Celecoxib modifies glomerular basement membrane, mesangium and podocytes in OVE26 mice, but ibuprofen is more detrimental

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Abstract

The role of COXs/PGs (cyclo-oxygenases/prostaglandins) in diabetic kidneys remains unclear. NSAIDs (non-steroidal anti-inflammatory drugs) that inhibit COXs/PGs are known for their renal toxicity, and COX-2 inhibitors worsen cardiovascular outcomes in susceptible individuals. Given the renal controversies concerning COX-2 inhibitors, we compared the effect of chronic NSAIDs (non-selective, ibuprofen; COX-2-selective, celecoxib) on diabetic kidneys in OVE26 mice from 8 weeks of age. Systolic BPs (blood pressures) were increased by NSAIDs in diabetic mice at 20 weeks, but were unchanged at 32 weeks. Although NSAIDs further increased diabetic kidney/body weight ratios, they did not affect albuminuria. Mesangial matrix was increased 2-fold by celecoxib but not ibuprofen. Electron microscopy revealed that NSAIDs reduced GBM (glomerular basement membrane) thickness and slit pore diameters. Although diabetics had increased glomerular diameters and reduced foot process densities, these were unaltered by NSAIDs. Celecoxib does not exacerbate the diabetic state, but PG inhibition may contribute to disease progression by modifying the GBM, mesangial area and podocyte structure in OVE26 mice. Despite these findings, celecoxib remains safer than a similar dose of ibuprofen. The present study substantiates the need to more closely consider selective COX-2 inhibitors such as celecoxib as alternatives to non-selective NSAIDs for therapeutic management in a setting of chronic kidney disease.

Key words: celecoxib, diabetic nephropathy, ibuprofen, OVE26 mouse, prostaglandin E₂

INTRODUCTION

COX (cyclo-oxygenase)-derived PGs (prostaglandins) are implicated in diabetic nephropathy, but their role has yet to be defined: initiating diabetic features or antagonizing other pathophysiological agents such as the RAS (renin–angiotensin system). Altogether PGs can regulate many aspects of diabetic kidney disease, including haemodynamics, growth, fibrosis, cell death and tubular transport. Therefore inhibition of COX/PGs by NSAIDs (non-steroidal anti-inflammatory drugs) will influence the course of diabetic nephropathy. Although selective COX-2 inhibitors were first expected to alleviate renal toxicities, their demise due to cardiovascular complications has led to limited therapeutic use. A great deal of emphasis has been placed on clarifying the beneficial and harmful responses associated with their use, giving incentive to examine the individual roles of COX in renal health and disease.

COX-2 selective NSAIDs are not less nephrotoxic than non-selective NSAIDS in the healthy kidney, but selective COX-2 inhibitors are protective in various models of nephropathy: reducing hyperfiltration, proteinuria, glomerulosclerosis, structural damage, interstitial fibrosis, disturbances in salt and water balance and macrophage infiltration [1–6]. The beneficial effects of short term NSAIDs in diabetic kidneys are controversial due to differences in the type of NSAID and duration of treatment [7,8]. Very few studies have evaluated the long-term use of COX-2 inhibitors and their impact on renal function in diabetes, with varying levels of nephroprotection and reductions in albuminuria [3,9–12], but all lacking a concomitant comparison of COX-2 inhibition with a non-selective NSAID. Despite the promising

Abbreviations: BP, blood pressure; COX, cyclo-oxygenase; GBM, glomerular basement membrane; NSAIDs, non-steroidal anti-inflammatory drugs; PG, prostaglandin; PGEM, PGE₂ metabolite; TGFβ, transforming growth factor β.

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outcomes of long-term studies with COX-2 inhibitors, current recommendations are to avoid celecoxib in diabetics and to limit the dose and duration of therapy. Our group also reported that chronic low-dose NSAIDs (ibuprofen and NS398) reduced albuminuria and hyperfiltration in diabetic mice [13], but it is not clear whether COX-2 inhibitors are harmful at higher doses. Therefore in the present study, we compared the effect of high-dose ibuprofen and celecoxib administered for 12–26 weeks to Type 1 diabetic OVE26 mice. This work provides important information with regards to the use of higher doses of celecoxib in diabetes and insight into the individual role of COX-1 and -2 in the diabetic kidney.

**MATERIALS AND METHODS**

**OVE26 mice and NSAIDs**

OVE26 mice are characterized by early-onset Type 1 diabetes [14], displaying many of the late-stage characteristics of diabetic nephropathy [15,16]. Male mice were used, n = 12–15 per group, comparing the non-selective NSAID ibuprofen and the COX-2 inhibitor celecoxib [17], from 8 weeks of age and continued for 12 or 26 weeks. NSAIDs were added to standard chow (prepared by Harlan Laboratories), administered at 15 and 50 mg/kg of body weight per day. Food consumption was assessed in a pilot study to adjust chow dosage, and monitored throughout the study. The protocol was approved by the University of Ottawa animal care committee. Standard protocols were utilized, as previously described [13,18], to measure kidney/body weights, weekly blood glucose, biweekly systolic BP (blood pressure) from 8 to 24 weeks of age (all mice) and at 32 weeks (12 or 26 weeks). NSAIDS were added to standard chow (prepared in FVB (Figure 1A). However, NSAIDs did not alter urinary albumin, levels ranging from 576.5 μg/g/24 h to 1289 μg/g/24 h at 24 weeks 24 h albumin excretion reached 601 ± 153 μg/g/24 h. By 32 weeks, albumin excretion in OVE26 mice (Table 1), but neither were affected by NSAID treatment. Urine albumin/creatinine was also increased 3-fold by 20 weeks of age. At 24 weeks 24 h albumin excretion reached 601 ± 153 μg/g/24 h in OVE26 mice compared with 48 ± 13 μg/g in FVB. NSAIDs did not alter urinary albumin, levels ranging from 576.5 ± 55 to 834.3 ± 96.1 μg/g/24 h. By 32 weeks, albumin excretion in OVE26 mice was 4430 ± 1289 μg/g/24 h compared with 478 ± 72 μg/g/24 h in FVB (Figure 1A). However, NSAIDs did not alter urine albumin. All measured urinary PGs showed increases in OVE26 mice compared with 55 to 6000 the GBM length was measured live with these images saved including the labelled measurement line. The peripheral open loops of entire glomeruli were sampled. Manual counting of foot processes along these measured lengths of GBM was done on the digital images and foot process density was calculated. The slit pore diamitragm diameters were measured at ×60000 in regions with perpendicularly oriented foot processes.

**Enzyme immunoassays**

Urine was collected and PGE2, PGEM (PGE2 metabolite), thromboxane B2 and the prostacyclin metabolite 6-keto-PGF1α were assessed by competitive enzyme immunoassays (Cayman Chemical) as described previously [13]. Samples were normalized to creatinine.

**Western blotting**

Cortical lysates were prepared in RIPA buffer, resolved by SDS/PAGE and transferred on to a nitrocellulose membrane, and incubated with different antibodies: anti-p21, anti-TGFβ (transforming growth factor β) (Santa Cruz Biotechnology) and anti-fibroectin (Sigma). ECL (enhanced chemiluminescence) was used to visualize the signals and normalized to β-actin for densitometry.

**Statistical analysis**

GraphPad Prism software for Windows Version 4 was used for data analysis, expressed as means ± S.E.M. Unpaired Student’s t tests, and one-way and two-way ANOVAs were performed.

**RESULTS**

**Albumin excretion and plasma creatinine are unaltered by NSAIDS, but urinary PGs are reduced**

At 20 weeks of age, blood glucose in all diabetic mice was 5-fold greater than FVB mice, and plasma creatinine increased from 18.4 ± 2.4 μmol/l in FVB to 29.0 ± 0.6 μmol/l in OVE26 mice (Table 1), but neither were affected by NSAID treatment. Urine albumin/creatinine was also increased 3-fold by 20 weeks of age. At 24 weeks 24 h albumin excretion reached 601 ± 153 μg/g/24 h. By 32 weeks, albumin excretion in OVE26 mice was 4430 ± 1289 μg/g/24 h compared with 478 ± 72 μg/g/24 h in FVB (Figure 1A). However, NSAIDs did not alter urine albumin. All measured urinary PGs showed increases in OVE26 by 3–4-fold of FVB. Celecoxib preferentially reduced PGE2 and PGEM compared with 6-keto-PGF1α and thromboxane B2. Only 50 mg of celecoxib significantly reduced PGE2 by 50% to 26.5 ± 8.8 pg/mg creatinine compared with 55.5 ± 6.1 pg/mg in untreated OVE26 mice. Ibuprofen did not affect diabetic PG excretion (Table 2). Of interest, mice treated with 50 mg of
Table 1 Characteristics of 20-week-old mice
Results are presented as means ± S.E.M., n = 12–15. *P < 0.05 compared with FVB; **P < 0.05 compared with OVE26.
FVB, untreated non-diabetic; 15I or 15C, non-diabetic with 15 mg of ibuprofen or celecoxib; 50C, non-diabetic with 50 mg of celecoxib; OVE26, untreated diabetic; O + 15I or O + 15C, diabetic with 15 mg of ibuprofen or celecoxib; O + 50C, diabetic mice with 50 mg of celecoxib, wt, weight.

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<th>FVB</th>
<th>15I</th>
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<th>O + 15I</th>
<th>O + 15C</th>
<th>O + 50C</th>
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<tr>
<td>Kidney wt (mg)</td>
<td>183.2 ± 7.7</td>
<td>181.7 ± 2.1</td>
<td>188.9 ± 2.1</td>
<td>190.2 ± 10.7</td>
<td>313.3 ± 17.8</td>
<td>371.6 ± 14.5</td>
<td>380.7 ± 20.1</td>
<td>400.6 ± 26.9</td>
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<td>Body wt (g)</td>
<td>28.4 ± 0.4</td>
<td>28.4 ± 0.5</td>
<td>29.9 ± 0.5</td>
<td>26.1 ± 0.7</td>
<td>24.3 ± 1.6</td>
<td>25.9 ± 0.7</td>
<td>24.5 ± 1.0</td>
<td>26.6 ± 0.6</td>
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<td>Kidney/body (×10^3)</td>
<td>6.4 ± 0.19</td>
<td>6.4 ± 0.07</td>
<td>6.3 ± 0.14</td>
<td>7.2 ± 0.35</td>
<td>12.96 ± 0.77</td>
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<td>15.53 ± 0.47</td>
<td>15.01 ± 0.85</td>
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<td>Glucose (mM)</td>
<td>12.8 ± 1.5</td>
<td>14.9 ± 0.7</td>
<td>14.0 ± 1.0</td>
<td>14.9 ± 0.7</td>
<td>59.8 ± 1.0</td>
<td>60.2 ± 2.7</td>
<td>57.9 ± 6.7</td>
<td>53.6 ± 3.6</td>
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<tr>
<td>Creatinine (µM)</td>
<td>18.4 ± 2.4</td>
<td>22.8 ± 1.4</td>
<td>20.3 ± 1.2</td>
<td>21.5 ± 2.0</td>
<td>29.0 ± 0.6</td>
<td>30.4 ± 1.1</td>
<td>30.7 ± 2.3</td>
<td>30.9 ± 0.6</td>
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<td>Systolic BP (mm Hg)</td>
<td>125.9 ± 3.9</td>
<td>122.9 ± 3.1</td>
<td>125.0 ± 3.1</td>
<td>134.6 ± 4.1</td>
<td>135.1 ± 5.3</td>
<td>150.4 ± 8.0</td>
<td>131.0 ± 4.0</td>
<td>156.1 ± 3.5</td>
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Table 2 Urinary PGs are reduced by celecoxib
Urinary PGs were measured by competitive enzyme immunoassays and were corrected for urinary creatinine (pg/mg of creatinine). Results are presented as means ± S.E.M., n = 4–5. *P < 0.05 compared with FVB; **P < 0.05 compared with OVE26.
FVB, untreated non-diabetic; 15I or 15C, non-diabetic with 15 mg of ibuprofen or celecoxib; 50C, non-diabetic with 50 mg of celecoxib; OVE26, untreated diabetic; O + 15I or O + 15C, diabetic with 15 mg of ibuprofen or celecoxib; O + 50C, diabetic mice with 50 mg of celecoxib.

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<th>O + 15C</th>
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<tr>
<td>PGE2</td>
<td>18.8 ± 1.3</td>
<td>17.3 ± 1.5</td>
<td>13.6 ± 0.6*</td>
<td>13.4 ± 0.9*</td>
<td>55.5 ± 6.1*</td>
<td>59.5 ± 9.1*</td>
<td>33.6 ± 9.9*</td>
<td>26.5 ± 8.8**</td>
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<tr>
<td>PGEM</td>
<td>29.8 ± 3.1</td>
<td>27.5 ± 2.3</td>
<td>21.4 ± 0.7</td>
<td>24.5 ± 2.5</td>
<td>124.4 ± 6.4*</td>
<td>148.4 ± 33.6*</td>
<td>112.0 ± 10.7</td>
<td>96.5 ± 21.8*</td>
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<tr>
<td>TXB2</td>
<td>20.2 ± 1.3</td>
<td>18.4 ± 2.0</td>
<td>15.1 ± 0.6</td>
<td>14.9 ± 0.8</td>
<td>65.2 ± 3.8*</td>
<td>67.8 ± 3.2*</td>
<td>67.6 ± 7.7</td>
<td>48.3 ± 5.8*</td>
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<tr>
<td>6-keto-PGF1α</td>
<td>23.4 ± 1.7</td>
<td>21.3 ± 2.2</td>
<td>16.1 ± 0.8*</td>
<td>16.3 ± 0.7*</td>
<td>78.1 ± 7.3*</td>
<td>80.6 ± 3.0*</td>
<td>74.9 ± 6.7*</td>
<td>55.8 ± 8.8***</td>
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Figure 1 Urinary albumin excretion and systolic BP are increased in OVE26 mice at 32 weeks
(A) 24 h urine collections were assayed for albumin and corrected for total urine volume. (B) Systolic BP was measured by tailcuff. Data are expressed as means ± S.E.M., *P < 0.05 compared with FVB. Legend for all Figures: FVB, untreated non-diabetic; 15I or 15C, non diabetic with 15 mg of ibuprofen or celecoxib; 50C, non diabetic with 50 mg of celecoxib; OVE26, untreated diabetic; O + 15I or O + 15C, diabetic with 15 mg of ibuprofen or celecoxib; O + 50C, diabetic mice with 50 mg of celecoxib.
ibuprofen either developed pyelonephritis, or died spontaneously with severe necrotizing pyelonephritis. Therefore the group comparisons were limited to the 15 mg ibuprofen dose.

**NSAIDS increase kidney weights and systolic BP in OVE26 mice**

Consistent with renal hypertrophic changes, kidney/body weight ratios increased 2-fold in diabetic mice (Table 1), with increased kidney weights but unchanged body weights. Celecoxib at 50 mg further increased kidney weights to 400.6 ± 26.9 mg, compared with 313.3 ± 17.8 mg in untreated OVE26 mice, without affecting body weights. Although systolic BPs were not significantly different in untreated OVE26 mice, measuring 135.1 ± 5.4 mmHg at 20 weeks, compared with 125.9 ± 4 mmHg in FVB mice, 50 mg of celecoxib in diabetic mice resulted in a significant increase in systolic BP to 156.1 ± 3.5 mmHg, whereas 15 mg of celecoxib had no effect (Table 1). At 20 weeks, BP also increased to 150.4 ± 8 mmHg by 15 mg ibuprofen in diabetic mice. BP was comparable in all FVB mice, regardless of NSAID treatment. At 32 weeks, BP was 116.1 ± 3.1 mmHg in FVB, and still unchanged in diabetic OVE26 mice; but was no longer increased in diabetic mice by NSAIDs (Figure 1B).

**Celecoxib increases mesangial area in OVE26 mice**

Morphological changes were assessed in all mice. Light microscopic impression revealed a mildly increased mesangium in OVE26 mice. No other glomerular changes were noted by simple light microscopy and glomerular and mesangial measurements...
were required. No interstitial fibrosis or tubular pathology was seen. Glomerular diameters increased by 18.4 and 19.5% in diabetic mice, at 20 and 32 weeks of age, respectively, but were unaltered by NSAIDs (Figure 2). Moreover, the relative mesangial area (ratio of mesangial to glomerular area) in OVE26 mice at 20 weeks numerically increased compared with FVB, but the increase was insignificant due to notable variability among mice (Figure 3A). However, by 32 weeks the relative mesangial area was significantly increased by 40% in OVE26 mice, from 13.7 ± 1.7 to 21.4 ± 2.1 pixels (Figure 3B), and further elevated by 50 mg of celecoxib to 39.1 ± 7.1 pixels in OVE26 mice. No effect was observed with 15 mg ibuprofen and celecoxib. Both 15 and 50 mg of celecoxib increased the relative mesangial area in FVB mice, to 30.2 ± 8.0 and 33.3 ± 7.1 pixels respectively.

**Celecoxib induces structural changes in the glomerulus**

As determined by electron microscopy, GBM thickness was unchanged in untreated diabetics compared with FVB, but celecoxib reduced GBM thickness by 30% at 20 weeks, in both non-diabetic and OVE26 mice (Figure 4). By 32 weeks, GBM thickness increased by 19% in OVE26 mice compared with FVB, but was unaffected by NSAIDs. Foot process densities were reduced in OVE26 mice at 20 and 32 weeks, by 26 and 36% respectively. Although NSAIDs did not affect this reduction in diabetic mice, both 15 and 50 mg of celecoxib reduced foot process densities in FVB mice by up to 30%, but again with no effect in diabetic mice. Although slit pore diameters were unaffected in 20 week OVE26 mice, 50 mg of celecoxib reduced slit diameters in FVB mice by 20% and by 23% in OVE26 mice (Figure 6). Structural changes in GBM, slit pores and mesangium are shown in Figure 7.

**TGFβ and p21 are reduced by celecoxib**

TGFβ is an important effector of renal change in diabetes. Celecoxib did not affect cortical TGFβ in non-diabetic mice, but 15 mg ibuprofen reduced levels to 0.5-fold of FVB. Cortical TGFβ in OVE26 mice increased 2.34 ± 0.46-fold (Figure 8A). Although 50 mg of celecoxib had no effect on this induction, TGFβ was significantly attenuated to 1.17 ± 0.08-fold by 15 mg of celecoxib in diabetic mice, but ibuprofen had no effect in OVE26 mice (Figure 8A).

The CDK (cyclin-dependent kinase) inhibitor p21 regulates diabetic hypertrophy downstream of TGFβ. NSAIDs did not affect cortical p21 in non-diabetic mice. Cortical p21 increased by 1.65 ± 0.31-fold in OVE26 mice (P = 0.06, n = 7), but 15 mg of celecoxib attenuated p21 to 0.84 ± 0.20-fold in FVB mice. p21 was not affected by 50 mg of celecoxib or ibuprofen in OVE26 mice (Figure 8B).

The development of glomerular and tubulointerstitial fibrosis is an important determinant of progressive kidney disease. We measured fibronectin in samples of renal cortex, and only 15 mg of celecoxib significantly reduced fibronectin in non-diabetic mice to 0.61 ± 0.12-fold of the control. In contrast, 15 mg ibuprofen increased fibronectin by 1.8-fold in non-diabetic FVB mice. Cortical fibronectin increased 2.59 ± 0.53-fold in OVE26 mice but was unaltered by celecoxib or ibuprofen (Figure 8C). Fibrotic changes were not detected up to 36 weeks of age, regardless of NSAID treatment.

**DISCUSSION**

The results of the present study show that, although celecoxib does not exacerbate the diabetic state, PG inhibition modifies GBM, mesangial area and podocyte structure. Urine albumin
Figure 5 Foot process densities are reduced by NSAIDs

Foot process densities at (A) 20 weeks and (B) 32 weeks, expressed as means ± S.E.M. of the number of foot processes/μm, n = 4–5. *P < 0.05 compared with FVB.

Figure 6 Slit pore diameters are reduced by Celecoxib

Slit pore diameters at (A) 20 weeks and (B) 32 weeks presented as means ± S.E.M. n = 4–5. *P < 0.05 against FVB and &P < 0.05 compared with OVE26.

was increased in diabetic mice but unaltered by NSAIDs. In previous reports, albuminuria improved by COX-2 inhibition in diabetic rats when administered later in the study, but not when given at the onset of diabetes [9]. We also observed elevated urinary PGE2, PG12 and TXA2 (thromboxane A2) in OVE26 mice, but comparing the pattern of PG inhibition by NSAIDs, COX-2 mainly produces PGE2 and prostacyclin; and in fact celecoxib preferentially lowered PGE2, as in previous reports on diabetic patients [7,8,19].

Systolic BP was comparable in FVB and OVE26 mice throughout the study, but NSAIDs increased BP in diabetic mice only, which could contribute to the glomerular phenotype observed in our mice, since hypertension is an important pathological factor in diabetes. Zheng et al. [14] showed that BP was elevated in OVE26 mice at 8 months of age. Although the mechanism and the link to the observed glomerular pathology have not been explored in our study, NSAIDS are associated with Na+ retention and oedema [17], which could certainly increase BP. Although we did not observe any adverse cardiovascular outcomes in our mice, a longer study protocol may have revealed other associated disturbances. There is emerging evidence emphasizing the contribution of COX-2 derived renal medullary prostacyclin in BP regulation [20]. Thus the increase in systolic BP in OVE26 mice treated with NSAIDs may result from altered systemic vasodilatory/vasoconstrictor PG balance, but could also originate in the kidney. Future studies should help to distinguish...
Figure 7  Structural alterations in glomeruli at 20 weeks
Electron microscopy images depicting glomerular structure at 20 weeks in (A) control and (B-E) mice with 50 mg of celecoxib. (A) Control (×20,000) with arrows showing slit pore diaphragm; (B) Thick GBM (×20,000) with mesangial expansion on edge indicated by asterisk; (C) Thin GBM (×20,000) indicated by arrow; (D) Thin irregular GBM (×10,000) with lamellation (arrows); (E) GBM (×15,000) with irregular structure (arrows). Scale bars are shown.
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Figure 8 Cortical TGFβ and p21 are reduced by celecoxib in OVE26 mice at 20 weeks

Representative Western blots showing cortical (A) TGFβ at 25–30 kDa (upper blot) normalized with β-actin (lower blot), (B) p21 at 20–25 kDa (upper blot) normalized with β-actin (lower blot) and (C) fibronectin (Fibro) at 220 kDa (upper blot) normalized with β-actin (lower blot). Densitometry showing means ± S.E.M. presented as fold-FVB with celecoxib (upper histogram) and ibuprofen (lower histogram), n = 4–7. *P < 0.05 compared with FVB.

the individual contribution of systemic and renal regulatory systems and could have great implications for pain management in the hypertensive population.

Renal growth is observed early in diabetes, and celecoxib further increased kidney weights in OVE26 mice. Although glomerular diameters were increased, they were unaffected by NSAIDs. The effect of NSAIDs on renal growth is controversial. In our study, an important cell cycle inhibitor, p21, was reduced by 15 mg of celecoxib and unaffected by ibuprofen, but kidney weights were only increased by 50 mg of celecoxib. The growth-reducing potential of celecoxib has been demonstrated in various cancers and inhibition of growth by celecoxib was due to increased p21 in polycystic kidneys [21]. Clearly renal growth responses depend on the dose of celecoxib and the cellular environment, with various effectors impacting diabetic growth. This substantiates the need to maintain NSAID dosage within certain limits, since the choice of dosage and duration could elicit very different effects on the diabetic environment.

TGFβ was also reduced by celecoxib in diabetic mice. This is not surprising since COX-2 increases TGFβ, the main growth factor promoting enlargement of diabetic kidneys [10]. In addition, in a previous study on liver fibrosis, celecoxib decreased TGFβ, induced metalloproteinase-2, and prevented collagen accumulation [22]. The effect of celecoxib and ibuprofen on TGFβ is interesting. Although celecoxib reduced TGFβ in diabetic mice and had no effect in non-diabetic mice, ibuprofen had the opposite effect, reducing TGFβ only in non-diabetic mice. These findings highlight the differences in the diabetic and non-diabetic environments with respect to changes in the relative levels of each enzyme (COX-2 is increased in diabetes and COX-1 reduced [13]), and individual contributions of COX-1 compared with COX-2. Considering the pathological role of TGFβ in diabetes, these responses warrant further clarification.

Cortical fibronectin did not change in response to NSAIDs in diabetic mice, although mesangial area was actually increased by celecoxib in OVE26 mice. The reasons for these discrepancies are not clear at this time, but confirm the multifactorial nature of the diabetic environment. However, in non-diabetic mice, celecoxib and ibuprofen had opposite effects on fibronectin levels, reducing and increasing fibronectin respectively. As discussed above, our findings emphasize the importance of taking into account different contributions of COX-1 and COX-2 in a non-diabetic compared with diabetic environment. Clearly future studies should focus on deciphering these specific differences to develop better guidelines for use of NSAIDs in patients with chronic kidney disease.

Most studies conducted indicate that chronic low-dose COX-2 inhibition is protective, with reduced markers of diabetic renal injury [10–12]. In our present study, we clearly demonstrate that at higher doses chronic NSAIDs contribute to the onset of glomerular changes in the filtration barrier that may ultimately worsen disease progression. Celecoxib resulted in GBM thinning, slit pore diameter and foot process density reduction and increased mesangial area, but at comparable doses, ibuprofen is more detrimental than celecoxib, causing severe necrotizing pyelonephritis. Interestingly, our celecoxib data are conflicting with recent
reports promoting the harmful role of COX-2 in mouse podocytes, whereby activation of the prorenin receptor by COX-2 overexpression predisposes to diabetic glomerular injury [11]. It is unclear how this is related to overexpression of podocyte COX-2, but more work is needed to account for the inconsistency.

NSAIDs caused GBM thinning. Although the glomerular changes observed are mostly consistent with typical diabetic manifestations, GBM thickening is classically reported in diabetes. However, GBM thinning or irregular thinning with atypical structure in diabetic glomeruli is reported [23–25], with a possible link to new vessel formation in glomerular capillaries in prolonged diabetes [23], and is associated with a better prognosis than GBM thickening. In our study, chronic PG inhibition is associated with GBM thinning in mice assessed at 20 weeks, and the mechanism warrants further investigation. However, by 32 weeks the thinning effect is no longer observed, most likely associated with changes in the diabetic environment such that many factors are involved in promoting thickening of the GBM and opposing any thinning effects of NSAIDs that were seen at 20 weeks.

Altogether our work confirms the renal responses to different celecoxib doses compared with ibuprofen, and highlights the precise structural changes in the glomerulus associated with chronic high-dose NSAIDs in a diabetic environment. A prolonged study period may have revealed a worsened diabetic phenotype. Although the study analysis was reduced by the fact that 50 mg of ibuprofen caused severe pyelonephritis in diabetic mice, the comparisons with 15 mg of ibuprofen shed light on the differences between selective and non-selective COX inhibition. It is possible that the elevation in BP might have contributed to the structural changes in NSAID-treated mice, but more work is needed to characterize the mechanisms involved.

**CLINICAL PERSPECTIVES**

- Diabetes remains an important cause of chronic kidney disease, and the current therapeutic strategies are unsuccessful in slowing disease progression. Studies using chronic low-dose NSAIDs indicate potential benefits in reducing markers of injury, but the current recommendations are to avoid NSAIDs in diabetic patients. There is also a lack of information on the effects of higher dose NSAIDs in diabetic kidneys.
- Although our results indicate that chronic high-dose celecoxib may contribute to disease progression by altering the GBM, mesangial area and podocyte structure, a comparable dose of ibuprofen caused a more detrimental response in OVE26 mice.
- The results of the study substantiate the need to more closely consider selective COX-2 inhibitors such as celecoxib as alternatives to non-selective NSAIDs for therapeutic management in a setting of chronic kidney disease.

**AUTHOR CONTRIBUTION**

Rania Nasrallah and Richard Hébert conceived and designed the research. Jacob Karsh provided the celecoxib and technical expertise for dosing. Rania Nasrallah performed all of the experiments. Rania Nasrallah and Susan Robertson analysed the data. Rania Nasrallah interpreted results, prepared the Figures and drafted the paper. Rania Nasrallah, Susan Robertson and Richard Hébert edited and revised the paper. Rania Nasrallah, Jacob Karsh, Susan Robertson and Richard Hébert approved the final version of the paper.

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