. It is of interest that these changes have been identified as early as the pre-vascularized S-shaped nephron. This implies that the potential mechanisms for altered glomerular development in this model are only indirectly a result of obstruction to urinary flow, and may, in fact, be directly related to the effect of ureteric duct obstruction on branching morphogenesis and consequently on vascularization of the developing glomerulus. In normal control fetal monkeys at similar gestational ages, podocyte apoptosis is virtually absent or undetected [5]. We, therefore, hypothesized that fetal urinary tract obstruc-
tion alters normal ureteric duct branching morphogenesis and vasculogenesis, affecting normal glomerular development and resulting in a paucity of normally functioning glomeruli.

**METHODS**

**Animals**

Normally cycling, adult female rhesus monkeys (*Macaca mulatta; N = 13*) with a history of prior pregnancy were bred and identified as pregnant using established methods [9]. Pregnancy in the rhesus monkey was divided into trimesters by 55 day increments, with 0 to 55 days gestation representing the first trimester, 56 to 110 days representing the second trimester, and 111 to 165 days the third trimester (term 165 ± 10 days). All pregnancies were sonographically assessed in order to confirm normal growth and development prior to obstruction. The dams were administered ketamine hydrochloride (10 mg/ kg) for these and subsequent ultrasound examinations.

**Fetal obstruction and sample collection**

Obstruction was induced in fetuses by ultrasound-guided injection of alginate beads into the fetal ureter as it exits the hilum at 75 ± 5 days gestation (early to mid-second trimester), as previously described [5]. At this gestational time point, the mean kidney length and width was 7.3 × 4.6 mm and the ureter was approximately 1 mm in diameter for one to two hours. The sections were then washed in PBS and counterstained with Carazzi’s hematoxylin or methyl green.

**Immunocytochemistry and lectin studies**

Specific histopathology and severity of renal dysplasia were defined by immunocytochemistry (ICC), as previously reported [5]. To study ureteric duct branching we evaluated cytokeratin immunoreactivity (CK-IR) and binding of the lectin *Dolichus biflorus* (DB). We and others have previously shown that CK-IR and DB binding are sensitive and specific markers for the differentiated ureteric duct epithelium in the fetal mammalian kidney [5, 8, 11–15].

Tissue sections of fetal kidney were deparaffinized in xylene, rehydrated in a series of decreasing alcohol concentrations, and then washed in phosphate-buffered saline (PBS). Endogenous peroxidase was inhibited with 1% hydrogen peroxide for 10 minutes at room temperature and washing the tissue sections with PBS terminated the reaction. The kidney sections were permeabilized by incubation with 0.03% trypsin for 5 minutes at room temperature and then washed in PBS for 10 minutes. The sections were incubated with a rabbit polyclonal antikeratin antibody (Dako, Carpinteria, CA, USA) in a 1:500 dilution overnight at 4°C. The sections were then washed in PBS before the incubation of a secondary antibody. For the diaminobenzidine tetrahydrochloride (DAB) method, a peroxidase conjugated secondary goat anti-rabbit IgG antibody (Jackson Immunoresearch Laboratories, Westgrove, PA, USA) was applied for two hours at room temperature. Immunoreactivity was identified with the avidin-biotin peroxidase complex (Vector Laboratories, Burlingame, CA, USA) and visualized using the DAB reaction. Sections were washed in PBS and counterstained with Carazzi’s hematoxylin or methyl green.

Sections were then dehydrated in a series of increasing concentrations of alcohol and mounted with Permount (Sigma, St. Louis, MO, USA).

To define ureteric duct DB binding, tissue sections of fetal kidney were deparaffinized, rehydrated, the endogenous peroxidase was inhibited with 1% hydrogen peroxide and the sections were permeabilized with 0.03% trypsin, as described above. Sections were then incubated in 0.03 mg/mL of biotinylated DB (Sigma) at room temperature for one to two hours. The sections were then washed in PBS and the lectin was visualized with the DAB. Sections were washed in PBS and counterstained with Carazzi’s hematoxylin or methyl green.

To study the effects of urinary obstruction and altered ureteric duct branching on glomerular vascularization, we defined alterations in glomerular capillary basement formation. Utilizing a type IV collagen antibody, we performed ICC utilizing the DAB technique described above. The tissue sections were treated with 0.1% pepsin (Roche Molecular Biochemicals, Quebec, Canada) at room temperature for 30 minutes before applying a 1:50 dilution of the type IV collagen antibody (RDI Research Diagnostics, Montclair, NJ, USA).

To determine cortical ureteric duct area, a digital imaging software package (Image Pro Plus; Media Cybernetics, Silver Spring, MD) was used to measure the exact surface area of CK-IR (which was specific to the ureteric duct epithelium) in a standardized unit of area (25612 μm²) that was applied to all outer cortical sampling of all kidneys from all animals. Ten random fields per kidney were sampled. Glomeruli were counted using a modification of the methods described by McVary and Maizels [16]. Total glomeruli per low power field (∼100 magnification) in non-overlapping areas were counted in at least 10 fields of at least four kidneys per group. Glomerular tuft surface area, not including Bowman’s space, was measured in periodic acid-Schiff (PAS)-stained kid-
ney sections using the Image Pro Plus software package to calculate the individual glomerular surface area in at least ten standard fields of at least four different kidneys from all groups of animals.

In order to compare our model to examples of human obstructive renal dysplasia, both fetal (N = 3, gestational ages 14 to 20 weeks) and postnatal (N = 6, ages 6 months to 2 years) kidney specimens of multicystic dysplasia were obtained, as previously described [17], and in accordance with Institutional Ethics Review Board approval. In addition, biopsy specimens from human infants undergoing diagnostic renal biopsy for obstructive renal dysplasia (N = 3, ages 2 weeks to 2 years) due to bladder outlet obstruction or posterior urethral valves were reviewed. Tissues were prepared and stained according to previously described techniques [17].

**RESULTS**

As we have previously described, ureteric obstruction in this fetal non-human primate model results in significant renal dysplasia, related both to the timing and to the length of obstruction [5]. To define the potential disruption of ureteric duct branching in this model, we studied a number of end points, including cortical ureteric duct formation, glomerular number, and glomerular capillary basement membrane formation.

The extent of ureteric duct branching was evaluated both qualitatively and quantitatively by utilizing CK-IR and DB lectin staining of the differentiated ureteric duct epithelium, which we have previously demonstrated to be specific and sensitive markers. Ductal epithelial expression of CK-IR was significantly reduced in the outer cortex of the kidneys which were obstructed at 70 days gestation as compared to control kidneys of the same gestational age and to the contralateral hypertrophied kidneys (Figs. 1 and 2).

In experimental mouse models of gene ablation, a primary defect in ureteric duct branching results in renal dysplasia, reduced glomerular number, and decreased nephron mass [18, 19]. In support of a defect in branching morphogenesis in this model of fetal obstructive renal dysplasia, there was an associated decrease in cortical glomerular number in obstructed developing kidneys as compared to control kidneys (Fig. 3A). Although less glomeruli were formed in the obstructed kidneys, the individual glomerular surface area was not statistically different from glomeruli in normal age-matched controls, however, glomerular surface area in the contralateral kidneys was significantly larger, an effect seen as early as 140 days gestation (Fig. 3B). In the obstructed animals at 150 days gestation, glomerular number appeared to correlate with total kidney weight, as both were reduced significantly when compared to age-matched controls (Fig. 3C). Contralateral kidneys were significantly larger and had larger glomerular surface areas than control kidneys, particularly at later gestational time points (Fig. 3 B, C). When compared to age-matched controls, however, the contralateral kidneys displayed fewer glomeruli and less cortical ureteric duct area per unit area studied (Figs. 2 and 3A). Although not shown, we suspect that the contralateral kidneys have a normal absolute glomerular endowment. The larger cortical area (and in fact the larger glomeruli) may explain why there are fewer glomeruli per unit area.

Next, the integrity of the glomerular capillary basement membrane was evaluated, which is normally derived from both the endothelial and podocyte cell layers. Examination of the pattern of distribution of type IV collagen immunoreactivity revealed substantial alterations in the glomeruli of the obstructed kidneys, reminiscent of experimental models of disrupted glomerular vasculogenesis and basement membrane formation, and consistent with a failure of normal glomerular endothelial cell development and induction (Fig. 4) [20, 21].

Examination of the human examples of cystic dysplasia highlighted important parallels with our fetal monkey model. In both the human and non-human primate models of fetal urinary tract obstruction, the histopathological changes consist of cystic transformation of glomeruli, tubules and collecting ducts, mesenchymal expansion, mesenchymal-myocyte transformation, and renal architectural disruption [22–25]. A salient feature of these human forms of obstructive dysplasia, as demonstrated in our non-human primate model, also appears to be marked disruption of normal glomerular development (Fig. 5). While long-term renal function in humans appears to be directly related to the severity of the dysplastic changes [26–28], the relative contribution of glomerular damage has not been emphasized in humans or in other experimental models.

**DISCUSSION**

In this fetal monkey model of unilateral ureteric obstruction, we have observed significant alterations in normal glomerular development. Our current findings suggest that these changes are associated with defective glomerular vascularization and podocyte cell death. It is significant that these changes were observed as early as the pre-vascularized S-shaped nephron [5], since this suggests that obstruction to urinary flow affects the communication of the ureteric duct ampulla with the metanephric blastema and subsequent recruitment of endo-
Fig. 1. Ureteric duct branching. (A and B) Control 150 days gestation fetal monkey kidney demonstrating ureteric duct formation up to the renal capsule, as depicted by cytokeratin immunoreactivity (CK-IR; A) and Dolichus biflorus (DB) binding (B; arrows; see Methods section for details). (C) Fetal monkey kidney obstructed at 70 days demonstrating a paucity of CK-IR and cortical ureteric duct formation when evaluated grossly at 150 days gestation, with significant cortical cystic dysplasia and a paucity of normal glomeruli (g). (D) Severe abnormalities in glomerular development are apparent at 150 days gestation after ureteric obstruction, including podocyte dropout (arrows) and abnormal glomerular vascular tuft development (magnifications A, B, and C ×100, D ×400). (Reproduction of this figure in color was made possible by Hoffman-LaRoche, Canada.)

Fig. 4. Glomerular basement membrane formation. (A) In the control, 150 days gestation, fetal monkey kidney there is elaborate glomerular vascular development with an intricate and defined glomerular basement membrane with apposition of podocytes (arrows) as demonstrated by type IV collagen immunoreactivity. (B) In the 150-days gestation fetal monkey kidney obstructed at 70 days, glomerular changes include abnormal tuft development with podocyte detachment and an underdevelopment of the glomerular capillary basement membrane (arrows) as shown by type IV collagen immunoreactivity (magnifications ×400). (Reproduction of this figure in color was made possible by Hoffman-LaRoche, Canada.)
Fig. 2. Cortical ureteric duct formation in (□) obstructed, (■) control, and (▲) contralateral kidneys. Total cortical ureteric duct area was measured by morphometry by calculating the total cytokeratin immunoreactivity (CK-IR) per unit area of cortex (Methods section). Total cortical ureteric duct area was significantly reduced in the obstructed kidneys compared to control kidneys of the same gestational age and when compared to the contralateral kidney at all time points studied. (N = 3 to 4 animals; *P < 0.05 for obstructed vs. control, and for contralateral vs. control; †P < 0.05 for obstructed vs. contralateral).

There were marked alterations in ureteric duct branching and a failure of normal glomerular development in this non-human primate model of fetal obstructive nephropathy. Kidneys obstructed in the early second trimester of pregnancy (equivalent to 16 to 18 weeks gestation in humans) displayed reduced cortical ureteric duct formation, demonstrated a reduced number of glomeruli, and were smaller. In addition, these changes were associated with what appears to be altered glomerular vasculogenesis and formation of the glomerular capillary basement membrane, as demonstrated by deficient type IV collagen expression in the glomerular tufts. Development of the normal glomerular blood supply resulted from the induction of endothelial cell precursors in the metanephros by the ureteric duct ampulla. Subsequent recruitment of the induced endothelial cells into the vascular cleft of the developing nephron.

Taken together, these data suggest that an important
consequence of acquired ureteric duct obstruction in the developing kidney is a defect in ureteric duct branching morphogenesis combined with cystic transformation of developed and functioning glomeruli. This branching deficit may lead to altered vasculogenesis of the glomerulus by interrupting normal glomerular endothelial cell induction and recruitment, resulting in abnormal glomerular basement membrane formation necessary for podocyte survival, as demonstrated in the laminin gene disruption studies [31]. This may explain the abundant podocyte apoptosis that occurs in this model as early as the pre-vascularized S-shaped nephron and in the developing glomerulus.

In humans the association of fetal compensatory growth in the kidney contralateral to a multicystic dysplastic kidney or to unilateral renal agenesis is well described [32–34]. Contralateral compensatory renal growth also has been demonstrated in a number of experimental models, including unilateral ureteric duct obstruction in fetal sheep [35]. In our fetal monkey model of unilateral ureteric obstruction, contralateral kidneys were significantly larger than control kidneys in later gestation, consistent with the observations in humans and in other experimental models. The mechanisms responsible for this fetal compensatory renal growth have yet to be adequately defined.

Our findings emphasize the impact of in utero urinary obstruction on normal glomerular development, and also highlight the importance of our non-human primate model as an accurate representation of human fetal urinary tract obstruction. In both circumstances, abnormal glomerular development is an important feature of dysplastic changes. These important similarities will lend this model to the study of the pathogenesis of human obstructive renal dysplasia, the identification of histological predictors of long-term renal function, as well as the optimal timing for therapeutic in utero intervention.

ACKNOWLEDGMENTS

This study was supported by National Institutes of Health grants #DK53711 and #RR00169.

Fig. 5. (A) Fourteen-week gestation human fetal kidney with multicystic dysplasia. (B) Postnatal biopsy specimen from a child with obstructive renal dysplasia due to bladder outlet obstruction or posterior urethral valves. In both cases, marked dysplastic changes have associated glomerular abnormalities, including under development of the tuft, reduced vascularization, and podocyte apoptosis and detachment (arrows) (magnifications ×400).

Reproduction of Figures 1 and 4 in color was made possible by Hoffman-LaRoche, Canada.

Reprint requests to Dr. Douglas G. Matsell, Children’s Hospital of Western Ontario, 2230-800 Commissioner’s Road East, London, Ontario, Canada N6C 2V5 E-mail: doug.matsell@lhsc.on.ca

REFERENCES


32. Glazebrook KN, McGrath FP, Steele BT: Prenatal compensatory renal growth Documentation with US. *Radiology* 189:733–735, 1993

