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Postnatal development and progression of renal dysplasia in cyclooxygenase-2 null mice

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Background. Genetic ablation of cyclooxygenase-2 (COX-2) resulted in cystic renal dysplasia and early death in adult mice. The ontologic development of the renal pathology and the biochemical and physiological abnormalities associated with the dysplasia are unknown.

Methods. Mice homozygous for a targeted deletion of COX-2 (−/−) were compared with wild-type littermates (+/+). Somatic and kidney growth and renal histology were studied at the day of birth and at a number of postnatal ages. Systolic blood pressure, urinalysis, urine osmolality, serum and urine chemistries, and inulin clearance were evaluated in adult animals.

Results. Beginning at postnatal day 10 (PN10), kidney growth was suppressed in −/− animals, while somatic growth and heart growth were unaffected. By PN10, −/− kidneys had thin nephrogenic cortexes and crowded, small, subcapsular glomeruli. The pathology increased with age with progressive outer cortical dysplasia, cystic subcapsular glomeruli, loss of proximal tubular mass, and tubular atrophy and cyst formation. Adult −/− kidneys had profound diffuse tubular cyst formation, outer cortical glomerular hypoplasia and periglomerular fibrosis, inner cortical nephron hypertrophy, and diffuse interstitial fibrosis. The glomerular filtration rate was reduced by more than 50% in −/− animals (6.82 ± 0.65 mL/min/kg) compared with wild-type controls (14.7 ± 1.01 mL/min/kg, $P < 0.001$). Plasma blood urea nitrogen and creatinine were elevated in null animals compared with controls. Blood pressure, urinalysis, urine osmolality, and other plasma chemistries were unaffected by the deletion of COX-2.

Conclusions. Deficiency of COX-2 results in progressive and specific renal architectural disruption and functional deterioration beginning in the final phases of nephrogenesis. Tissue-specific and time-dependent expression of COX-2 appears necessary for normal postnatal renal development and the maintenance of normal renal architecture and function.

Key words: nephrogenesis, eicosanoids, cystic renal dysplasia, postnatal kidney development, *Ptgs2* gene, toxic COX-2 blockade.

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Prostaglandins, most widely known as inflammatory mediators, also regulate vascular contractility and salt and water balance within the kidney. Prostaglandins, prostacyclin, and thromboxane are produced from the intermediate prostaglandin H_2 by the conversion of membrane-derived arachidonic acid by the enzymatic activity of prostaglandin G_2/H_2 synthase, also referred to as cyclooxygenase (COX). COX exists as two isoforms, COX-1 and COX-2, which are produced from two different genes (in the mouse, *Ptgs1* and *Ptgs2*). COX-1 is ubiquitously expressed throughout most mammalian tissues, primarily in stomach, platelets, and vasculature. Within the kidney, it is reportedly expressed within the vascular endothelium, medullary collecting ducts, and medullary interstitium [1, 2]. The production of prostanoids by COX-1, thought to be the primary mechanism of prostanoid production within the kidney, is usually constitutive with fairly constant levels of enzyme activity [1]. Unlike COX-1, COX-2 is undetectable in most tissues, but can be induced by a variety of inflammatory cytokines in macrophages, brain, chondrocytes, fibroblasts, and synovial cells [1]. Within the rat kidney, COX-2 has been localized to macula densa, cortical thick ascending limb, and papillary interstitium and is induced by salt restriction [3, 4].

In 1995, both isoforms of COX were independently inactivated in mice using homologous recombination. Disruption of the *COX-1* gene resulted in decreased platelet aggregation and a decreased inflammatory response to arachidonic acid [5]. No obvious renal abnormalities were seen. Deletion of *COX-2*, however, produced a strikingly different phenotype [6, 7]. While inflammatory responses were surprisingly normal, severe renal pathology was present in adult homozygous null mutant (−/−) animals. These animals often died early, and histopathologic examination of the kidneys revealed profound cystic dysplasia.

While these genetic manipulations clearly indicate that COX-2 activity is critical for the maintenance of normal renal architecture, the time of presentation and physiological consequences of these abnormalities is unknown.

The object of the current studies was to determine the ontologic development and progression of the renal dysplasia seen in COX-2–deficient mice and to assess the biochemical and physiological abnormalities associated with the pathology.

METHODS

Animals

Breeding colonies of two previously described mouse lines carrying targeted deletions of the *COX-2* gene were established at the University of Virginia (Charlottesville, VA, USA). The first line (line 1), derived at the University of North Carolina (Chapel Hill, NC, USA), contains a 104 bp deletion of the *Ptgs2* gene in exon 8 that eliminates the nucleotides encoding the crucial Tyr-371 and His-374, both necessary for COX activity [6]. The second line (line 2), derived by Dinchuk et al [7], was purchased from the Induced Mutant Resource of Jackson Laboratories (Bar Harbor, ME, USA). This line contains a 220 bp deletion within exon 1 of the *Ptgs2* gene. Both lines were initially established as 129/C57BL6 chimeras and were interbred into C57BL6 wild-type mice obtained from Hilltop Labs (Scottsdale, PA, USA) to establish the colonies at the University of Virginia. F2 and F3 heterozygous pairs were bred to obtain litters of pups containing wild-type (+/+), heterozygous (+/−), and homozygous mutant (−/−) littermates. Approximately equal numbers of mice from lines 1 and 2 were used in the following studies. Animals were sacrificed at the day of birth ($N = 46$) and at postnatal days 3 (PN3; $N = 65$), 7 ($N = 52$), 10 ($N = 44$), 14 ($N = 87$), 28 ($N = 47$), and 42 ($N = 50$) for comparison of genotype expression, body weight, total kidney weight, heart weight, and renal histology. Kidneys from previously studied PN56 (8 weeks) and PN112 (16 weeks) adult animals were also examined for pathologic changes. Adult mice (ages 8 to 24 weeks) were utilized for determination of blood pressure, random urine osmolality, and inulin clearance, as described later in this article. Separate groups of adult mice were housed in metabolic cages for four days for documentation of water intake and collection of 24-hour urine samples for volume and electrolyte determination. The animals were then sacrificed for determination of serum electrolytes as described later in this article. All animals were maintained on Tekland 7012 rodent diet and had free access to tap water. Pups were nursed by their dams and were weaned at three weeks of age. All procedures were performed with the approval of the University of Virginia Animal Research Committee.

Genotype analysis

Genotypes of mice from line 1 were determined by Southern analysis as previously described [6]. Following *SacI* digestion of tail DNA, hybridization with a ^{32}P-labeled 1.9 kb COX-2 cDNA probe detected the unaltered gene as a band at 2.6 kb, while the targeted gene was detected at 3.8 kb. Line 2 was genotyped by detection using a polymerase chain reaction strategy. Using three primers, two specific for the unaltered *Ptgs2* gene (A, 5′-GCC ACC TCC GCT GCC ACC TCT GCG A-3′, and B, 5′-CAT ACA TTC CCC ACG GTT TTG A-3′) and another specific for the inserted neomycin-resistance cassette (C, 5′-GAT TCG CAG CGC ATC GCC TTC-3′), thermal cycling amplification was performed by melting at 94°C for 45 seconds, annealing at 60°C for 1 minute, and extension at 72°C for 1 minute for 33 cycles. The wild-type gene was detected by gel electrophoresis as a band at 800 bp, while the targeted gene was detected at 430 bp.

Urinalysis and urine osmolality

Random urine samples were obtained from adult mice ($N = 7$ each genotype, ages 14 to 20 weeks) for urinalysis using Multistix® 10 SG reagent strips (Bayer, Elkhart, IN, USA). Urine osmolality was determined by vapor pressure osmometry (Wescor, Logan, UT, USA).

Serum and urine chemistries

Plasma samples obtained at the time of sacrifice (ages 14 to 18 weeks) were utilized for analysis of sodium, potassium, chloride, total CO_2, and glucose using a NOVA 16 analyzer (NOVA Biomedical, Waltham, MA, USA). Twenty-four–hour urine collections were assayed similarly for sodium, potassium, and chloride. Plasma creatinine was measured by the alkaline picrate colorimetric assay (Sigma #555-A; Sigma Chemical Co., St. Louis, MO, USA), and blood urea nitrogen (BUN) was measured colorimetrically using diacetyl monoxime (Sigma #535-A).

Blood pressures

Awake systolic blood pressures were measured by tail cuff in adult animals ($N = 12$, each genotype) ages 8 to 14 weeks using a Visitech BP 2000 Blood Pressure Analysis System (Apex, NC, USA) [8]. All animals had two cycles of 10 measurements recorded per day for a minimum of three days. Those animals undergoing inulin clearance studies (described later in this article) had anesthetized intra-arterial blood pressures ascertained at the beginning of the procedure.

Tissue preparation and light microscopy

At the time of animal euthanasia using halothane, the kidneys were rapidly removed, weighed, hemisected, and fixed in Bouin's solution or 10% buffered formalin. Following embedding in paraffin, 5 to 7 μm sections were prepared and stained with hematoxylin and eosin, periodic acid-Schiff (PAS), or Masson's trichrome and were examined under direct microscopy. A minimum of two sections, at least 100 μm apart, were examined for each kidney.

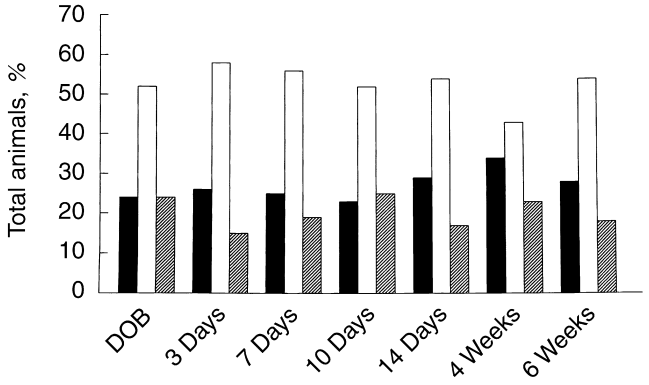


Fig. 1. Distribution of genotypes at the *Ptgs2* locus in mice sacrificed at varying ages during the first six weeks of life. Wild-type (+/+; ■), heterozygous (+/−; □), and homozygous null (−/−; ▨) genotypes are represented in the expected 1:2:1 ratios at all time points.

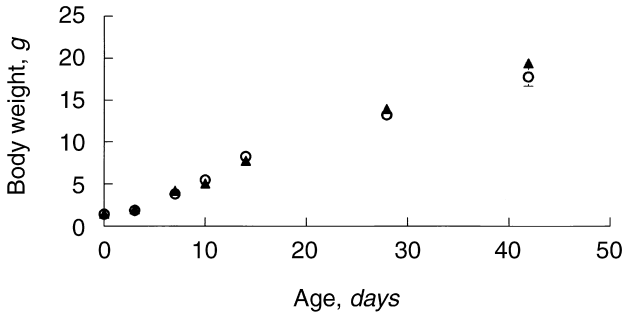


Fig. 2. Somatic growth of wild-type (+/+; ▲) and COX-2 homozygous null mutant (−/−; ○) mice during the first six weeks of life. No significant differences in body weight are apparent at any age.

Inulin clearance

Adult mice (16 to 24 weeks of age) had a glomerular filtration rate (GFR) measured by tritiated inulin clearance as previously described [9]. Wild-type ($N = 5$) and homozygous null ($N = 6$) animals were anesthetized with intraperitoneal pentobarbital (70 mg/kg) and were placed on a thermostatically controlled heating table. Under continuous oxygen flow by tracheostomy tube, a carotid artery was cannulated for blood withdrawal and mean arterial blood pressure monitoring. A jugular venous catheter was used for continuous infusion of ^{3}H-inulin (New England Nuclear, Danvers, MA, USA) at a dose of 1000 μCi/min/100 g body weight. A suprapubic bladder catheter was surgically placed for urine collection. After a 45-minute equilibration period, two 20-minute urine collections were made, and 0.1 mL blood collections were made at the start of each period and at the end of the second urine collection. Blood samples were replaced with 4% bovine serum albumin. The radioactivity of plasma and urine aliquots was measured in a Beckman LS6500 scintillation counter (Beckman Instruments, Irvine, CA, USA), and inulin clearance was calculated as the product of urine flow and urine to plasma activity ratio (UV/P) corrected for body weight.

Statistical analysis

Results were expressed as mean ± SE. Chi-square analysis was used to compare the distribution of genotypic expression at the targeted locus to the 1:2:1 ratio of +/+:+/−:−/− animals expected by normal Mendelian inheritance. Comparisons of body and organ weights, blood pressure, blood and urine chemistries, and inulin clearance were made between wild-type and homozygous mutant mice using Student's *t*-test. No differences in any of the study variables were identified between the two lines of mice, and the data have been consolidated to reflect only wild-type (+/+), heterozygous (+/−), and homozygous mutant (−/−) genotypes without differentiating between the two targeting constructs.

RESULTS

Genotypic ratios

Matings between heterozygous pairs produced newborn progeny carrying all three expected genotypes in the expected Mendelian proportions of 1:2:1 wild-type: heterozygote:homozygous mutant (Fig. 1). The normal distribution of genotypes did not vary from expected throughout the first six weeks of life (χ^2, $P = 0.38$). The results indicate that a deficiency of COX-2 does not result in fetal loss or in early postnatal death. Of the 90 homozygous null animals studied in the first six weeks of life, there were 35 males and 55 females (χ^2, $P = 0.177$), suggesting that through early life, male and female animals with COX-2 deficiency survive equally well. During adulthood, however, there was clearly a difference in long-term survivability based on genotype. Of animals allowed to survive longer than six weeks of age, there were no spontaneous deaths in wild-type animals, while 12 of 58 homozygous null mice (20.7%) died between the ages of 7 and 23 weeks. Of these animals, 8 were female and 4 were male.

Somatic and organ growth

The body weights of animals at ages of birth through 42 days are shown in Figure 2. There are no significant differences between the somatic growth of homozygous null mutant mice and their wild-type littermates at any of the ages studied, suggesting that COX-2 deficiency does not affect normal postnatal growth.

Figure 3 shows the effect of COX-2 genotype on heart growth during the first six weeks of life. With the exception of birth, there are no differences in heart weight: body weight ratios during postnatal growth and early adulthood. The significance of higher heart weight:body weight ratios in newborn knockout pups is unclear.

The effect of COX-2 deficiency on kidney growth is

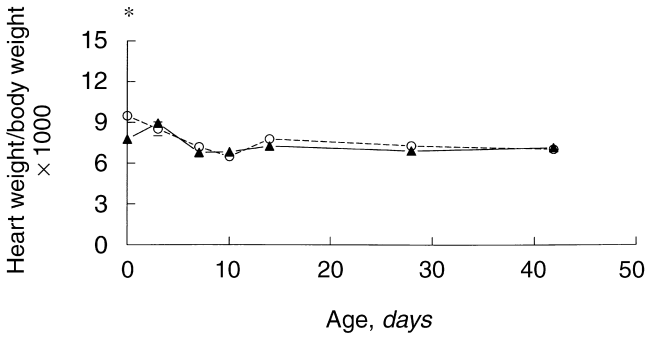


Fig. 3. Heart growth of wild-type (+/+; ▲) and COX-2 homozygous null mutant (−/−; ○) mice during the first six weeks of life. During postnatal development, heart growth is unaffected by genotype at the *Ptgs2* locus.

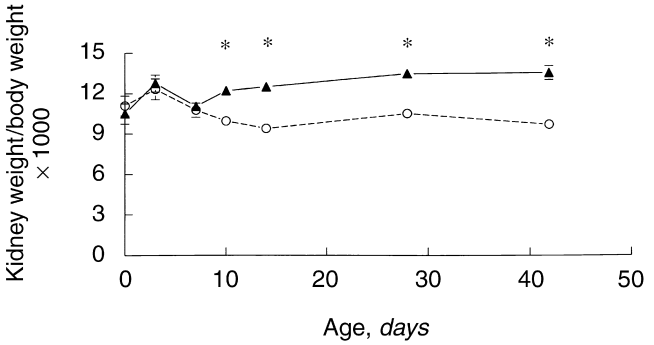


Fig. 4. Kidney growth of wild-type (+/+; ▲) and COX-2 homozygous null mutant (−/−; ○) mice during the first six weeks of life. By postnatal day 10 (PN10; $*P < 0.001$), kidney growth is suppressed in COX-2–deficient animals compared with their wild-type littermates. This organ-specific growth suppression persists throughout the remainder of development.

shown in Figure 4. Beginning at PN10, total kidney mass is markedly suppressed in homozygous null animals as compared with their wild-type littermates ($P < 0.001$). This growth suppression persists throughout the remainder of the preweanling period and early adulthood without significant change.

Renal histopathology

No differences in renal morphology were detected between COX-2 −/− mice and their +/+ controls on the day of birth, PN3, and PN7 (data not shown). By PN10, early cystic changes were identifiable in some −/− kidneys affecting several different tubule sections and glomeruli, and crowded, small, subcapsular glomeruli were noted (Fig. 5). A distinctive lack of the normal proximal tubular mantle was also noted in −/− animals. Not all PN10 −/− kidneys exhibited identifiable abnormalities, suggesting that the rate of pathologic progression may vary slightly among animals. However, by PN14, all COX-2–deficient kidneys could be differentiated from their wild-type littermates. Kidneys from PN14 and PN28 −/− animals showed progressive outer cortical dysplasia with cystic subcapsular glomeruli, loss of proximal tubular mass and brush-border definition, and tubular atrophy and cyst formation (Fig. 6). Hypertrophy of juxtamedullary glomeruli and tubules was notable by PN28. The severity of pathological changes continued to increase with age. Adult (PN42) homozygous null kidneys had profound diffuse tubular dilation and cyst formation, outer cortical glomerular hypoplasia and periglomerular fibrosis, focally variable glomerular sclerosis, inner cortical nephron hypertrophy, and diffuse interstitial fibrosis (Fig. 7). At no time point was significant inflammatory infiltrate or vascular pathology seen. Some degree of variability in the severity of adult pathology was noted among littermates, with some animals showing more severe cystic degeneration than others (Fig. 8). These differences were not related to gender or targeting strategy.

Blood pressures

No significant differences in awake systolic blood pressures were found in COX-2–deficient animals (100.7 ± 2.4 mm Hg, $N = 12$) as compared with wild-type controls (96.4 ± 1.3 mm Hg, $N = 12$), suggesting that under unstressed conditions, COX-2 deficiency does not affect resting blood pressure. Likewise, in anesthetized animals at the initiation of the measurements for inulin clearances, no differences were seen in intra-arterial systolic pressures in COX-2–deficient animals (87.3 ± 7.8 mm Hg, $N = 6$) as compared with wild-type controls (73.2 ± 10.2 mm Hg, $N = 5$).

Daily water intake and urine output

Twenty-four–hour urine collections ($N = 3$ each animal) were obtained, and daily water intake was quantitated after overnight equilibration in metabolic cages. Table 1 shows that COX-2 deficiency does not cause significant alterations in drinking behaviors and urine output under nonstressed conditions.

Urinalysis and urine osmolality

Table 2 illustrates that no significant abnormalities were found on random urinalysis in either genotype. Likewise, spot urine osmolalities did not differ between the groups (+/+ = 1573 ± 252 mOsm/kg vs. −/− = 1172 ± 163 mOsm/kg, $P = 0.21$). These results suggest that COX-2 deficiency does not result in clinically detectable hematuria, proteinuria, glycosuria, or extreme alterations in urinary concentrating capacity.

Plasma and urine chemistries

Table 3 includes the plasma electrolytes, urea nitrogen, and creatinine values obtained from adult COX-2–deficient mice and their wild-type controls. Plasma sodium, potassium, bicarbonate, and chloride were not different

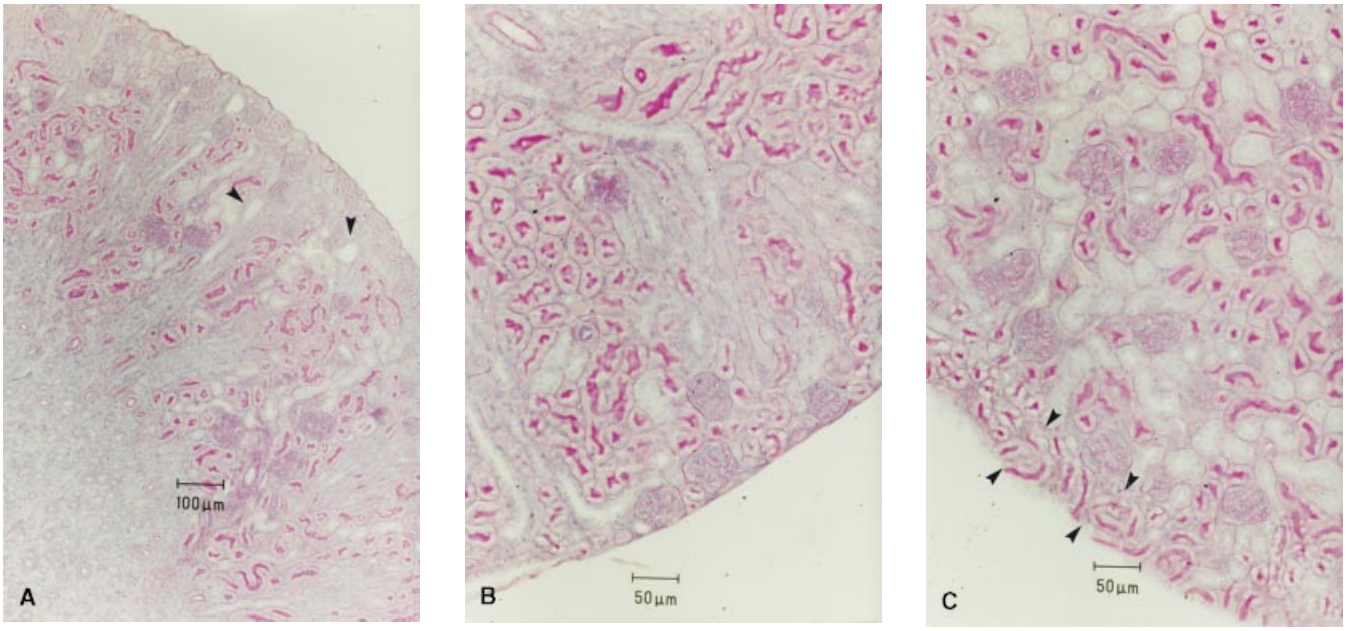


Fig. 5. Renal morphology at PN10 (PAS staining). (*A*) Low-power view of −/− kidney with tubular dilation and early cystic changes (arrowheads). (*B*) High-power view of −/− kidney showing crowding of glomeruli in the subcapsular region with loss of normal proximal tubular mantle. (*C*) Wild-type littermate control. The subcapsular proximal tubular mantle is marked by arrows.

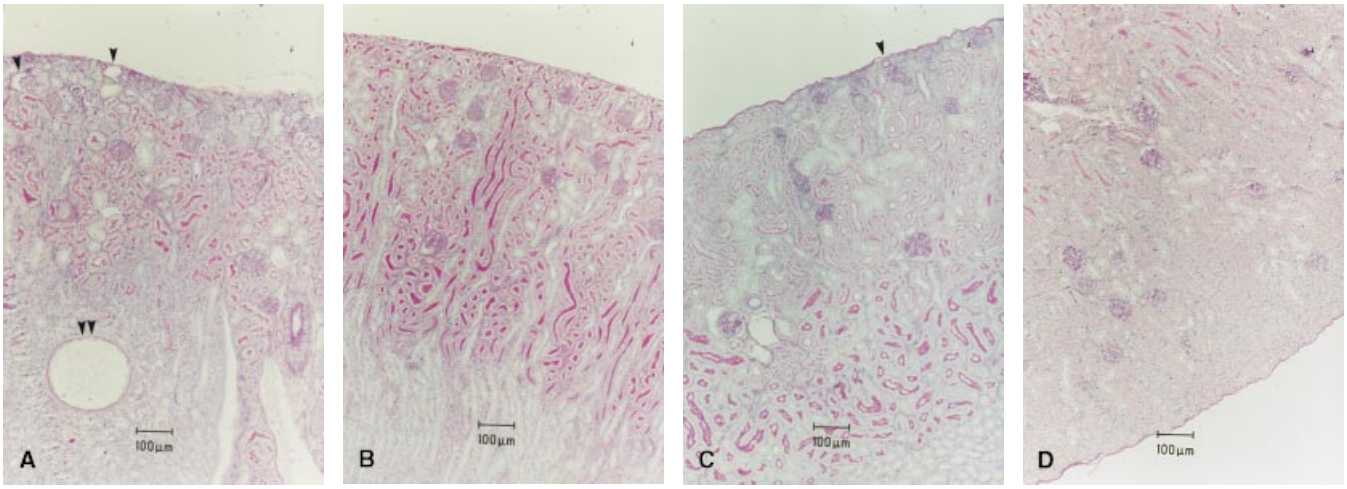


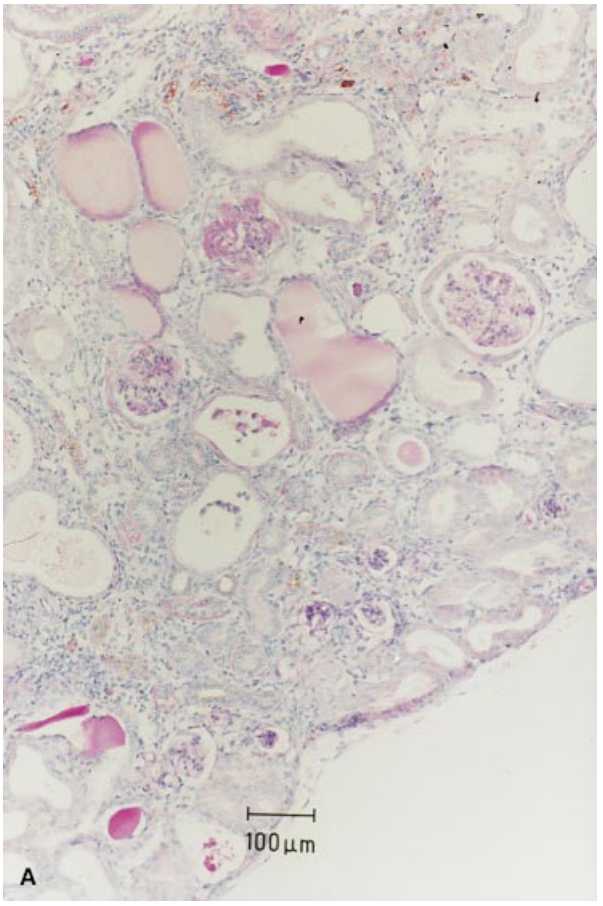
Fig. 6. Renal morphology at two and four weeks of age (PAS staining). (*A*) Severely affected −/− kidney at PN14 showing cortical disorganization, loss of subcapsular tubular mass, glomerular cysts (arrowhead), and massive tubular cysts (double arrowheads). (*B*) Wild-type littermate control at PN14. (*C*) PN28 −/− kidney. Note the loss of outer cortical tubular mass, tubular atrophy (arrowhead), and hypoplasia of outer glomeruli compared with hyperplasia of juxtamedullary glomeruli. (*D*) Wild-type littermate control at PN28.

between the two groups, while both BUN and creatinine were significantly elevated in animals lacking COX-2. Plasma BUN was approximately 2.5-fold higher (79.2 ± 7.5 vs. 29.1 ± 0.7 mg/dL, $P < 0.001$), and creatinine was approximately 1.5-fold higher (0.49 ± 0.06 vs. 0.29 ± 0.04 mg/dL, $P = 0.014$) in knockout animals compared with their wild-type controls. These results thus document that renal insufficiency is indeed a consequence of the pathologic abnormalities seen in this disorder.

Table 4 shows the daily urinary excretion of sodium, potassium, and chloride for COX-2–deficient mice and their wild-type controls. When allowed free access to a normal diet and tap water, there were no significant differences in electrolyte excretion rates between the two groups.

Inulin clearance

Genotype at the *Ptgs2* locus had profound effects on GFR as measured by inulin clearance (Fig. 9). The GFR of animals deficient in COX-2 was reduced approximately 50% (6.82 ± 0.65 mL/min/kg body weight, $N = 6$) com-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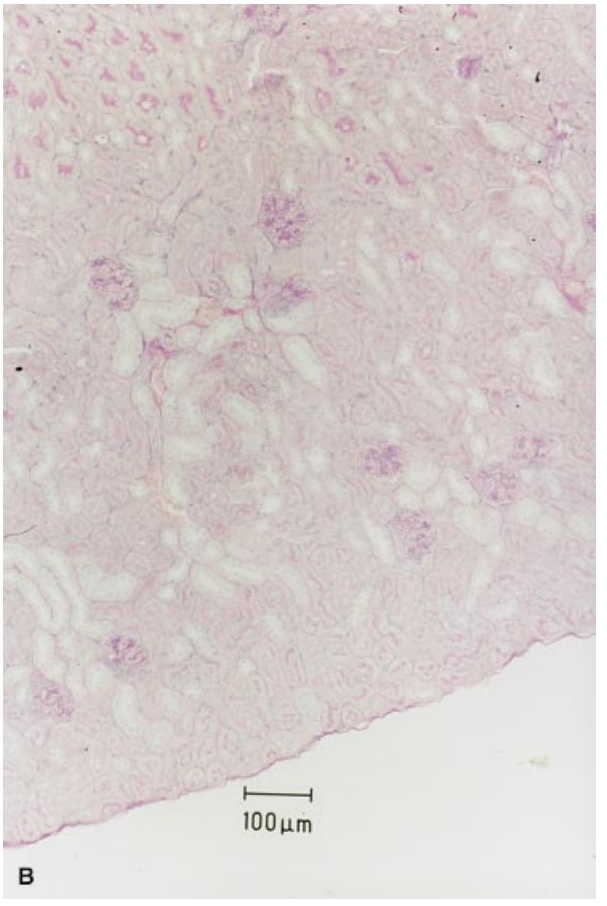
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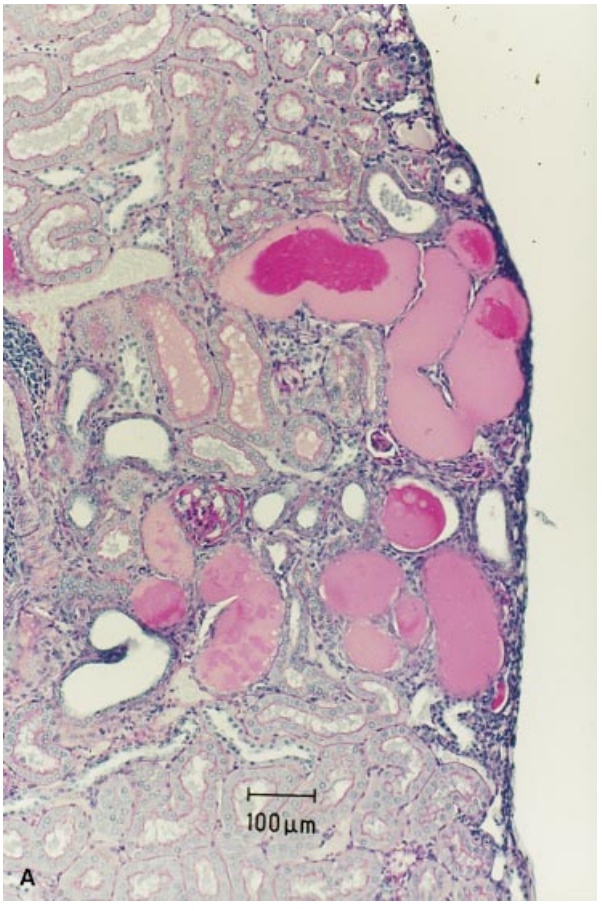
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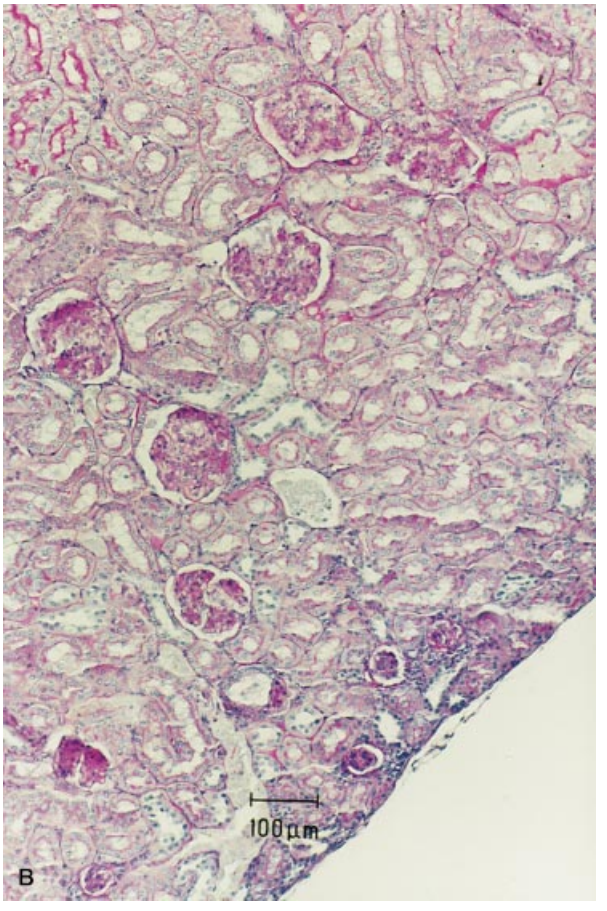
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Fig. 7. Renal morphology in adult animals (PAS staining). (*A*) Sixteen-week-old −/− kidney showing widespread loss of architectural integrity, outer cortical glomerular hypoplasia, inner glomerular hypertrophy and sclerosis, and interstitial fibrosis. (*B*) Wild-type littermate control.

pared with normal wild-type controls (14.7 ± 1.01 mL/min/kg body weight, $N = 5$, $P < 0.001$).

DISCUSSION

While the COXs and their prostanoid products have important roles in cytoprotection, vascular homeostasis, platelet function, and control of renal blood flow and water balance, the independent functions of COX-1 and COX-2 in the kidney have remained elusive. Isozyme-specific pharmacologic inhibitors are currently in clinical trials [10], but in the past have not been adequate for differentiation of the functions of the two isoforms with respect to renal function. Targeted deletion of the genes by homologous recombination has provided excellent insights into the importance of these enzymes in the kidney. Clearly, our studies show that functional COX-2 is necessary to achieve a normal mature renal phenotype and maintain normal renal excretory activities, but is not necessary for early renal differentiation or fetal and early postnatal survival. After maturation, COX-2 deficiency clearly results in lethality in some animals, with approximately 20% of homozygous null animals dying between the ages of 7 and 23 weeks. While Dinchuk et al also found a high incidence of neonatal deaths not seen in our breeding colony [7], the average lifespan of −/− animals was reported to be 3.5 months in their report, which is consistent with our findings. Similarly, the initial report from our line of mice noted normal survival to weaning but a number of deaths at approximately eight weeks of age [6]. Morham et al noted an unusually low number of male progeny of F1 matings carrying one or two mutated alleles [6]. The F3 and F4 generations described in this report contained a normal distribution of male and female animals in all genotypes, suggesting that—at least in subsequent generations—there is no effect of COX-2 deficiency on gender survival.

This report documents an organ-specific renal growth failure induced by deletion of the *Ptgs2* gene. While heart growth and body growth were unaffected, COX-2 deficiency resulted in an arrest of kidney growth beginning at PN10. These results complement similar findings by Kömhoff et al in which pharmacologic inhibition of COX-2 resulted in impaired renal cortical development and reduced glomerular diameter [11]. Further studies are necessary to determine whether this growth failure is diffuse throughout the kidney or specific to particular cell types or nephron structures. Dinchuk et al reported a 50% incidence of diffuse myocardial fibrosis in their adult null mice [7]. Although no microscopic analysis was performed on the hearts in this report, there were clearly no differences in cardiac mass seen within the first six weeks of life.

Histologic analysis of the kidneys from infant, young, and adult mice deficient in COX-2 documented a profound progression of cystic dysplasia over time. Consistent with Morham et al's report [6], the kidneys of newborn and three-day-old mice appeared normal. Dinchuk et al reported impressive cystic dysplasia at PN2 [7], a finding not documented in the F3 and F4 generation progeny of the current study. Other knockout models have shown variations in the phenotype with subsequent inbreeding [12], suggesting that the remaining genetic environment of the mouse may impact the final phenotype. Whether the abnormalities caused by deletion of *Ptgs2* will improve, worsen, or remain unchanged with further inbreeding remains to be seen. Certainly, there is no evidence that the final pathway of progressive renal dysplasia and insufficiency in these mice is significantly alleviated by the third and fourth generations.

It is important to consider the fact that prenatal and early PN kidney development is normal in these mice even though no COX-2 has been present in the mouse itself throughout development of the animal. These results have recently been confirmed using a pharmacologic approach of prenatal and early postnatal COX-2 inhibition with SC58236. In these studies, Kömhoff et al noted that treatment throughout gestation caused no definable renal abnormalities at birth, while persistence of therapy through weaning resulted in histologic abnormalities at PN21 [11]. Several possibilities exist that might explain this phenomenon. COX-2 may not normally be expressed during early nephrogenesis in the mouse, but expression may be required at a later time point to tailor the final stages of renal architectural development appropriately. However, COX-2 immunoreactivity has been found as early as E16 in the developing rat with increasing expres-

Fig. 8. Phenotypic variability in adult (8-week-old) −/− littermates. (*A*) Severe cystic degeneration with glomerular sclerosis and loss. (*B*) Littermate of (A) without significant cystic changes. Outer cortical glomerular hypoplasia and inner nephron hypertrophy are consistently demonstrated.

Table 1. Daily water intake and urine output

	Wild-type (+/+) $N = 6$	Homozygous null (−/−) $N = 4$
Water intake *cc/g/day*	0.2655 ± 0.022	0.2650 ± 0.015
Urine output *cc/g/day*	0.0605 ± 0.0063	0.0688 ± 0.008

Table 2. Urinalysis and urine osmolality

Urine	Wild-type (+/+) $N = 7$	Homozygous null (−/−) $N = 7$
Blood	Negative	Negative
Protein	Trace to 1+	Trace to 1+
Specific gravity	1.015 ± 0.002	1.015 ± 0.002
Glucose	Negative	Negative
pH	7.31 ± 0.06	7.23 ± 0.21
Ketone	Negative	Negative
Bilirubin	Negative	Negative
Osmolality *mOsm/kg*	1573 ± 252	1172 ± 163

Table 3. Plasma chemistries

	Wild-type (+/+) $N = 7$	Homozygous null (−/−) $N = 5$
Sodium *mEq/L*	152 ± 1	149 ± 3
Potassium *mEq/L*	3.7 ± 0.4	4.0 ± 0.2
Chloride *mEq/L*	125 ± 0.4	125 ± 0.6
Total CO_2 *mEq/L*	15.3 ± 0.7	15.4 ± 0.6
Glucose *mg/dL*	171 ± 6	184 ± 16
BUN *mg/dL*	29.1 ± 0.7 ($N = 10$)	79.2 ± 7.5[a] ($N = 13$)
Creatinine *mg/dL*	0.29 ± 0.04 ($N = 10$)	0.49 ± 0.06[b] ($N = 11$)

[a] $P < 0.001$ vs. +/+
[b] $P = 0.014$ vs. +/+

Table 4. Urinary electrolyte excretion rates

	Wild-type (+/+) $N = 5$	Homozygous null (−/−) $N = 4$
Sodium *mEq/kg/day*	8.59 ± 1.85	8.04 ± 1.94
Potassium *mEq/kg/day*	12.71 ± 2.77	10.10 ± 1.88
Chloride *mEq/kg/day*	17.02 ± 3.66	13.33 ± 2.72

sion as ontogeny and maturation progress [3]. These results suggest the intriguing hypothesis that the function of COX-2 in early renal development is not mandatory, while later expression is necessary for terminal processing. Alternative explanations for normal prenatal development include maintenance of normal eicosanoid production by local COX-1 expression or circulation of maternally produced prostanoids, either of which could support normal development. An additional explanation for the development of progressive cystic dysplasia in the later stages of renal development includes the potential toxic effects of HETEs, leukotrienes, and cytochrome P450 metabolites that may be elevated in the absence of COX-2.

The initial pathologic abnormalities seen in the −/− animals included cyst formation in multiple nephron segments, crowding of differentiating glomeruli, and poor tubule differentiation within a thin nephrogenic cortex. These abnormalities suggest that a variety of cell types are affected by COX-2 deficiency, although a cascade effect resulting from the alteration of a single precursor cell cannot be excluded. It is interesting to note that the progressive renal destruction in COX-2–deficient mice is both similar and different to the abnormalities seen in several murine kidney disease models. The *cpk* mouse and the *bpk* mouse, both models of recessive cystic disease, develop proximal tubular cysts in the late stages of gestation and develop collecting duct cysts in the first postnatal week [13, 14]. Both lines die with massive renal enlargement and renal insufficiency by 25 to 28 days of postnatal life. Mice lacking the bcl-2 protooncogene also have abnormal renal development and cystic degeneration [15]. These kidneys are hypoplastic at birth and exhibit cystic changes in all nephron segments beginning in the first week of life [15]. COX-2–deficient mice have been suggested as a model of oligomeganephronia because of the hypertrophy of the relatively unaffected juxtamedullary nephrons [6]. The only described mouse model of this disorder is the Os/+ mouse, an autosomal dominant mutation, that results in prenatal renal hypoplasia, progressive hypertrophy of remaining nephrons, without cystic degeneration, and oligosyndactyly [16]. COX-2–deficient mice, in contrast, have normal prenatal renal growth, postnatal pathology, including cysts, and an autosomal recessive inheritance pattern. It appears that deficiency of COX-2 results in a unique form of renal maldevelopment that should provide important clues as to the role of COX-2 in this complex process.

The pathologic changes seen in COX-2 deficient kidneys result in surprisingly few alterations in physiologic markers. Blood pressures are not different between +/+ and −/− animals, suggesting that prostanoid products of COX-2 activity do not regulate baseline blood pressure or that compensatory mechanisms completely abolish the appearance of any effects. Likewise, baseline sodium and water balance is unaffected by COX-2 deficiency. Given the known roles of distal nephron prostanoid activity to enhance sodium and water excretion, it was anticipated that deletion of COX-2 would result in salt and water retention, much like that seen with nonsteroidal anti-inflammatory drug (NSAID) use. Whether salt and water reabsorption is "normalized" by the destruction of normal tubular architecture has not been determined. In fact, the only definable difference in baseline renal function seen in COX-2–deficient mice is decreased GFR and resultant uremia. Given the degree of cystic degeneration seen in these kidneys, this loss of functional capac-

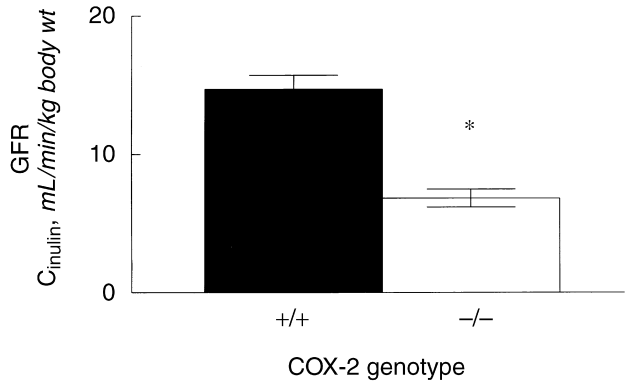

Fig. 9. Glomerular filtration rate in an adult wild-type (+/+) and COX-2 homozygous null mutant (−/−) mice. A loss of COX-2 expression results in approximately 50% loss of renal functional capacity.

ity is certainly expected. The otherwise normal electrolyte values seen in these animals may reflect the fact that acidosis and hyperkalemia may be apparent only in the final stages of disease, just before death.

In summary, genetic inactivation of the *COX-2* gene results in progressive organ-specific postnatal renal dysplasia initially affecting the final generations of nephrons and eventually involving the entire kidney. While prenatal and early postnatal renal development is apparently normal, by 10 days of postnatal life, outer cortical nephrogenesis is impaired, manifested by loss of proximal tubular mass, arrested glomerular growth and early cystic changes. Over time, cystic degeneration progresses, and inner cortical nephrons hypertrophy, presumably in response to hyperfiltration. Finally, all normal renal architecture is destroyed by cystic changes and glomerular fibrosis. These histologic changes are accompanied by a loss of GFR without changes in blood pressure. These results indicate that functional COX-2 is necessary to achieve a normal mature renal phenotype and maintain normal renal excretory activities, but is not necessary for early renal differentiation or fetal and early postnatal survival. The exact mechanisms by which COX-2 and/or its prostanoid products modulate renal development remain unknown.

Perspectives

Congenital renal hypoplasias and dysplasias are common etiologies of renal insufficiency leading to end-stage renal disease in children. While modern mutant mouse technologies have clearly shown the importance of a number of transcription factors, growth factors, hormones, cell adhesion molecules, and matrix proteins in the complex process of renal development, the specific causes of human renal dysplasias remain poorly defined. The pathologies seen in COX-2–deficient mice and mice treated with perinatal COX-2 inhibitors are similar to those seen in several human infants with renal failure secondary to long-term NSAIDs in utero [17]. In addition, persistent renal insufficiency has been noted in patients receiving chronic ibuprofen for Bartter's syndrome [18]. It is therefore important to understand how COX-2 regulates renal development both as a means to understand renal dysplasia and to protect the developing kidney more adequately from the potentially permanent toxic effects of COX-2 blockade.

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