Influence of genetic background and gender on hypertension and renal failure in COX-2-deficient mice

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Submitted 14 June 2004; accepted in final form 3 November 2004

Yang, Tianxin, Yuning G. Huang, Wenling Ye, Pernille Hansen, Jurgen B. Schnermann, and Josephine P. Briggs. Influence of genetic background and gender on hypertension and renal failure in COX-2-deficient mice. Am J Physiol Renal Physiol 288: F1125–F1132, 2005.—The present study was undertaken to determine whether the severity of renal failure or hypertension in homozygous cyclooxygenase (COX)2-deficient (COX-2−/−) mice affected by genetic background or gender. COX-2 deletion was introduced into three congenic genetic backgrounds. 129/Sv (129/COX-2−/−), C57/BL6 (C57/COX-2−/−), and BALB/c (BALB/COX-2−/−), by backcrossing the original mixed-background knockout mice with the respective inbred strains for 9 or 10 generations. Evaluation of the severity of hypertension and renal failure was performed in knockout and wild-type mice at the age of 2.5–3.5 mo. Blood pressure measured by tail-cuff plethysmography was significantly elevated in the male 129/COX-2−/− mice (165.8 ± 9.2 vs. 116 ± 5.1 mmHg, P < 0.05), and to a much lesser extent in the female 129/COX-2−/− mice (127.4 ± 3.3 vs. 102.4 ± 3.3), whereas it was unchanged in the C57- or BALB/COX-2−/− mice regardless of gender. Urinary excretion of albumin, determined by ELA, was remarkably increased in the 129/COX-2−/− (16.4 ± 4.1 vs. 0.16 ± 0.043 mg albumin/mg creatinine, P < 0.001), and to a lesser extent in the male C57/COX-2−/− mice (0.595 ± 0.416 vs. 0.068 ± 0.019). Albumin excretion was not elevated in the male BALB/COX-2−/− or in female COX-2−/− mice on any of the three genetic backgrounds. Histological analysis showed abundant protein casts, dilated tubules, and infiltration of inflammatory cells in the male 129/COX-2−/− mice, but not in COX-2−/− mice in other strains or gender. However, the presence of small glomeruli in the nephrogenic zone was observed in all strains of COX-2 knockout mice, regardless of genetic background and gender. Therefore, we conclude that the severity of hypertension and renal failure in COX-2-deficient mice is influenced by genetic background and gender, whereas the incomplete maturation of outer cortical nephrons appears to be independent of genetic background effects.

Cyclooxygenase, also called PGH synthase, is a rate-limiting enzyme catalyzing the metabolism of arachidonic acid to PGH2, the common precursor for bioactive prostaglandins (PGs). COX exists in two distinct isoforms, the constitutive COX-1 and the inducible isoform COX-2 (37). COX-1 is expressed in a wide variety of tissues, and its expression level doesn’t appear to undergo robust changes under most experimental conditions. COX-1 mediates housekeeping functions such as platelet aggregation and cytoprotection in the gastrointestinal tract. Some evidence suggests that COX-1-derived PGs may exert vasoconstrictive and sodium-retaining effects (1, 31), but its exact function in the kidney has largely remained elusive. In contrast, COX-2 is much more restricted in its expression and its abundance is highly inducible by growth factors and cytokines as well as physiological stimuli. In the kidney, COX-2 is constitutively expressed in the macula densa and thick ascending limb cells as well as in renal medullary cells in which the expression undergoes dynamic changes under changing states of sodium and water balance (4, 12, 39). COX-2 has been shown to influence renin secretion (6, 40) and renal hemodynamics (21, 22, 29, 32, 33). As in many other areas of research, gene knockout technology has provided powerful tools for studying the functional roles of the two COX isoforms. COX-1 knockout (KO) mice have a virtually normal phenotype, and there are no obvious renal structural abnormalities (19). In contrast, COX-2 KO mice show marked renal pathology, including hydropplasia of the nephrogenic zone and tubular atrophy, starting at postnatal periods (8, 27).

Although the kidney abnormalities in COX-2 KO mice were observed consistently in a number of studies, the severity of the renal dysfunction appears to be variable. COX-2−/− mice, as generated by Dinchuk et al. (8), develop severe renal abnormalities, including an overabundance of immature small glomeruli and multiple foci of dystrophic tubules in the subcapsular zone at postnatal day 2 (PND2), and the average life span of these mice has been reported to be only ~3 mo. COX-2−/− mice subsequently developed by Morham et al. (27) showed a milder renal pathology, with 75% of the mice having a life span of longer than 6 mo. Since the gene pool of the two COX-2−/− strains represents a mixture of C57/BL6 and 129/Sv DNA, it seems possible that differences in the genetic background may account for the phenotypic variations. Despite the reduced renal function, blood pressure in these mice is usually normal and gender differences in the progression of the disease have not been reported.

The present study was undertaken to determine whether the severity of renal failure or hypertension in COX-2 KO mice is affected by genetic background or gender. COX-2 deletion was introduced into 129/Sv, C57/BL6, and Balb/C congenic genetic backgrounds by backcrossing the original KO mice with the respective inbred strains for 9 or 10 generations. Remarkable differences in the severity of renal failure and hypertension were observed among different strains and between genders, with the most severe pheno-

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type being found in male COX-2 KO mice on 129 genetic background.

METHODS

Establishment of COX-2 congenic lines. COX-2−/− mice on a mixed 129/C57 background were originally generated by Dinchuk et al. (8), and the breeder pairs were obtained from Jackson Laboratories. Inbred 129, C57, and BALB mice were purchased from the same vendor. Using the conventional backcross breeding strategy, congenic COX-2 KO strains were generated (3, 16) by crossing male heterozygous COX-2−/− mice with females of each of the inbred recipient strains. After genotyping, male heterozygous offspring were chosen as breeders and again crossed with females of the inbred strains. This breeding strategy was repeated over 3 yr for 9 generations of backcross breeders and again crossed with females of the inbred strains. Plasma blood urea nitrogen (BUN) was determined by the SYNCHRON LX system (LX-20, Beckman Coulter), which detects the changes in the solution conductivity after enzymatic conversion of a nonionic species (urea) to an ionic species (NH₄⁺). Plasma creatinine was determined by the Jaffe method.

Statistical analysis. Values shown represent means ± SE. Statistical analysis was performed by ANOVA and Bonferroni’s tests with a P value of <0.05 being considered statistically significant.

RESULTS

Systolic blood pressure. Wild-type and homozygous COX-2 mice on various genetic backgrounds in the 2.5- to 3.5-mo age range were subjected to systolic blood pressure measurement using a tail-cuff computerized system. As shown in Fig. 1, blood pressure was significantly elevated in the male 129/COX-2−/− (165.8 ± 9.2 vs. 116 ± 5.1 mmHg, P < 0.05) and to a much lesser extent in the female 129/COX-2−/− (127.4 ± 3.3 vs. 102.4 ± 3.3) mice, but C57/COX-2−/− or BALB/COX-2−/− mice regardless of gender were normotensive.

Albuminuria. Adult mice were placed in metabolic cages, and 24-h urine was collected for determination of urinary albumin and creatinine. To eliminate the influence of urine evaporation, the ratio of urinary albumin vs. creatinine is presented. Urinary excretion of albumin, determined by ELA, was significantly increased in the male 129/COX-2−/− mice (16.4 ± 4.1 vs. 0.16 ± 0.043 mg albumin/mg creatinine, P < 0.001), and to a lesser extent in the male C57/COX-2−/− mice (0.595 ± 0.416 vs. 0.068 ± 0.019), whereas there was no albuminuria in the male BALB/COX-2−/− or in female COX-2−/− mice on any of the three genetic backgrounds (Fig. 2).
Renal morphological analysis. Examples of renal histological sections from male and female adult mice are shown in Figs. 3 and 4. Small glomeruli in the superficial cortex throughout adulthood, a hallmark of the postnatal abnormalities in kidney maturation of COX-2 KO mice, were observed in all congenic COX-2 $-/-$ mice, regardless of strain and gender. However, the severity of kidney injury varied markedly between different strains and genders, with the most severe injury occurring in the male 129/COX-2 $-/-$ mice. As shown in Fig. 3, renal histology of male 129/COX-2 $-/-$ mice exhibited large amounts of protein casts, dilation of tubules, and infiltration of inflammatory cells. In contrast, these pathological changes were much milder in the female counterparts of the same strain or in the BALB/COX-2 $-/-$ or C57/COX-2 $-/-$ strain. Furthermore, an injury score system was used for semiquantitative analysis of the major pathological parameters as described in previous studies (20). The score was based on a 1–5 scale, with higher numbers indicating more severe injury. As shown in Fig. 5, the highest injury score was found in the male 129/COX-2 $-/-$ mice.

Plasma chemistry. Plasma BUN was significantly increased in all congenic COX-2 $-/-$ mice regardless of genetic background and gender (Fig. 6). The greatest increase was found in the male 129/COX-2 $-/-$ mice, but the plasma BUN variations in the different congenic strains and genders were not as striking as the histological changes or albuminuria. Surprisingly, plasma creatinine, determined by the Jaffe method, was not significantly increased in any strains of the congenic KO mice despite the clear evidence of renal failure in these mice (data not shown). Plasma Na, K, and Cl concentrations were not significantly altered in the three strains of congenic KO mice when the animals were kept on a normal-sodium diet (data not shown).

Examination of gender difference in young 129 mice. Considering the remarkable gender difference in 129 strain adults, we performed further studies to determine the time frame for the occurrence of this phenomenon. Specifically, we attempted to compare the renal phenotype between the genders at various postnatal periods including PND7 and 14. Because visible identification of the gender in the pups was difficult, we performed PCR analysis of the SMCX gene, which shows a sex-related polymorphism (Fig. 7). To validate the method, PCR of the SMCX gene was performed on tail DNA of four male and four female mice at weaning. The PCR products were detected as a single band in females while as two bands in males, thus permitting a clear gender distinction. As shown in Fig. 8, the abnormality of postnatal kidney development, as evidenced by the presence of increased numbers of small glomeruli in the superficial cortex, was detected in the 129/COX-2 $-/-$ mice at PND7 and was more pronounced at PND14. However, no gender differences in renal histology or
in plasma BUN (Fig. 9) were detected in the young 129/COX-2−/− mice.

**DISCUSSION**

COX-2 KO mice developed by two independent groups of investigators develop a comparable renal phenotype starting at an early postnatal period (8, 27). The most striking abnormality consists of a persistence of hypotrophic tubules in the nephrogenic zone with a large number of immature glomeruli and dystrophic tubules. A similar renal phenotype can also be produced by pharmacological blockade of COX-2 (17). These observations suggest a critical role of COX-2 in postnatal kidney development. However, the severity of the renal pathology in COX-2−/− mice varies considerably, suggesting the existence of modifying factors. To investigate the possible influence of the genetic background on the renal phenotype induced by COX-2 deficiency, we developed various congenic strains of COX-2-deficient mice using the backcross breeding strategy. Three congenic strains in a C57/BL6, 129/Sv, or BALB/c background were generated by crossing the original COX-2−/− mice into the respective inbred strains for 9 or 10 generations. The entire breeding procedure took ~3–4 yr, a typical generation time with the conventional backcross protocol (3, 16). To our knowledge, this is the first report on the generation of congenic strains of COX-2−/− mice. These models provide a novel tool for the study of genetic factors that may affect the development of chronic renal disease.

A persistent presence of immature glomeruli in the superficial cortex was observed in COX-2−/− mice in all three congenic strains as previously described in the mixed background C57/129 strain. Regardless of genetic background or gender, the impairment of nephrogenesis in COX-2-deficient mice appears to eventually progress to chronic renal failure, as evidenced by the rise in plasma BUN. These findings demonstrate that COX-2 deficiency alone is sufficient to initiate chronic renal failure, and they suggest that COX-2 plays an important role in the maintenance of renal structure and function. This raises the possibility that COX-2 may be a candidate gene for congenital diseases of kidney development in humans. In support of this notion, the renal pathology of the COX-2−/− mice shares some degree of similarity with that associated with perinatal exposure to nonsteroidal anti-inflammatory drugs in humans (15, 34).
The pathogenesis of chronic renal disease (end-stage renal disease) is multifactorial, and the factors that initiate the disease likely differ from those that contribute to its progression. Elucidation of each of these factors in human studies is difficult but can be achieved using genetic mouse models. Our studies provide evidence that renal failure is clearly initiated by COX-2 deletion, whereas its progression is affected by different factors, including genetic background and gender. Comparing the phenotype of various congenic strains within the same gender reveals remarkable strain-dependent variations. Despite chronic renal failure, male BALB/COX-2−/− mice do not develop hypertension or albuminuria, an observation similar to that in COX-2−/− mice on a mixed genetic background (28). Male C57/COX-2−/− mice show mild proteinuria but no hypertension. In contrast, male 129/COX-2−/− mice develop malignant hypertension and overt proteinuria associated with severe renal fibrosis. In a somewhat milder form, a similar pattern of strain-dependent variations was also observed in female mice. Our findings are in good agreement with the report of Ma et al. (25) on the occurrence of hypertension and proteinuria induced by 5⁄6 or 7⁄8 nephrectomy in the 129/Sv strain compared with C57/BL6 mice. Taken together, these observations demonstrate that the genetic background is an important factor determining susceptibility to the development of hypertension and chronic renal failure in COX-2−/− mice.

Comparing the gender-specific phenotype in the same strains of mice revealed marked differences with substantially more pronounced renal injury and hypertension in male than in female animals, particularly in the 129 strain. The malignant hypertension, overt proteinuria, and severe renal injuries in male 129/COX-2−/− mice contrast remarkably with the mild hypertension, normal proteinuria, and well-preserved renal structures in the female counterparts. In further investigations of this phenomenon in the 129 strain at various postnatal periods, we found that the remarkable gender difference observed in the adult was absent in early postnatal periods. This age-dependent gender difference represents definitive evidence for the role of gender in the progression of renal failure rather than in the development of the primary renal abnormality. Indeed, chronic renal diseases especially those associated with hypertension tend to be more progressive in men than in women (11, 26, 35). Furthermore, the incidence of hypertension-related renal and cardiovascular disease increases sharply after menopause (14).

The mechanism underlying the susceptibility to hypertension and renal failure in the male mice of the 129 strain is not clear. Some evidence suggests that this could be related to the number of renin genes. Two renin genes (Ren-1 and Ren-2, resulting in four renin gene copies) are harbored by 129 mice, whereas C57 and BALB mice only have one renin gene (Ren-1, resulting in two renin gene copies). Ren-1 is expressed at the highest levels in the juxtaglomerular apparatus of the kidney, whereas Ren-2 is highly expressed in the submaxillary gland as well as in other organ systems including the kidney. Wang et al. (6) examined plasma renin concentration in the offspring of crosses of C57Bl6/j and 129Ola mice to evaluate the contribution of one or two renin genes to total plasma renin in a controlled genetic background. Plasma renin concentration was 100-fold higher in mice with two renin genes than in those with one renin gene. In addition, Ren-2 has been found to be an androgen-responsive gene and is expected to be more activated in males than in females (13, 38). In line with this notion, genetic deletion of the Ren-1 gene in 129/Sv mice produces hypotension in females but not in males (7), indicating involvement of Ren-2 in a sexually dimorphic mechanism in the
control of blood pressure. These findings suggest that enhanced activity of the renin-angiotensin system resulting from the androgen-activated Ren-2 gene may be in part responsible for the susceptibility to the renal phenotype in the male 129 strain. However, this notion is not supported by a recent observation that plasma renin is lower in C57/BL6 mice with one renin gene than in 129/Sv mice with two renin genes (24).

Although blood pressure and urinary albumin excretion varied strikingly with genetic background and gender, we observed only modest variations in plasma BUN. These findings suggest that different renal injury parameters may be under the control of different genetic factors. In agreement with this notion, linkage analysis performed in the fawn-hooded rat has identified that albuminuria and glomerulosclerosis are influenced by RF1 and RF2 loci, whereas hypertension resides at a different locus, called bphf (5, 30, 36). Future linkage analysis studies on congenic COX-2/H11002/H11002/H11002 mice will be necessary to identify the chromosome locus or loci responsible for the phenotypic variations.

The present study permits a comparison between plasma BUN and creatinine as estimates of renal function in mice. In general, plasma BUN in the various strains of congenic COX-2/H11002/H11002/H11002 mice correlates with the extent of the renal pathological changes. In contrast, plasma creatinine, measured by the Jaffe method, did not increase in any of the COX-2-deficient strains, including male 129/COX-2/H11002/H11002 mice. A recent study by Dunn et al. (9) documents that plasma creatinine measured by high-performance liquid chromatography but not the Jaffe method accurately reflects renal function in mice. Taken together, the Jaffe method

Fig. 8. Microscopy of HE-stained kidney sections in 7- and 14-day-old COX-2/H11002/H11002 and wild-type mice of the 129/Sv strain. The rectangular box encloses glomeruli in the subcapsular cortical region in COX-2/H11002/H11002 mice but not in wild-type controls. PND, postnatal day.

Fig. 9. Plasma BUN in 14-day-old young mice of the 129/Sv strain. Under general anesthesia, blood samples were collected from wild-type (WT; n = 8, pooled males and females), male 129/COX-2/H11002/H11002 (n = 6), and female 129/COX-2/H11002/H11002 mice. Values are means ± SE. There was no statistically significant difference in plasma BUN between male and female 129/COX-2/H11002/H11002 mice. *P < 0.05 vs. WT.
should not be considered as an accurate method for the determination of plasma creatinine in mice.

It should be pointed out that it remains to be determined whether the congenic COX-2 KO mice, especially those of the 129 strain, develop abnormalities in organs other than the kidney. It has been shown that 35% of COX-2−/− mice on a mixed genetic background of C57/6J and 129/Ola die of patent ductus arteriosus within 48 h after birth, although the majority of the COX-2−/− mice have normal closure of the ductus arteriosus and live to adulthood (23). Furthermore, perinatal death has not been reported consistently in studies of COX-2−/− mice. Norwood et al. (28) have observed that the ratios of wild-type, heterozygous, and homozygous COX-2 mice on the same mixed genetic background (crossing +/− males with +/− females) were 1:2:1 as expected. Our studies agree with this report in that our breeding records do not reveal obvious perinatal death of 129/COX-2 KO mice. In this regard, the litter size of 129/COX-2 KO colonies (crossing female COX-2−/− males with female COX-2+/− mice, the ratio of homozygous and heterozygous mice at 1 mo of age was roughly 1:1 as predicted (average number per litter: 4.5 ± 0.5 in −/− group vs. 3.7 ± 0.6 in +/− group). The reason for the variable survival and possibly variable incidence of patent ductus arteriosus in COX-2 KO mice in different genetic backgrounds is unclear but could be again related to differences in genetic backgrounds. In summary, in the present study we propagated the COX-2−/− null mutation in three congenic mice strains, 129/Sv, C57/BL6, and BALB/c, using the conventional backcross strategy. All COX-2−/− mice developed abnormalities of postnatal kidney developmental and chronic renal failure, regardless of genetic background and gender. However, the severity of the pheno-type is significantly influenced by genetic background and gender, with most severe hypertension and renal injury being observed in the male 129 strain. Our findings reinforce the dominant role of COX-2 in the maintenance of kidney structure and function and identify genetic background and gender as modifying factors for the progression of hypertension and chronic renal failure.

ACKNOWLEDGMENTS

We thank Dr. Donald E. Kohan (Div. of Nephrology, Univ. of Utah) for a critical reading of the manuscript.

GRANTS

The work was supported by intramural funds of the National Institute of Diabetes and Digestive and Kidney Diseases and National Institutes of Health Grants DK-064981, DK-066592, and HL-079453 (to T. Yang).

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