Selective COX-2 inhibition markedly slows disease progression and attenuates altered prostanoid production in Han:SPRD-cy rats with inherited kidney disease

Deepa Sankaran,1 Neda Bankovic-Calic,2 Malcolm R. Ogborn,1,2,4 Gary Crow,3 and Harold M. Aukema1,4

Departments of 1Human Nutritional Sciences, 2Pediatrics and Child Health, and 3Animal Sciences, University of Manitoba, and 4Manitoba Institute of Child Health, Winnipeg, Manitoba, Canada

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Cyclooxygenase (COX) exists as two major isoforms, COX-1 and COX-2. Previously, COX-1 was considered to be constitutively expressed and have general "housekeeping" functions, whereas COX-2 was believed to be inducible and involved in inflammatory responses (10). Now it is known that COX-2 also is constitutively expressed in the mammalian kidney and that its metabolites, including prostanoids, play an integral role in the regulation of renal hemodynamics, the renin-angiotensin system, nephronogenesis, and renal pathogenesis (10). Prostanoids orchestrate their effects on renal function via a number of membrane-bound G protein-coupled receptors that display stimulatory and inhibitory actions. For example, PGE2 has vasodilator and vasoconstrictor effects, depending on the type of E-prostanoid receptors present. In addition to hemodynamic effects, prostanoids also can influence inflammation (PGE2), renal interstitial fibrosis, and cell growth and death (PGI2) (4, 29, 35).

Elevated COX-2 enzyme activity has been reported in a number of renal disorders and nephritic models (27, 31, 32, 55, 57), including the rat model of polycystic kidney disease (PKD) (58). In animal models of renal disease, as well as in humans with elevated COX-2 activity, COX-2 inhibition often has beneficial effects (3, 9, 11, 13, 16, 31, 42, 53, 56). However, pharmacological blockade of COX-2 decreased glomerular filtration rate in salt-depleted and elderly subjects (40, 51) and was detrimental in a rat model of glomerulonephritis (30). Nevertheless, the effect of selective blockade of COX-2 in cystic kidneys has not been ascertained.

The Han:SPRD-cy rat is an established model of autosomal dominant PKD, in which heterozygotes develop progressive cystic change, renal interstitial fibrosis, inflammation, and oxidative damage, as well as hypertension and uremia, in adult life (12). We recently demonstrated that renal disease selectively alters the prostanoid profile and ratios in the Han:SPRD-cy rat (58). The thromboxane (TX) B2 (TXB2)-to-PG ratios were higher in diseased than in normal kidneys, suggesting that the presence of cystic kidney disease shifts the balance of renal prostanoids, which may contribute to disease progression. In addition, PGI2 was altered to a greater extent than PGE2 by renal disease. Although renal COX-1 and COX-2 activities were elevated in diseased rats, COX-2 activity was predominately in normal and diseased Han:SPRD-cy rats. The previous study indicated that no specific prostanoids were associated with these isoforms. Thus we undertook the present study to determine whether administration of a selective COX-2 inhibitor in vivo would ameliorate renal cystic disease progression, as well as disease-associated alterations in renal tissue injury and prostanoid production, in these rats. This study also elucidates further the role of COX-2 and its metabolites in the pathogenesis of disease in the Han:SPRD-cy rat model of PKD.

METHODS

Animals. All animal procedures were examined and approved by the University of Manitoba Animal Care and Use Committee and were carried out in accordance with the guidelines of the Canadian Council on Animal Care. Han:SPRD-cy [also known as PKD/Mhm (cy+/cy)] rats were derived from our breeding colony, which originated from the colony of Dr. Benjamin Cowley (University of Kansas Medical Center, Kansas City, KS) (12). Weanling male Han:SPRD-cy heterozygotes were randomly assigned to a control diet consisting of the AIN-93G rodent diet (38) or the same diet to which NS-398 (3 mg kg body wt−1·day−1) or a control diet without NS-398 for 7 wk. In diseased rats, selective COX-2 inhibition resulted in 18% and 67% reduction in cystic expansion and interstitial fibrosis, respectively, but no change in renal function. NS-398 also ameliorated disease-associated pathologies, such as renal inflammation, cell proliferation, and oxidant injury (by 33, 38, and 59%, respectively). Kidney disease was associated with elevated renal COX-1 and COX-2 enzyme activities, and NS-398 blunted the increase in COX-2 enzyme activity (as indicated by 21 and 28% lower COX-1 and COX-2 activities, respectively). NS-398 reduced urinary excretion of prostanoid metabolites in diseased rats. In summary, COX-2 inhibition attenuated renal injury, reduced the elevated renal COX-2 activity, and ameliorated disease-related alterations in prostanoid production in this rat model of chronic renal disease.

Address for reprint requests and other correspondence: H. M. Aukema, H506 Duff Roblin Bldg., Dept. of Human Nutritional Sciences, Univ. of Manitoba, Winnipeg, MB, Canada R3T 2N2 (e-mail: aukema@umanitoba.ca).

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COX-1 activity only minimally, even at >200 mg/kg body wt (17). NS-398 was mixed with the diet to achieve a dose of 3 mg·kg body wt
day−1. To achieve this dose and on the basis of previously
determined food intake data, weaning rats were given NS-398 at 25
mg/kg diet for the first 2 wk of the study and then switched to 36
mg/kg diet for the remainder of the study. The adequate dose was
ensured by determination of food intake (i.e., routine weighing of food
cups) and weekly measurement of body weight of all rats. The dose of
NS-398 was based on previous studies that showed effective inhibi-
tion of COX-2 in the rat for up to 24 h with use of a similar dose (18,
33). Diet ingredients, except NS-398 (Cayman Chemical, Ann Arbor,
MI), were purchased from Dyets (Bethlehem, PA) or Harlan Teklad
(Madison, WI).

Animals were fed the control and experimental diets for 7 wk (i.e.,
until they were 10 wk of age). At 9 wk of age, rats were placed in
metabolic cages for collection of metabolic data, and, after an accli-
matization period of 2 days, 24-h food and water intakes were
measured. Urine was collected and stored at −80°C for later analyses.
At the end of the feeding period, rats were killed, and trunk blood was
collected to obtain serum. Left kidneys were removed, weighed, and
sectioned in half longitudinally across the hilum. One half of the
left kidney tissue was fixed in 10% buffered formalin for morphological
and histological analyses. The other half of the left kidney and the
right kidney were snap frozen in liquid nitrogen and stored at −80°C
for later analyses.

Histology and immunohistochemistry. The left kidney was embed-
ded in paraffin, cut into 5-μm sections, and processed using our
previously described methods for histological and immunohistochem-
ical analyses (36). Sections for cyst area measurement were stained
with hematoxylin and eosin and those for quantitative analysis of
fibrosis with sirius red (an adaptation of Masson's trichrome stain),
which permits image analysis measurement using a standard incan-
descent microscope light source. Renal cell proliferation, inflamma-
tion, and oxidant injury were assessed using 1:50 dilutions of an
anti-mouse proliferating cell nuclear antigen (PCNA) antibody
(M0879, Dako, Carpentry, CA), a mouse anti-rat monocyte/macroph-
age mononclonal antibody (MAb 1435, Chemicon International,
Temecula, CA), and a rabbit anti-Cu2+-oxidized LDL polyclonal
antibody (Ab 3230, Chemicon International, respectively). The
EnVision Plus system (model K4008, Dako) was used for secondary
detection. Omission of the incubation step with primary antibody was
used as a negative control for all antigens.

Image analysis and morphometric assessment. Quantitative histo-
logical analysis of the left kidney was performed using our previously
described methods (36, 43). Briefly, after they were captured using a
SPOT Junior charge-coupled device camera by random stage move-
ment through the sections, images were analyzed using Image Pro
version 4.5 (Media Cybernetics, Silver Spring, MD). An average of 25
measurements, starting from a random field of tissue from kidney
cross sections, were collected for all histomorphometric analyses. All
measurements were carried out in a blinded fashion. The portion of
tissue section occupied by tubular lumen or cyst was determined for
cyst area measurements. Renal interstitial fibrosis and oxidant injury
were measured by densitometry, whereas the number of cells that
stained positive for PCNA, as well as macrophages, was counted
using Image Pro version 4.5, as described previously (36, 43). Renal
cyst area measurements are expressed relative to kidney weight-to-
body weight ratios. PCNA-positive cells in tubular epithelium were
counted, insomuch as these represent the base cell type of cysts.
Measurements of fibrosis, oxidized LDL, and other cellular markers
were corrected to solid tissue areas of sections to avoid underestima-
tion of these variables due to empty cystic areas in these sections.

Immunoblotting. Half of the left kidney was lyophilized, and 30 mg
were homogenized in ice-cold homogenization buffer containing
protease inhibitors, as described elsewhere (1, 58). After centrifuga-
tion of the kidney homogenate, the resulting pellet was resuspended
in buffer containing 1% Triton X-100 and recentrifuged. The resulting
supernatant contains COX-2 protein. Protein concentrations of these
fractons were determined using the Bradford method. After SDS-
PAGE, COX-2 was detected with a 1:250 dilution of the primary
antibody (catalog no. 160106, Cayman Chemical) followed by incu-
bation with a peroxidase-conjugated secondary antibody at a dilution
of 1:20,000. ChemiGlow (Alpha Innotech, San Leandro, CA) sub-
strate was used to visualize the immunoreactive bands, which were
then analyzed and quantified on the Fluorochem FC digital imaging
system (Alpha Innotech) (1, 58).

Real-time RT-PCR. Total RNA was extracted from 10–20 mg of
lyophilized kidneys using TRizol, as described elsewhere (58). DNA
was removed by treatment with DNase I (Invitrogen, Carlsbad, CA)
for 15 min at room temperature. One-step RT-PCR was performed on
0.5 μg of total RNA using the QuantiTect SYBR Green RT-PCR kit
(Qiagen, Mississauga, ON, Canada). Oligonucleotide sequences for
the rat COX-1 and COX-2 primers, generated using Primer 3 soft-
ware, have been described elsewhere (58). Real-time RT-PCR was
performed on a SmartCycler II (Cepheid, Sunnyvale, CA) with the
following protocol: reverse transcription at 50°C for 30 min, PCR
activation at 95°C for 15 min, and 40 PCR cycles at 94°C for 15 s,
55°C for 30 s, and 72°C for 30 s. Relative amounts of mRNA were
determined by comparison of cycle threshold (Ct) numbers for equal
amounts of RNA subjected to RT-PCR and calculation of differences
in gene expression using the following formula: 2ΔΔCt (58). Data are
expressed as a relative percentage of the control normal COX-2
mRNA levels.

Prostanoid production and COX activity. Tissue processing for the
determination of prostanoids has been described elsewhere (58).
Briefly, 60 mg of lyophilized tissue from the right kidney were
homogenized with ice-cold fresh Tyrode buffer. Protein concentra-
tions of all fractions were determined using the Bradford method. For
determination of endogenous prostanoid levels, steady-state prosta-
noid production in vitro, and potential COX activity, aliquots were
incubated under the following times and conditions: 1) 0 min for
determination of endogenous levels of prostanoids and background
levels for conditions 2–4, 2) 60 min at 37°C for determination of
steady-state in vitro prostanoid production, 3) 10 min at 37°C for
determination of prostanoid production by total COX activity, and
4) 10 min at 37°C with 0.1 μM SC-560, a selective COX-1 inhibitor
(Cayman Chemical) for determination of prostanoid production by
COX-2 activity. COX-1 activity was determined as the difference
between total COX (condition 3) and COX-2 (condition 4) activity.
Criteria for selection of incubation times and level of inhibitor have
been published elsewhere (58). Briefly, when kidney homogenates
were incubated with SC-560, COX activity was inhibited between 1
and 10 μM, which corresponds to IC50 of 6.3 μM for COX-2. In
contrast, at 0.009 μM (IC50 for COX-1), there was no inhibition of
COX-1 activity in a whole rat blood assay. This also was confirmed
using the selective COX-2 inhibitor niflumic acid, which has a COX-2
IC50 of 0.1 μM and a COX-1 IC50 of 16 μM. Because the isoform
selectivity was greater for SC-560 than for niflumic acid, 0.1 μM
SC-560 was chosen as the selective inhibitor to determine COX
activities. Time-course studies of prostanoid production showed that
prostanoid production increased linearly for the first 10 min, reached
steady-state levels by 30–40 min, and remained at that level until
80 min. From these time-course studies, a 10-min incubation period
was used for the COX activity assays and a 60-min period was used
to determine steady-state levels of prostanoids. At the end of all
incubation periods, reactions were stopped with fresh ice-cold acetyl
salicylic acid (5 mmol/l final concentration). Samples were centri-
fuged, and the supernatant was collected and stored at −80°C for the
determination of PGE2 and the stable metabolites of PGI2 (6-keto-
PGF1α) and TXA2 (TXB2) using commercial enzyme immunoassay
kits (Cayman Chemical).

Urinary TXB2, 6-keto-PGF1α, and PGE2 (a stable urinary metab-
olite of PGE2) were also analyzed using enzyme immunoassay kits
(Cayman Chemical) according to the manufacturer's instructions.
Table 1. Body weight, kidney weight, and renal function in normal and diseased Han:SPRD-cy rats given NS-398 in the diet for 7 wk

<table>
<thead>
<tr>
<th></th>
<th>Control (+/+) (n = 13)</th>
<th>NS-398 (+/+) (n = 9)</th>
<th>NS-398 Cy/+(n = 16)</th>
<th>Effects (P Value)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Body wt, g</td>
<td>385±4</td>
<td>362±4</td>
<td>387±5</td>
<td>Genotype (P &lt; 0.0001)</td>
</tr>
<tr>
<td>Kidney wt, g/100 g body wt</td>
<td>0.73±0.1</td>
<td>2.50±0.1</td>
<td>0.77±0.1</td>
<td>Genotype (P &lt; 0.0001)</td>
</tr>
<tr>
<td>Serum creatinine, µmol/l</td>
<td>54.6±3.5</td>
<td>101.9±3.5</td>
<td>50.8±4.2</td>
<td>Genotype (P &lt; 0.0001)</td>
</tr>
<tr>
<td>Serum urea nitrogen, mmol/l</td>
<td>4.2±1.3</td>
<td>28.5±1.4</td>
<td>3.7±1.5</td>
<td>Genotype (P &lt; 0.0001)</td>
</tr>
<tr>
<td>Creatinine clearance, ml/min/100 g body wt⁻¹</td>
<td>0.34±0.02</td>
<td>0.16±0.03</td>
<td>0.41±0.03</td>
<td>Genotype (P &lt; 0.0001)</td>
</tr>
<tr>
<td>Urinary protein, mg/24 h</td>
<td>5.7±0.5</td>
<td>5.7±0.5</td>
<td>4.4±0.8</td>
<td>None</td>
</tr>
</tbody>
</table>

Values are means ± SE. +/+, Normal; Cy/+, diseased.

Statistical analyses. Data were analyzed using SAS software (SAS, Cary, NC) as a completely randomized design with a 2 × 2 factorial set of treatments [2 genetic groups (+/+ and Cy/+) and 2 diets (control and NS-398)]. Diagnostic tests were performed to evaluate assumptions about the residuals, in particular, normality and the uniformity of their variance across treatment groups. Data were normalized using a logarithmic transformation where necessary. Where variance was unequal across treatment groups, the SAS Mixed procedure was used. Post hoc t-tests were performed if the interaction effect was significant at P < 0.10. P < 0.05 was used to determine significance of main or treatment effects.

RESULTS

Selective COX-2 inhibition with NS-398 ameliorates indexes of renal injury but does not alter renal function in diseased Han:SPRD-cy rats. During the study period, all animals thrived on the diets and displayed normal growth patterns, including a slight (7%) growth depression associated with disease (Table 1). Food intake data indicated that rats treated with NS-398 were ingesting 3–5 mg NS-398·kg body wt⁻¹·day⁻¹ (data not shown). NS-398 administration resulted in reduced urinary excretion and renal production of prostanoids (see below), indicating that the dose of NS-398 effectively inhibited COX-2 activity in vivo.

The effect of selective COX-2 inhibition with NS-398 on parameters of renal injury was determined morphologically and immunohistochemically. Cystic expansion, the primary defect in Han:SPRD-cy rats, was 18% less with drug treatment for 7 wk (Fig. 1). Interstitial fibrosis was reduced by 67% in kidneys of NS-398-treated rats (Fig. 2). Disease-associated pathologies, such as renal inflammation, cell proliferation, and oxidant injury, also were ameliorated with selective COX-2 blockade. Renal macrophage infiltration, a marker of inflammation, was 33% lower in NS-398-treated than in untreated diseased rats (Fig. 3). Selective COX-2 inhibitor treatment also resulted in a reduction in renal cell proliferation, with 38% fewer PCNA-positive cells in kidney sections of NS-398-treated than control rats (Fig. 4). Furthermore, oxidative damage (as determined by the presence of oxidized LDL) was reduced by 59% in the kidneys of NS-398-treated rats compared with rats given NS-398-free diets (Fig. 5).

At the end of the study period, kidneys were larger, creatinine clearance rates were lower, and renal function was worse overall in rats with renal disease than in their normal counterparts (Table 1). The reduced creatinine clearance was associated with elevated serum creatinine and urea levels (Table 1). In contrast to the effects on renal injury, worsened renal function in diseased Han:SPRD-cy rats was not attenuated by NS-398. Neither genotype nor selective COX-2 inhibition significantly affected proteinuria (Table 1).

COX-2 inhibition results in elevated COX-2 gene and protein expression in Han:SPRD-cy rats. Consistent with previously reported COX-2 mRNA expression in Han:SPRD-cy rat kidneys (58), COX-2 mRNA levels were 80–86% lower in kidneys from diseased rats than in kidneys from their normal unaffected counterparts (Table 2). NS-398 partially reversed these changes by increasing renal COX-2 gene expression by 14–66% in normal and diseased rats. Renal COX-2 protein levels, as measured by Western immunoblotting analyses, reflected the relative COX-2 gene levels. COX-2 enzyme levels were reduced 92% in diseased rat kidneys compared with normal rat kidneys. As with gene expression, selective COX-2 inhibition with NS-398 upregulated immunoreactive protein expression of COX-2 in normal and diseased kidneys by 84% and 64%, respectively.

Selective COX-2 inhibition attenuates disease-associated increases in endogenous renal prostanoids. In normal kidneys, PGE₂ and 6-keto-PGF₁α were the predominant prostanoids, accounting for ~93% of the endogenous and in vitro steady-state prostanoid levels, whereas TXB₂ accounted for 7% (Table 3). Endogenous and in vitro steady-state levels of all three prostanoids were significantly higher in diseased than in normal kidneys. In diseased kidneys, PGE₂ and 6-keto-PGF₁α were the predominant prostanoids, accounting for ~93% of the endogenous and in vitro steady-state prostanoid levels, whereas TXB₂ accounted for 7% (Table 3).
constituted 87–89% of the total endogenous and steady-state prostanoid levels and TXB2 accounted for 11–13%.

Selective COX-2 inhibition with NS-398 blunted the disease-associated elevation in endogenous prostanoid levels, with the effect being significant for PGE2, TXB2, and total prostanoid levels (Table 3). Renal TXB2 levels were 21% lower, PGE2 levels were 28% lower, and total prostanoid levels were 29% lower overall in diseased NS-398-treated than untreated rats. Although NS-398 did not significantly alter in vitro steady-state levels of the prostanoids (Table 3), its effect on these levels followed a trend similar to that of the endogenous prostanoid concentrations.

Selective COX-2 inhibition attenuates disease-associated increases in COX activities and prostanoid production. COX activities in normal and diseased kidneys mirrored the endogenous prostanoid levels. Total COX, COX-1, and COX-2 (Table 4) activities were significantly greater in kidneys from diseased than normal rats, irrespective of the prostanoid measured. In diseased rats, NS-398 attenuated this disease-induced increase in total COX and COX-2 activities as measured by total prostanoid production, as well as TXB2 and PGE2 (Table 4). Consistent with selectivity of the drug, NS-398 did not have a significant effect on renal COX-1 activity (Table 4).
The TXB2-to-PG ratios for all conditions, except COX-1 activity (for which very low values preclude the importance of these ratios), were calculated to determine which prostanoid exhibited the greatest relative difference between diseased and normal rats. The TXB2-to-PGE2 ratio was higher in diseased than in normal rat kidneys when calculated under any of the conditions (Fig. 6A). The TXB2-to-6-keto-PGF1α ratio (Fig. 6B) also was elevated by disease, but the relative increase was significant (P < 0.05) only for prostanoids resulting from COX-2 activity. The 6-keto-PGF1α-to-PGE2 ratio (Fig. 6C) was higher in diseased than in normal rats when measured under all conditions. Thus the change in the relative levels of prostanoids in diseased kidneys is as follows: TXB2 > 6-keto-PGF1α > PGE2. Renal prostanoid ratios in rats given NS-398 and control diets also were calculated to determine selective prostanoid production by COX isoforms. Interestingly, selective COX-2 inhibition with NS-398 did not significantly alter relative renal prostanoid ratios (Fig. 6).

Selective COX-2 inhibition decreases urinary prostanoid metabolite excretion even further in Han:SPRD-cy rats. Similar to the renal prostanoid profile, urinary PGEM and 6-keto-PGF1α were the predominant prostanoids excreted by normal and diseased rats: 92–96% in normal and 86–89% in PKD rats (Table 5). Urinary levels of TXB2 accounted for 4–8% of total prostanoids in normal and 11–14% in PKD animals (Table 5).

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Urinary prostaglandin levels were significantly lower in diseased rats than in their normal counterparts, ranging from a twofold (TXB2 and PGEM) to a sevenfold decrease (6-keto-PGF1α) in urinary prostaglandin excretion. Preferential COX-2 inhibition resulted in a further reduction of urinary PGE2 and 6-keto-PGF1α excretion in normal and diseased rats. NS-398 treatment resulted in 31–58% lower PGE2 and 31–48% lower 6-keto-PGF1α levels in the urine (Table 5). NS-398 treatment did not alter urinary TXB2 levels, although they tended to be lower in treated diseased rats.

**DISCUSSION**

Selective COX-2 inhibition by NS-398 significantly ameliorated renal injury in Han:SPRD-cy rats with inherited cystic kidney disease. Treatment with NS-398 resulted in less cyst growth and interstitial fibrosis, as well as fewer PCNA-positive cells, decreased macrophage counts, and less oxidant injury, in the kidneys of diseased rats, indicating that COX-2 plays a significant role in the pathology and progression of chronic renal injury in Han:SPRD-cy rats. These observations are consistent with the previously reported renoprotective effects of selective COX-2 inhibitors in other forms of chronic renal injury (3, 9, 11, 13, 16, 31, 42, 56). Moreover, the attenuation of inflammatory and proliferative components of this renal disease by NS-398 is concurrent with the established anti-inflammatory effects of COX-2 inhibition, as well as previously reported antiproliferative (5, 60, 63) and antifibrotic benefits of NS-398 (25).

Despite the lack of an effect on renal function, these histological changes are likely to have long-term beneficial effects on disease progression. Cyst expansion is a sensitive marker of disease progression. Cyst expansion is a sensitive marker of disease progression. Cyst expansion is a sensitive marker of disease progression.

**Table 2. Relative renal COX-2 mRNA and protein expression in Han:SPRD-cy rats given NS-398 in the diet for 7 wk**

<table>
<thead>
<tr>
<th>COX-2 mRNA</th>
<th>Control +/- (n = 9)</th>
<th>Cy/+ (n = 9)</th>
<th>NS-398 +/- (n = 9)</th>
<th>Cy/+ (n = 9)</th>
<th>Effects (P Value)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>100±11.1</td>
<td>13.6±14.2</td>
<td>113.6±15.3</td>
<td>22.5±11.5</td>
<td>Genotype (P &lt; 0.0001), drug (P = 0.0246)</td>
</tr>
<tr>
<td>COX-2 protein</td>
<td>100±12.3*</td>
<td>8.2±1.4†</td>
<td>184.4±21.0‡</td>
<td>13.5±1.1§</td>
<td>Interaction (P = 0.0116)</td>
</tr>
</tbody>
</table>

Values are means ± SE, expressed as percentage of normal control. Means with different symbols (*, †, ‡, §) are significantly different (P < 0.05).

**Table 3. Endogenous and in vitro steady-state prostaglandin levels in normal and diseased Han:SPRD-cy rats given NS-398 in the diet for 7 wk**

<table>
<thead>
<tr>
<th>Endogenous (0 min)</th>
<th>Control +/- (n = 9)</th>
<th>Cy/+ (n = 9)</th>
<th>NS-398 +/- (n = 8)</th>
<th>Cy/+ (n = 9)</th>
<th>Effects (P Value)</th>
</tr>
</thead>
<tbody>
<tr>
<td>TXB2 0.10±0.04†</td>
<td>0.66±0.04</td>
<td>0.12±0.04*</td>
<td>0.52±0.04§</td>
<td>Interaction (P = 0.0544)</td>
<td></td>
</tr>
<tr>
<td>PGE2 0.75±0.21*</td>
<td>2.28±0.22†</td>
<td>0.96±0.22*</td>
<td>1.64±0.21‡</td>
<td>Interaction (P = 0.0607)</td>
<td></td>
</tr>
<tr>
<td>6-Keto-PGF1α 0.59±0.28</td>
<td>2.69±0.28</td>
<td>0.56±0.30</td>
<td>2.01±0.28</td>
<td>Genotype (P &lt; 0.0001)</td>
<td></td>
</tr>
<tr>
<td>Total 1.44±0.45*</td>
<td>5.91±0.48‡</td>
<td>1.64±0.48*</td>
<td>4.18±0.45§</td>
<td>Interaction (P = 0.0474)</td>
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<table>
<thead>
<tr>
<th>In vitro steady state (60 min)</th>
<th>Control +/- (n = 9)</th>
<th>Cy/+ (n = 9)</th>
<th>NS-398 +/- (n = 8)</th>
<th>Cy/+ (n = 9)</th>
<th>Effects (P Value)</th>
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<tbody>
<tr>
<td>TXB2 0.43±0.54</td>
<td>6.28±0.54</td>
<td>0.50±0.57</td>
<td>5.16±0.54</td>
<td>Genotype (P &lt; 0.0001)</td>
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<tr>
<td>PGE2 1.91±0.45</td>
<td>5.82±0.47</td>
<td>2.54±0.47</td>
<td>5.47±0.45</td>
<td>Genotype (P &lt; 0.0001)</td>
<td></td>
</tr>
<tr>
<td>6-Keto-PGF1α 4.17±3.27</td>
<td>38.03±3.27</td>
<td>3.72±3.47</td>
<td>32.73±3.27</td>
<td>Genotype (P &lt; 0.0001)</td>
<td></td>
</tr>
<tr>
<td>Total 6.51±3.86</td>
<td>50.19±4.09</td>
<td>6.76±4.09</td>
<td>43.35±3.86</td>
<td>Genotype (P &lt; 0.0001)</td>
<td></td>
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</tbody>
</table>

Values are means ± SE, expressed in ng/mg protein. TXB2, thromboxane B2. Means with different symbols (*, †, ‡, §) are significantly different (P < 0.05).
COX-2 dependent. The present study shows that this PGE2 synthesis by Sprague-Dawley rat kidneys was found to be predominantly antagonism in normal rats, as shown by reduced urinary prostanoids can be reduced by selective COX-2 inhibitors on whole body prostanoid synthesis (8, 14, 34, 48). By well-characterized inhibitory effects of specific COX-2 inhibitors on whole renal production of prostacyclin (39). The inhibitory effect of PGEM reflects whole body synthesis of PGE2 (46), whereas renal production of prostacyclin (39). The inhibitory effect of NS-398 on PGEM and 6-keto-PGF1α reflects systemic, as well as renal, production of prostacyclin (39). The inhibitory effect of NS-398 on PGE2 and 6-keto-PGF1α excretion confirms the well-characterized inhibitory effects of specific COX-2 inhibitors on whole body prostanoid synthesis (8, 14, 34, 48). By comparison, urinary excretion of TXB2 has been shown to reflect intrarenal synthesis of TXB2 (37), and the lack of an NS-398 treatment effect on TXB2 excretion in normal rats is consistent with the data on renal TXB2 production.

The primary feature of PKD is tubular cystic expansion associated with abnormal proliferation of renal tubular epithelial cells. Cyst epithelia are hyperproliferative and lose polarized cell architecture (59). In vitro studies of cyst epithelial cells demonstrate a significant role for cAMP in the proliferation of cyst mural cells, as well as in cyst fluid secretion (2, 24, 62). In addition, elevated renal and urine levels of cAMP have been reported in animal models of PKD (20, 61). Consistent with the premise that adenyl cyclase agonists have the potential to accelerate cyst growth in PKD, in studies using agents that block adenyl cyclase activation, a dramatic reduction in cyst formation and growth in animal models of inherited renal cystic disorders was reported (20). PGI2 and PGE2 are adenyl cyclase agonists that result in elevated cAMP and TXB2 levels, respectively. Similar to the effect of COX-2 inhibition on PGE2 levels, NS-398 did not alter renal 6-keto-PGF1α or TXB2 levels in normal rats but decreased 6-keto-PGF1α and TXB2 levels in diseased rat kidneys. Along with the finding that renal COX-2 activity accounts for most of the COX isoform activity, these results suggest that, similar to renal PGE2, renal synthesis of 6-keto-PGF1α and TXB2 is primarily COX-2-dependent and prostanoid pools resistant to COX-2 inhibition are present in normal kidneys, whereas those susceptible to COX-2 inhibition are present in diseased kidneys. This inference is consistent with studies that demonstrated the efficacy of COX-2 inhibitors for prostanoids synthesized by inflamed tissue compared with those produced by normal tissue (18, 33).

In contrast to renal prostanoid production, whole body synthesis of prostanoids can be reduced by selective COX-2 antagonism in normal rats, as shown by reduced urinary excretion of PGE2 and 6-keto-PGF1α. Urinary excretion of PGEM reflects whole body synthesis of PGE2 (46), whereas urinary excretion of 6-keto-PGF1α reflects systemic, as well as renal, production of prostacyclin (39). The inhibitory effect of NS-398 on PGEM and 6-keto-PGF1α excretion confirms the well-characterized inhibitory effects of specific COX-2 inhibitors on whole body prostanoid synthesis (8, 14, 34, 48). By comparison, urinary excretion of TXB2 has been shown to reflect intrarenal synthesis of TXB2 (37), and the lack of an

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### Table 4. Renal COX, COX-1, and COX-2 activities as determined by PGE2, 6-keto-PGF1α, TXB2, and total prostanoid production in normal and diseased Han:SPRD-cy rats given NS-398 in the diet for 7 wk

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>NS-398</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>++/+(n=9)</td>
<td>Cy/+ (n=9)</td>
</tr>
<tr>
<td><strong>COX activity</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>TXB2</td>
<td>0.03±0.03</td>
<td>0.46±0.03</td>
</tr>
<tr>
<td>PGE2</td>
<td>0.10±0.02*</td>
<td>0.33±0.02†</td>
</tr>
<tr>
<td>6-Keto-PGF1α</td>
<td>0.20±0.16</td>
<td>2.28±0.16</td>
</tr>
<tr>
<td>Total</td>
<td>0.33±0.18*</td>
<td>3.10±0.19†</td>
</tr>
<tr>
<td><strong>COX-1 activity</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>TXB2</td>
<td>0.00±0.00</td>
<td>0.03±0.01</td>
</tr>
<tr>
<td>PGE2</td>
<td>0.00±0.00</td>
<td>0.06±0.02</td>
</tr>
<tr>
<td>6-Keto-PGF1α</td>
<td>0.02±0.01</td>
<td>0.19±0.12</td>
</tr>
<tr>
<td>Total</td>
<td>0.02±0.01</td>
<td>0.30±0.14</td>
</tr>
<tr>
<td><strong>COX-2 activity</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>TXB2</td>
<td>0.03±0.03*</td>
<td>0.44±0.03†</td>
</tr>
<tr>
<td>PGE2</td>
<td>0.10±0.02*</td>
<td>0.28±0.02‡</td>
</tr>
<tr>
<td>6-Keto-PGF1α</td>
<td>0.23±0.17</td>
<td>2.16±0.17</td>
</tr>
<tr>
<td>Total</td>
<td>0.36±0.19*</td>
<td>2.96±0.21†</td>
</tr>
</tbody>
</table>

Values are means ± SE, expressed in ng·mg protein−1·min−1. COX, cyclooxygenase. Means with different symbols (*, †, ‡) are significantly different (P < 0.05).
activity induces renal COX-2 expression (15, 16, 28). Earlier studies also demonstrated that renal cytosolic PLA2 protein levels are elevated in rats with PKD (1, 58). The present findings of elevated renal prostanoid levels suggest that the activity of this enzyme is also elevated.

In conclusion, our study provides clear evidence of a role for COX-2 aberrations in the pathology and progression of renal disease in the Han:SPRD-cy rat. Selective COX-2 inhibition significantly ameliorates characteristic features of cystic renal disease (i.e., cystic growth and interstitial fibrosis), as well as

Table 5. Urinary prostanoid excretion in normal and diseased Han:SPRD-cy rats given NS-398 in the diet for 7 wk

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>NS-398</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>+/- (n = 13)</td>
<td>Cy/+ (n = 12)</td>
</tr>
<tr>
<td>Urinary TXB2</td>
<td>4.24±0.59</td>
<td>2.78±0.51</td>
</tr>
<tr>
<td>Urinary PGE2</td>
<td>11.16±0.48</td>
<td>7.04±0.52</td>
</tr>
<tr>
<td>Urinary 6-Keto PGF1α</td>
<td>83.36±4.44</td>
<td>13.02±4.62</td>
</tr>
</tbody>
</table>

Values are means ± SE, expressed in ng/24 h.
disease-associated pathologies (e.g., inflammation, oxidant injury, and cell proliferation), without adversely affecting renal function. Further studies on the dose, potential risks, and long-term effects of COX-2 inhibition in this disorder are warranted.

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