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ORIGINAL ARTICLE

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Moderate hyperuricaemia ameliorated kidney damage in a low-renin model of experimental renal insufficiency

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Abstract

Uric acid has promoted renal fibrosis and inflammation in experimental studies, but some studies have shown nephroprotective effects due to alleviated oxidative stress. We studied the influence of experimental hyperuricaemia in surgically 5/6 nephrectomized rats. Three weeks after subtotal nephrectomy or sham operation, the rats were allocated to control diet or 2.0% oxonic acid (uricase inhibitor) diet for 9 weeks. Then blood, urine and tissue samples were taken, and renal morphology and oxidative stress were examined. Inflammation and fibrosis were evaluated using immunohistochemistry and real-time PCR (RT-PCR). Remnant kidney rats ingesting normal or oxonic acid diet presented with ~60% reduction of creatinine clearance and suppressed plasma renin activity. Oxonic acid diet increased plasma uric acid levels by >80 μmol/L. In remnant kidney rats, moderate hyperuricaemia decreased glomerulosclerosis, tubulointerstitial damage and kidney mast cell count, without influencing the fibrosis marker collagen I messenger RNA (mRNA) content. In both sham-operated and 5/6 nephrectomized rats, the mast cell product 11-epi-prostaglandin-$F_{2\alpha}$ excretion to the urine and kidney tissue cyclooxygenase-2 (COX-2) levels were decreased. To conclude, hyperuricaemic remnant kidney rats displayed improved kidney morphology and reduced markers of oxidative stress and inflammation. Thus, moderately elevated plasma uric acid had beneficial effects on the kidney in this low-renin model of experimental renal insufficiency.

KEYWORDS
experimental renal insufficiency, hyperuricaemia, kidney morphology, oxonic acid

1 | INTRODUCTION

Hyperuricaemia is a common finding in chronic renal insufficiency (CRI), mainly due to decreased renal uric acid (UA) excretion caused by reduced glomerular filtration and tubulointerstitial damage.[1] Hyperuricaemia may be injurious to the kidneys and the cardiovascular system, but whether hyperuricaemia is a culprit or merely a marker of renal injury remains a matter of debate.[2]

Experimental and clinical studies have linked hyperuricaemia with enhanced cardiovascular complications and progression of CRI. In animal models, oxonic acid (Oxo)-induced hyperuricaemia has been associated with stimulation of the renin–angiotensin system (RAS), endothelial dysfunction, oxidative stress and salt-sensitive hypertension.[3–6] In patients with chronic kidney disease, hyperuricaemia correlated with the severity of glomerulosclerosis and interstitial fibrosis in renal biopsy.[7,8] In contrast, rats receiving intraperitoneal UA infusions presented with antihypertensive and nephroprotective effects, probably due to the antioxidant effects of UA.[9–11] A protective role for UA during acute ischaemic stroke has also been reported.[12–15]

In the absence of previous renal impairment, harmful hyperuricaemia-associated morphological changes have been reported in rat kidneys.[5,16–18] A diet with 2% of the uricase inhibitor Oxo was found to increase collagen deposition, macrophage infiltration, juxtaglomerular renin staining and media–lumen ratio of afferent arterioles and induce glomerular hypertrophy in rat kidneys.[16–18] When Oxo was given daily by gastric gavage to rats for 5 weeks, afferent arteriolopathy and signs of increased intrarenal oxidative stress were observed.[5] In experimental models of CRI, two reports associated hyperuricaemia with harmful effects on the kidneys. In rats subjected to surgical 5/6 nephrectomy (NX), 2% Oxo diet for 6 weeks induced afferent arteriolopathy, enhanced glomerulosclerosis and interstitial fibrosis and increased cyclooxygenase-2 (COX-2) and renin expression in preglomerular vessels.[6] In the ligation type of 5/6 NX (removal of the right kidney and selective ligation of two to three branches of left renal artery), which is a high-renin model of CRI, daily administration of Oxo by gastric gavage for 5 weeks induced renal cortical vasoconstriction and afferent arteriole thickening.[19]

Previously, we found both harmful[20] and beneficial effects[9] in response to hyperuricaemia induced by 2.0% Oxo feeding in rats subjected to surgical 5/6 NX, which is a low-renin model of CRI.[20] Hyperuricaemia increased circulating RAS activity and promoted urinary K^+ loss,[20] but also reduced oxidative stress in vivo as shown by increased plasma antioxidant capacity and decreased urinary 8-iso-prostaglandin-$F_{2\alpha}$ excretion, and improved nitric oxide (NO)-mediated vasorelaxation in the carotid artery.[9] The influences of experimental hyperuricaemia on the kidney and cardiovascular system may depend on the model that was examined, which may partially explain the discrepancies between various studies.[21] These discrepancies may have been due to the differences in modelling schemes between the studies.

In the present study, the hypothesis whether moderate pharmacologically induced experimental hyperuricaemia influences kidney structure was further tested. We determined renal histology and markers of inflammation and fibrosis in 5/6 NX and sham-operated rats that ingested 2.0% Oxo diet for 9 weeks. Before allocation to study groups, our protocol included a 3-week recovery period after the NX surgery to reduce potential selection bias caused by deviations in the degree of renal insufficiency before the 2.0% Oxo diet. In the remnant kidney model of CRI, experimental hyperuricaemia improved kidney morphology, reduced oxidative stress and decreased markers of inflammation.

2 | METHODS

2.1 | Animals and experimental design

Male Sprague–Dawley rats were housed in an animal laboratory with free access to water and chow (Lactamin R34, AnalyCen, Linköping, Sweden) containing 0.9% calcium, 0.8% phosphorus, 0.27% sodium, 0.2% magnesium, 0.6% potassium, 16.5% protein, 4.0% fat, 58% nitrogen-free extract, 3.5% fibre, 6.0% ash, 10% water and 12 550-kJ/kg energy. At the age of 8 weeks (study week 0), the rats were anaesthetized with ketamine (Parke-Davis Scandinavia AB, Solna, Sweden) plus diazepam (Orion Pharma Ltd., Espoo, Finland) using intraperitoneal doses 75 and 2.5 mg/kg, respectively, and NX ($n = 24$) was carried out by the removal of upper and lower poles of the left kidney and the whole right kidney. The kidneys of the Sham rats ($n = 24$) were decapsulated. Anaesthesia, antibiotics and treatment of postoperative pain were as previously reported.[22,23]

Three weeks after the operations, rats were assigned to four groups ($n = 12$ in each): Sham, Sham + Oxo, NX and NX + Oxo. Group sizes were based on our previous experience in this model.[9,20] To ensure corresponding levels of blood pressure (BP) and renal function, the groups were formed so that mean systolic BPs, 24-h urine volumes and body weights in the Sham and Sham + Oxo, and NX and NX + Oxo groups, respectively, were similar. The 2.0% Oxo (Sigma-Aldrich Chemical Co., St. Louis, MO, USA) diet was fed to Sham + Oxo and NX + Oxo groups for 9 weeks, while the Sham and NX groups continued normal diet. Systolic BP was measured at 28°C by the tail-cuff method as the averages of five recordings in each rat (Model 129 BP Meter; IITC Inc., Woodland Hills, CA, USA). The 24-h urine output was collected in metabolic cages at study weeks 3 and 12, and urine samples were stored at −80°C until analyses.

After 9 weeks of the Oxo diet, the rats were anaesthetized with urethane (1.3 g/kg), and blood samples

from cannulated carotid artery were drawn into chilled tubes with heparin or EDTA as anticoagulants. Unexpectedly, cardiac arrest occurred in three Sham group rats, and therefore, no blood samples were obtained from these animals. The kidneys were harvested, kidney halves were frozen in isopentane at −40°C and stored at −80°C. The other kidney halves were fixed in 4% formaldehyde for 24 h and embedded in paraffin. The study was approved by the Animal Experimentation Committee of the University of Tampere and the Provincial Government of Western Finland Department of Social Affairs and Health, Finland (decision LSLH-2003-9718/Ym-23), and conforms to the Guiding Principles for Research Involving Animals. The study was conducted in accordance with the Basic & Clinical Pharmacology & Toxicology policy for experimental and clinical studies.[24]

2.2 | Blood and plasma determinations

All blood and plasma samples were analysed in a blinded fashion. Plasma creatinine was measured using a standard clinical chemical method (Cobas Integra 800 Clinical Chemical Analyzer, Roche Diagnostics, Basel, Switzerland). UA was measured using an enzymatic colorimetric method[25] and plasma renin activity using a GammaCoat assay (DiaSorin SpA, Saluggia, Italy). All other determinations were carried out as described earlier.[22,23]

2.3 | Kidney morphology and immunohistochemistry

Five-micrometre-thick kidney sections were stained with haematoxylin–eosin, periodic acid Schiff (PAS), toluidine blue or immunohistochemistry and processed for light microscopic evaluation. All kidney morphology analyses were performed by an expert (J.My.) blinded to the groups and treatments.

2.3.1 | Glomerulosclerosis (haematoxylin–eosin and PAS stain)

One hundred glomeruli from each rat were examined at a magnification of ×400 and scored from 0 to 5[26]: 0 = normal; 1 = mesangial expansion or basement membrane thickening; 2 = segmental sclerosis in <25% of the tuft; 3 = segmental sclerosis in 25–50% of the tuft; 4 = diffuse sclerosis in >50% of the tuft; and 5 = diffuse glomerulosclerosis, tuft obliteration and collapse. The damage index for each rat was calculated as a mean of the scores.

2.3.2 | Tubulointerstitial damage (haematoxylin–eosin and PAS stain)

Injury consisting of tubular atrophy, dilatation, casts, interstitial inflammation and fibrosis was assessed in 10 kidney fields at a magnification of ×100.[26] Damage scoring was performed from 0 to 4: 0 = normal; 1 = lesions <25% of the area; 2 = lesions in 25–50% of the area; 3 = lesions in >50% of the area; and 4 = lesions covering the whole area.

2.3.3 | Arteriosclerosis index (PAS stain)

Small arteries were identified from kidney samples magnified ×400 and graded from 0 to 2: 0 = no hyaline thickening; 1 = mild to moderate hyaline thickening in at least one arteriole; and 2 = moderate or severe hyaline thickening in more than one arteriole.[27]

2.3.4 | Kidney mast cells (toluidine blue stain)

Mast cell abundance correlates with renal disease severity.[28] Toluidine blue staining was applied for mast cell identification and quantification. The number of the purple-stained mast cells was counted at a magnification of ×400 and related to kidney tissue area.

2.3.5 | Immunohistochemistry COX-2 and smooth muscle actin (SMA)

Increased COX-2 synthesis has been linked with tissue damage in hyperuricaemia.[6] For the staining of COX-2, a 1:200 dilution of monoclonal anti-COX-2 IgG antibody (RRID AB397602, clone 33, BD Biosciences, San Diego, CA, USA) and, for SMA, a 1:200 dilution of monoclonal anti-SMA IgG antibody (RRID AB2223500, code M0851, Dako Denmark A/S, Glostrup, Denmark) were used. Immunostaining was performed using the Ventana BenchMark LT Automated IHC Stainer (Ventana Medical Systems, AZ, USA) with the ultraView Universal DAB detection kit (catalogue no. 760-500, Ventana Medical Systems) as previously described.[29] The immunohistochemistry staining in kidney tissue was analysed by V.K. and A.E. blinded to the study groups and treatments. Tubulointerstitial

COX-2 staining was scored 0 to 3: 0 = no cells stained; 1 = faint immunoreactivity; 2 = moderate positive staining; and 3 = strong positive staining. Cell positivity (percentage of positive cells) was defined: 0 = no cells stained; 1 = 1–25% positive cells; 2 = 26–75% positive cells; and 3 = >75% positive cells. The results of both analyses were combined for the final score. In the glomeruli, the numbers of COX-2-positive cells were counted and related to tissue area. Staining of SMA was evaluated in an attempt to identify afferent arterioles from the efferent arterioles.[17]

2.4 | Kidney haem oxygenase-1 (HO-1) and collagen I messenger RNA (mRNA) with quantitative real-time PCR (RT-PCR)

Total RNA was isolated from rat kidney tissue using TRIzol reagent (Invitrogen, Carlsbad, CA, USA), and reverse transcription of RNA was performed using M-MLV reverse transcriptase (Invitrogen). The expressions of collagen I, oxidative stress indicator HO-1[30–32] and housekeeping control 18S mRNAs were studied by quantitative RT-PCR using ABI PRISM 7000 sequence detection system (Applied Biosystems, Foster City, CA, USA). The following primer sequences were used for collagen I and HO-1: collagen I: forward 5′-TGCAAGAACAGCGTAGCCTACAT-3′ and reverse 5′-AGCGTGCTGTA GGTGAATCGA-3′ (product size 125 bp, accession number NM_053304.1); HO-1: forward 5′-CACAAAGACCAGAGTCCCTCACAG-3′ and reverse 5′-AAATTCCCACTGCCACGGT-3′ (product size 187 bp, accession number NM_012580.2). PCR reactions for collagen I and HO-1 were performed in duplicate in a 25-μl final volume containing 1× SYBR Green Master Mix (Applied Biosystems) and 300 nM of primers. PCR reactions for 18S were performed in duplicate in a 25-μl final volume containing 1× TaqMan Master Mix (Applied Biosystems) and 1× 18S TaqMan Gene Expression Assay primer and probe mix (RRID Hs999999_s1, Applied Biosystems). PCR cycling conditions were 10 min at 95°C and 40 cycles of 20 s at 95°C and 1 min at 60°C. Data were analysed using the absolute standard curve method. 18S was used for normalizing the results as the unnormalized expression of 18S mRNA did not differ between the experimental groups (data not shown).

2.5 | Urine 11-epi-prostaglandin-$F_{2\alpha}$

The concentration of 11-epi-prostaglandin-$F_{2\alpha}$, a mast cell-derived metabolite of prostaglandin D_2 (PGD2),[33,34] was determined from urine collected in metabolic cages after selective solid-phase extraction by radioimmunoassay as previously described.[33]

2.6 | Data presentation and analysis of results

For normally distributed variables, statistical analyses were carried out using one-way and two-way analyses of variance (ANOVAs), as appropriate. For variables with skewed distribution, the Kruskal–Wallis test was applied, with the Mann–Whitney *U* test in the post hoc analyses. Spearman's two-tailed correlation coefficients (r_S) were calculated. Differences between the groups were considered significant when $P < 0.05$, and the Bonferroni correction was applied in the post hoc analyses. The results were expressed as means and standard errors of the mean (S.E.M.s), or as medians, 25th–75th percentiles and ranges. Unless otherwise indicated, the *P* values in the text refer to one-way ANOVA. The statistics were performed using IBM SPSS version 26.0 (Armonk, NY, USA).

3 | RESULTS

3.1 | Animal data

In the beginning of the 2% Oxo diet (study week 3), body weights and systolic BPs were similar in the study groups (Table 1). At study week 12, however, body weights were lower in the NX + Oxo group *versus* NX rats, and two-way ANOVA analysis showed a significant lowering effect of 2% Oxo feeding on body weight ($P = 0.004$). During the follow-up, the two NX rat groups displayed a modest elevation of BP when compared with the two Sham groups (two-way ANOVA $P = 0.041$), while Oxo diet had no statistically significant influence on BP. Urine outputs were similarly higher in both NX groups at study weeks 3 and 12 than in the Sham groups. The surgically removed renal tissue weight was similar in both NX groups. The body weight-adjusted total kidney tissue weight was lower in the NX groups than in the Sham groups, but the remnant left kidney weight in the NX rats was higher than the left kidney weight in the Sham rats (Table 1).

3.2 | Laboratory determinations

The 2% Oxo diet elevated plasma UA levels ~2.5 to 3 times in the NX and Sham rats, respectively (Table 1). Plasma creatinine was elevated by 35–42 μmol/L, and

TABLE 1 Experimental group data and laboratory findings at study weeks 3 and 12

	Sham (n = 9–12)	Sham + Oxo (n = 12)	NX (n = 11–12)	NX + Oxo (n = 12)
Body weight (g)				
Week 3	339 ± 6	338 ± 7	333 ± 8	332 ± 7
Week 12	433 ± 8	412 ± 11[#]	448 ± 10	411 ± 8[†,#]
Systolic blood pressure (mmHg)				
Week 3	120 ± 4	121 ± 5	127 ± 5	125 ± 5
Week 12	134 ± 7	136 ± 5	142 ± 6[‡]	152 ± 4[‡]
Urine volume (ml/24 h)				
Week 3	13.5 ± 0.6	13.8 ± 1.1	31.8 ± 1.8*	31.8 ± 2.6*
Week 12	25.2 ± 1.7	25.8 ± 1.8	53.3 ± 3.8*	49.3 ± 3.9*
Renal tissue removal during 5/6 NX				
Right kidney (g)	n.a.	n.a.	1.53 ± 0.05	1.55 ± 0.04
Left kidney parts (g)	n.a.	n.a.	0.64 ± 0.02	0.61 ± 0.02
Total kidney tissue (g/kg)	n.a.	n.a.	7.67 ± 0.17	7.50 ± 0.089
Final renal tissue weight (g)				
To body weight (g/kg)	6.41 ± 0.18	5.95 ± 0.08	5.24 ± 0.26*	4.97 ± 0.27*
Right kidney (g)	1.30 ± 0.05	1.22 ± 0.03	Removed	Removed
Left kidney (g)	1.37 ± 0.04	1.23 ± 0.03	2.34 ± 0.12*	2.06 ± 0.12*
Laboratory determinations at week 12				
Uric acid (μmol/L)	36 ± 11	117 ± 21*	63 ± 19	152 ± 19*,[†]
Creatinine (μmol/L)	40 ± 5	49 ± 3	82 ± 3*	83 ± 8*
Creatinine clearance (ml/min)	2.9 ± 0.4	2.0 ± 0.2*	1.2 ± 0.1*	1.2 ± 0.1*
Renin activity (ng/ml/h)	27.3 (22.8–30.9)	31.2 (27.8–41.4)	2.0 (0.3–3.1)*	5.0 (2.7–9.9)*
Phosphate (mmol/L)	1.4 ± 0.1	1.5 ± 0.1	1.9 ± 0.2[‡]	1.9 ± 0.2[‡]
Calcium (mmol/L)	2.42 ± 0.02	2.36 ± 0.02	2.42 ± 0.04	2.42 ± 0.02
Sodium (mmol/L)	136.5 ± 0.5	137.3 ± 0.6	136.7 ± 0.9	137.0 ± 0.5
Potassium (mmol/L)	4.1 ± 0.1	3.8 ± 0.1	4.3 ± 0.2	4.4 ± 0.2
Kidney tissue collagen I mRNA copies (× 10^4/ng total RNA)	8.0 ± 0.8	6.6 ± 0.7	11.0 ± 1.9[‡]	10.3 ± 1.9[‡]

Note: Values are mean ± S.E.M. or median (25th–75th percentile).

Abbreviations: n.a., not applicable; NX, 5/6 nephrectomized rat; Oxo, 2.0% oxonic acid diet; Sham, sham-operated rat.

*$P < 0.05$ compared with the Sham group.

[†]$P < 0.05$ compared with the NX group.

[#]$P < 0.05$ oxonic acid-treated groups *versus* untreated groups (two-way ANOVA).

[‡]$P < 0.05$ NX groups compared with the Sham groups (two-way ANOVA).

creatinine clearance decreased by ~60% in the NX rats (corresponding to G3b class of decreased glomerular filtration, i.e. moderately to severely decreased renal function[35]), while hyperuricaemia reduced creatinine clearance by ~30% in the Sham rats. Plasma creatinine concentrations in the rats correlated significantly with urine volumes ($r_S = 0.689$, $P < 0.001$). Suppressed plasma renin activity and moderate phosphate retention were observed in both NX groups, whereas the plasma concentrations of calcium, sodium and potassium were corresponding in all groups (Table 1). The 24-h urinary protein excretion was clearly increased in both NX groups (Figure 1A).

3.3 | Renal histology

The indices of arteriosclerosis (Figure 1B), glomerulosclerosis (Figure 1C) and tubulointerstitial damage (Figure 1D) were increased in the NX group, while all

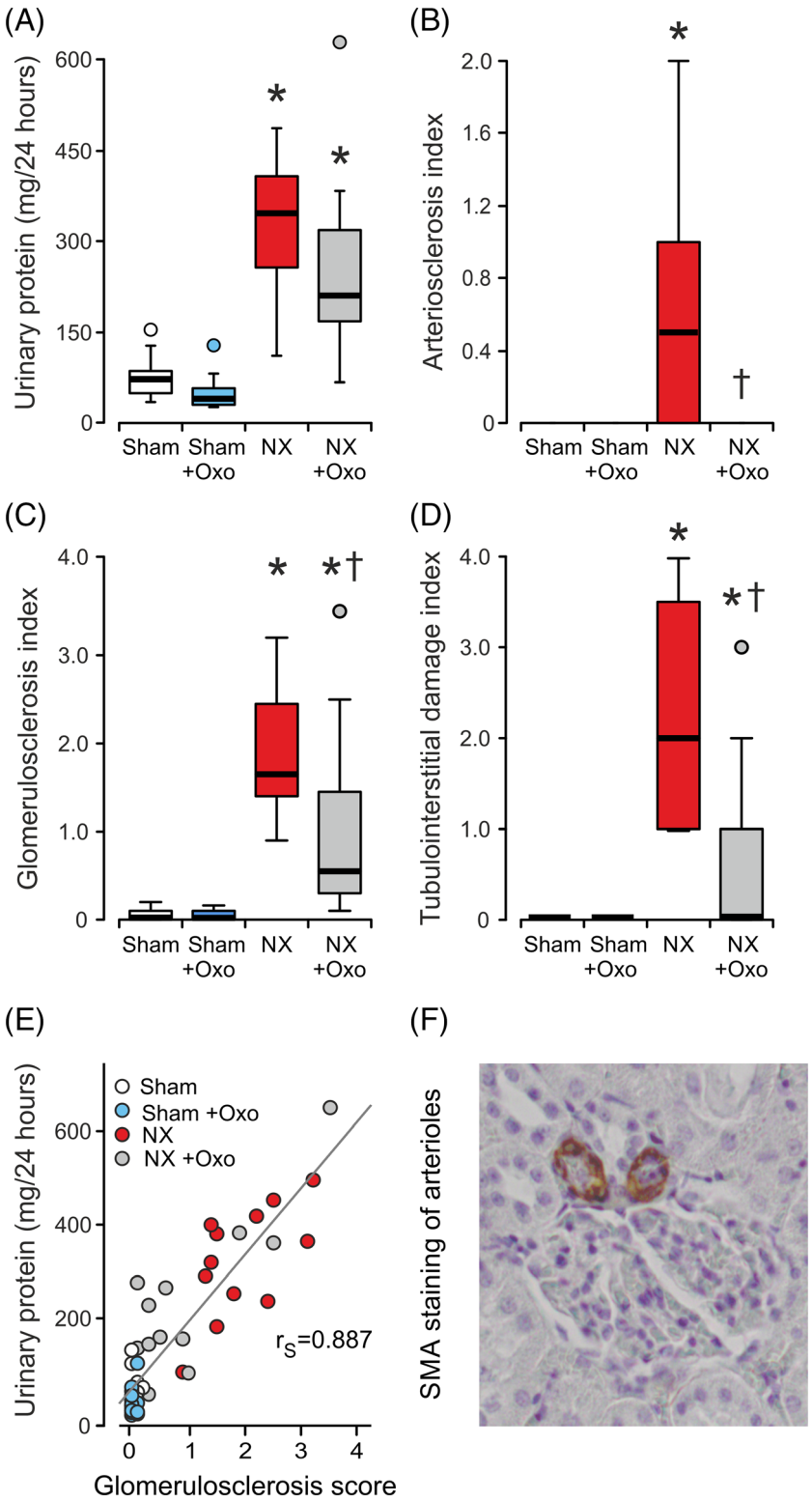

FIGURE 1 Urinary protein excretion during study week 12 (A), kidney arteriosclerosis index (B), glomerulosclerosis index (C), tubulointerstitial damage index (D), correlation between urinary protein excretion and glomerulosclerosis score (E) and representative photomicrograph of smooth muscle actin (SMA) staining of the glomerular arterioles (F) in the study groups ($n = 11–12$ in each group). NX, 5/6 nephrectomized rat; Oxo, 2.0% oxonic acid diet; Sham, sham-operated rat. Values are median (thick line), 25th–75th percentile (box) and range (whiskers), and outliers are depicted as small circles. *$P < 0.05$ *versus* Sham. †$P < 0.05$ *versus* NX

these indices of renal damage were alleviated in the NX + Oxo group when compared with the NX group. The glomerulosclerosis score also significantly correlated with the 24-h urinary protein excretion (Figure 1E).

Staining of SMA was evaluated in an attempt to identify afferent arterioles, as afferent arteriolopathy has been previously reported in a setting where rats were put on 2% Oxo diet immediately after surgical 5/6 NX operation and followed for 6 weeks.[6,17] We observed a clear SMA staining of the arteries that were adjacent to the glomeruli (Figure 1F). However, we could not reliably differentiate the afferent arterioles from the efferent

arterioles with the present techniques. Therefore, further analysis of the preglomerular arterioles was not performed.

3.4 | Markers of inflammation, oxidative stress and collagen I in the kidney

Already 3 weeks after the NX operation when the 2% Oxo diet commenced (study week 3), the 24-h excretion of 11-epi-prostaglandin-$F_{2\alpha}$ to the urine was ~1.6 times higher in the NX groups than in the Sham groups (Figure 2A). At study week 12, the 24-h urinary 11-epi-prostaglandin-$F_{2\alpha}$ excretion was 2.3 times higher in the NX group than in the Sham group, whereas the excretion was reduced by more than 70% in both groups ingesting the 2% Oxo diet (Figure 2B).

Kidney tissue HO-1 mRNA content was higher in both NX groups than in the Sham groups (Figure 2C). The number of mast cells in the kidney tissue was elevated after subtotal NX, while the mast number was significantly lower after the Oxo diet in the remnant kidney rats (Figure 2D). A significant direct correlation between the kidney mast cell count and 24-h urinary 11-epi-prostaglandin-$F_{2\alpha}$ excretion ($r_S = 0.415$, $P = 0.003$) was also observed.

A clear staining of both glomerular and tubulointerstitial COX-2 was observed in the kidney sections (Figure 3A,B). Tubulointerstitial COX-2 score was lower in the Sham + Oxo group than in the Sham group, and in the NX + Oxo group than in the NX group (Figure 3C). The COX-2 staining was abundant in tubuli with thick epithelium corresponding to the ascending limb of the loop of Henle.[36] In the glomeruli, the number of COX-2-positive cells was lower in the Sham + Oxo group than in the Sham group, while lowest COX-2-positive cell numbers were observed in the glomeruli of the NX groups (Figure 3D). The present surgical remnant kidney model was associated with increased kidney tissue collagen I mRNA expression (two-way ANOVA $P = 0.022$), but collagen I mRNA expression was not influenced by the Oxo diet (Table 1).

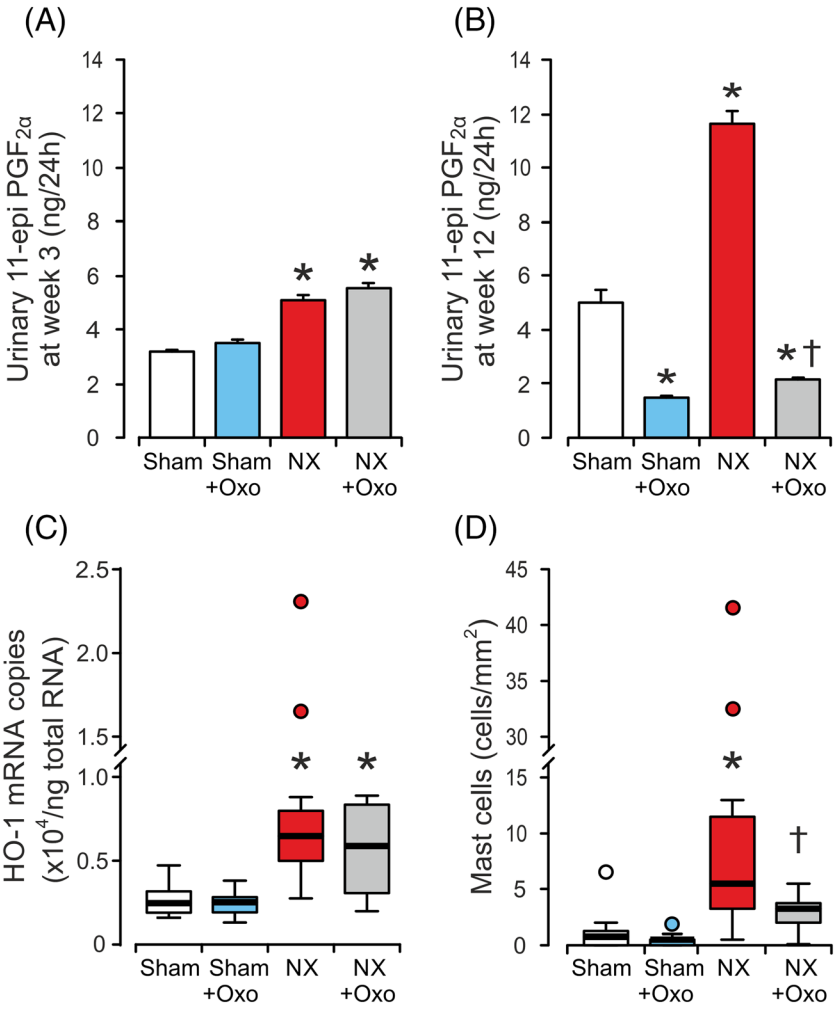

FIGURE 2 The 24-h urinary excretion of 11-epi-prostaglandin $F_{2\alpha}$ during study week 3 (A) and study week 12 (B), kidney haem oxygenase-1 (HO-1) mRNA expression (C) and kidney mast cell content (D) in the study groups ($n = 12$ in each group). Groups as in Figure 1. Values are mean ± S.E.M., median (thick line), 25th–75th percentile (box) and range (whiskers), and outliers are depicted as small circles. *$P < 0.05$ *versus* Sham. †$P < 0.05$ *versus* NX

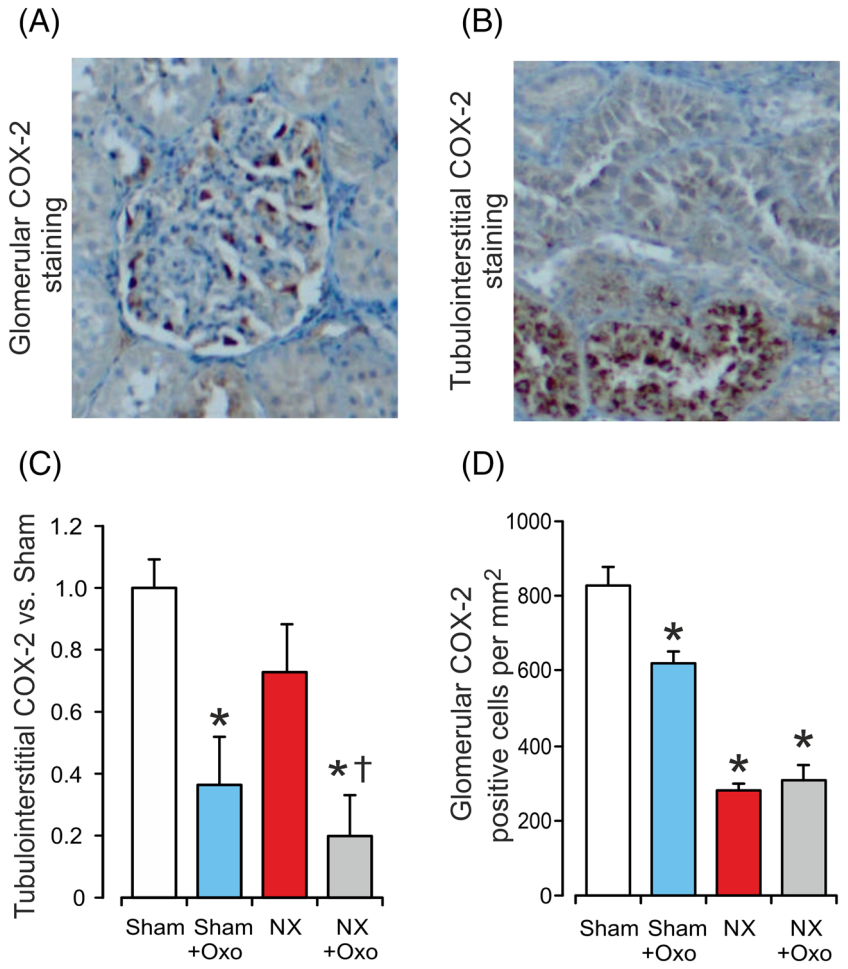

FIGURE 3 Representative photomicrographs of the immunohistochemical staining of glomerular (A) and tubulointerstitial (B) cyclooxygenase-2 (COX-2), tubulointerstitial COX-2 score (C) and number of COX-2-positive cells in the glomeruli (D) in the study groups ($n = 9$–11 in each group). Groups as in Figure 1. Values are mean ± S.E.M. *$P < 0.05$ *versus* Sham. †$P < 0.05$ *versus* NX

4 | DISCUSSION

Here, we examined the effects of 2% Oxo diet-induced hyperuricaemia on kidney morphology, inflammation and markers of oxidative stress in a low-renin model of experimental CRI. Previously, we found that 2% Oxo diet elevated plasma renin and aldosterone, but in parallel improved NO-mediated vasorelaxation in the carotid artery, and reduced oxidative stress in vivo in remnant kidney rats.[9,20] The current results showed that moderately elevated plasma UA level was associated with favourable changes in kidney histology and reduced markers of inflammation in NX rats.

During 2% Oxo feeding, a moderate rise in circulating UA levels is achieved due to inhibition of the hepatic enzyme uricase that metabolizes UA to its final end-product allantoin.[16] Subsequently, plasma UA levels are elevated to concentrations that are closer to those observed in humans. The present 2.5–3 times elevations of UA levels induced by the Oxo diet well correspond to previous findings.[6,16,17,19] Pharmacological lowering of serum UA levels was not included in the present protocol, as several studies have shown that the xanthine oxidase (XO) inhibitors allopurinol and febuxostat effectively prevent the effects of Oxo diet in rats.[5,16,17,19] Notably, the therapeutic effects of XO inhibitors have not been solely related to reduced UA concentrations but also to the anti-oxidative and anti-inflammatory properties of these compounds.[37] The 5/6 NX model has also been characterized by reduced tissue XO activity and a compensatory increase in intestinal UA excretion.[38] These mechanisms may explain why plasma UA was not significantly higher in the NX rats on the normal diet than in Sham rats on the normal diet.

To reduce the risk of selection bias in the current study, the rats were allocated to groups 3 weeks after the operations. The groups were constructed so that BPs, 24-h urine volumes and body weights were similar in the NX and NX + Oxo groups and in the Sham and Sham + Oxo groups, respectively. The 5/6 NX rat model is characterized by immediate and long-term increases in urine volumes,[20,39] probably resulting from high filtration load in the remaining glomeruli and concentrating defect in the remaining nephrons, while the reduction in fractional proximal fluid reabsorption is clearest in the early phase after the renal insult.[39] Of note, in the study by

Kang et al., the division to groups ($n = 4–5$ rats per group) was done immediately after the 5/6 NX operation and was based solely on the amount of the removed kidney tissue.[6] The present renal insufficiency 12 weeks after the NX operation was documented by elevated plasma creatinine, reduced creatinine clearance, hyperphosphataemia and increased urinary protein excretion,[19] while urine volumes were also found to correlate well with the degree of renal insufficiency. The histology showed increased indices of arteriosclerosis, glomerulosclerosis and tubulointerstitial damage in the NX group. The reliability of the histological findings is supported by the good correlation between the glomerulosclerosis score and 24-h urinary protein excretion. Surgical subtotal NX results in marked glomerular hypertrophy so that the size of the glomeruli is increased almost three times when compared with sham-operated controls.[38] The hypertrophy of the remnant kidney can be attributed to compensatory tissue growth in an attempt to compensate for the reduced renal function.[38,40] The NX rats presented with low plasma renin activity probably due to the associated volume load, corresponding to previous findings in rats subjected to surgical renal ablation.[20] Systolic BP was only modestly elevated 12 weeks after renal ablation, as more marked hypertension develops only later in the course impaired renal function in this surgical low-renin model.[41,42]

Previously, the harmful effects of high UA concentrations in renal tissue have been attributed to the deposition of non-soluble monosodium urate crystals in renal tubules (gouty nephropathy).[43] Intracellularly, UA may also mediate biological effects that may play a role in the development of subclinical "non-gouty" types of renal and cardiovascular disease.[43] Excess generation of reactive oxygen species (ROS) has been suggested to play a central role in the UA-induced renal disease.[5] The interaction between UA and ROS is complex, as the synthesis of UA from its purine and pyrimidine nucleotide precursors is catalysed by two xanthine oxidoreductase enzymes: xanthine dehydrogenase and XO. In ischaemic states such as CRI, the latter is the predominant catalyser creating ROS, mainly superoxide anion, as a by-product in the UA synthesis. In cell cultures, UA can inhibit renal production of NO synthase, a catalysing enzyme in NO generation.[16] The reaction between ROS and NO may result in renal NO depletion and afferent artery vasoconstriction, which is an essential step in renal fibrosis.[17] On the other hand, the antioxidant properties of UA are widely accepted. By scavenging superoxide anions, UA can prevent it from reacting with NO and thus inhibit the formation of the toxic peroxynitrite.[10,44] Also, the reaction of UA with peroxynitrite yields a nitrated UA derivate, which has vasodilatory effects.[45] Finally, UA can counter oxidant-induced renal injury by preventing the inactivation of extracellular superoxide dismutase, an enzyme that provides tissue protection by catalysing the dismutation of superoxide radical into oxygen and hydrogen peroxide.[46,47]

We found that the number of mast cells was elevated in remnant kidneys, while mast cell quantity was reduced after the Oxo diet. Kidney mast cell density is known to correlate with the severity of renal disease.[28] Various aetiologies, such as several forms of nephropathies and renovascular ischaemia that cause glomerular damage and interstitial fibrosis, are associated with mast cell abundance in the kidney.[28] Mast cells can aggravate tissue damage and fibrosis by recruiting leucocytes, profibrogenic cytokines, proteases and growth factors and also by directly stimulating collagen synthesis.[28] In an experimental rat model, a close association between mast cell density and oxidative stress in the kidney, as indicated by superoxide anion generation, was previously reported.[48] We also assessed mast cell activity by measuring the quantity of mast cell-derived PGD_2 metabolite, 11-epi-prostaglandin-$F_{2\alpha}$, in the urine.[33,34] Due to the long half-life and stability, 11-epi-prostaglandin-$F_{2\alpha}$ is a convenient way to evaluate mast cell activity in vivo.[33,34,49] The present findings of kidney mast cell density and urinary 24-h 11-epi-prostaglandin-$F_{2\alpha}$ excretion were congruent, and a direct correlation between these variables was observed. Possible explanations to the reduced mast cell infiltration and activity in the renal tissue of hyperuricaemic NX rats are decreased amounts of ROS and increased NO bioavailability,[9] as both of these factors can reduce tissue inflammation and inhibit mast cell degranulation.[48,50]

Whether the actions of UA are detrimental or beneficial may depend on the distribution of UA between the intracellular and extracellular compartments.[43] Extracellularly, the antioxidant properties predominate, whereas intracellularly, UA may be a pro-oxidant.[43] For instance, the free radical scavenging capability of plasma UA appears to have favourable effects on kidney tissue in CRI.[51] In contrast, the blockade of UA entry into the renal tubular cells by the organic anion transporter inhibitor probenecid prevented epithelial-to-mesenchymal transition, an event contributing to progressive tubular fibrosis.[52] We found that kidney tissue HO-1 mRNA content was higher in both NX groups than in the Sham groups but did not differ between the Sham and Sham + Oxo groups, or between the NX and NX + Oxo groups. These findings support the view that the present Oxo diet did not cause oxidative stress even at the cellular level in vivo. Tissue HO-1 content serves as an index of oxidant stress in humans and in animal models of renal disease.[30–32] By converting cell toxic haem to biliverdin in a reaction that liberates carbon monoxide (CO) and iron, HO-1

counteracts the oxidant burden. Inactivation of haem by HO-1 prevents it from inducing lipid peroxidation, and ROS and hydrogen peroxide generation in tubular epithelial cells, while the reaction by-products biliverdin and CO in low concentrations possess antioxidant and vasodilatory effects.[53] In CRI, biliverdin and CO can even help to preserve normal glomerular filtration rate and sodium handling by suppressing tubule-glomerular feedback and afferent arteriolar vasoconstriction.[31]

In the mammalian kidneys, COX-2 has been mainly localized to the macula densa, cortical thick ascending limb and medullary interstitial cells.[36] Increased juxtaglomerular renin and preglomerular arterial COX-2 production have been suggested to contribute to smooth muscle cell proliferation and renal arteriolar obliteration in experimental hyperuricaemia.[6] However, in the present study, the number of glomerular COX-2-positive cells was reduced by 2% Oxo feeding in the Sham rats and was equally further reduced in both NX groups. The explanation for the reduced number of glomerular COX-2-positive cells in both NX groups remains unknown, but may be related to the glomerular hypertrophy and hyperfiltration caused by surgical subtotal NX.[20,38] We found that experimental hyperuricaemia suppressed tubulointerstitial COX-2 protein staining. These findings suggest reduced COX-2-derived inflammatory influences in the kidneys after the 2% Oxo diet. Lower kidney tissue COX-2 content is in line with the beneficial effects of experimental hyperuricaemia on renal histology in the NX rats. Of note, in addition to mast cells, 11-epi-prostaglandin-$F_{2\alpha}$ can also originate from prostanoids synthetized via COX-2. Therefore, reduced 11-epi-prostaglandin-$F_{2\alpha}$ excretion may also reflect reduced total COX-2 content in the kidneys of the hyperuricaemic rats.[54]

Immunohistochemical staining of SMA was done in order to examine the renal preglomerular arterioles, as thickening of the afferent arterioles has been suggested to trigger UA-induced renal fibrosis.[17] The renal arterioles were identified adjacent to glomeruli, but we were unable to reliably differentiate the afferent from the efferent arterioles. Therefore, the present results are inconclusive with respect to preglomerular small artery structure. Increased interstitial collagen deposition has been suggested to mediate UA-mediated renal fibrosis.[16] In the present study, collagen I mRNA expression was elevated in CRI but was not influenced by moderate hyperuricaemia. Although several previous studies have shown that the effects of Oxo feeding are prevented by pharmacological lowering of UA,[5,16,17,19] the possibility remains that some of the present effects were caused by Oxo itself and not by UA.

The causal role of UA in the progression of renal disease has been questioned by Mendelian randomization studies.[55,56] A comprehensive review concluded that the causal association of UA with a range of health outcomes is evident only in gout and nephrolithiasis.[57] In haemodialysis patients, lower UA levels were independently associated with higher all-cause and cardiovascular mortality,[58] while in patients with end-stage renal disease not receiving dialysis or receiving peritoneal dialysis, higher serum UA was associated with higher mortality.[53] There is also evidence that patients genetically predisposed to hypouricaemia present with an elevated risk of renal disease.[59] The optimal range of circulating UA levels in various health conditions warrants further research.

5 | CONCLUSIONS

Consistent with previous reports, the surgical remnant kidney low-renin model of CRI was characterized by renal scarring and increased proteinuria. These pathological alterations were related to increased renal inflammation, fibrosis and oxidative stress, as indicated by increased mast cell infiltration and activation, elevated collagen I mRNA and elevated HO-1 mRNA in the kidneys of the NX rats. Nine weeks of 2% Oxo diet increased plasma UA concentrations and improved renal histology with a parallel reduction in local and urinary markers of inflammation in the remnant kidney rats. These findings indicate that elevated UA levels, which increase the antioxidant capacity in plasma,[9] do not always cause histological and functional impairment of the kidneys.

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CONFLICTS OF INTEREST

The authors declare that they have no competing interests.

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