Semicarbazide-sensitive amine oxidase (SSAO) inhibition ameliorates kidney fibrosis in a unilateral ureteral obstruction murine model

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The histological hallmarks of end-stage renal disease, glomerulosclerosis and tubulointerstitial fibrosis, are the products of a multifaceted cellular response to both acute and chronic insults. Pathology within the tubulointerstitium is recognized as a prognostic marker (20) and a more reliable histological predictor of kidney disease progression than is glomerular pathology (3). Inflammation is also recognized to play a pathogenic role in the majority of causes of chronic kidney disease (CKD) (11). The dynamic process of injury and repair in CKD results in the pathological accumulation of inflammatory cells, loss of peritubular capillaries, and excessive deposition of extracellular matrix (ECM) (2, 15). Currently available therapeutics are largely limited to agents that inhibit the renin-angiotensin-aldosterone system (RAAS) and are only partially effective in delaying the progression of renal disease (25). Recent evidence suggests that combination therapies [with either a renin inhibitor or a combination of angiotensin-converting enzyme inhibitor (ACEi) or an angiotensin receptor blocker (ARB)] show limited benefit but confer more adverse events (6, 17). As the etiology of CKD progression is multifactorial (4, 12), combination therapy with different classes of agents that target independent pathogenic pathways is a rational strategy for designing future treatments for patients with CKD.

Semicarbazide-sensitive amine oxidase (SSAO) is an enzyme predominantly located in the endothelium and leukocytes. SSAO is highly active in the endothelial cells of highly vascularized tissues, including the kidney (21). It is also abundant in fat tissue, the liver, and gonads. SSAO is unique among other endothelial-expressed adhesins as it is also an ectoenzyme. A soluble form of SSAO is present in plasma (13) and is known as vascular adhesion protein (VAP)-1 (23). It is well known to regulate two key inflammatory processes which are integral to progressive renal pathology. First, the soluble end products of its enzymatic cleavage, hydrogen peroxide and reactive aldehydes, are highly reactive and lead to the formation of protein cross-linking and oxidative stress (8) Second, SSAO mediates the transmigration of intraluminal leukocytes into sites of tissue inflammation, which is initially a protective reparative process, but if persistent, can lead to chronic inflammatory cell accumulation. In combination, these processes contribute to the development of kidney fibrosis (27). Hence we hypothesize that inhibition of SSAO activity may be of therapeutic potential in the prevention of kidney fibrosis.

Previous studies in murine models have studied the pathological role of SSAO in fibrotic disease, including liver fibrosis (28), chronic obstructive pulmonary disease, and other models of pulmonary fibrosis and vascular remodeling (14). However, its role in kidney disease remains largely unknown. This study is the first to examine the role of SSAO inhibitors (SSAOi) in...
an acute model of kidney fibrosis using a unilateral ureteric obstruction (UOO) model.

METHODS

UOO Animal Model

In-house-bred male C57BL/6 mice, 6–8 wk of age, each weighing 22–26 g, were used in this study. Briefly, animals were anesthetized by 2% isoflurane gas (1 part oxygen, 2 parts nitrogen). Through a flank incision, the left ureter was exposed and completely ligated at two points using fine suture material (4-0 silk) as previously described (24). Sham animals underwent the same surgical intervention with manipulation of the kidney except for ureteric ligation. PXS4728A, a highly selective SSAAOI with high oral bioavailability (Pharmaxis), was administered by daily oral gavage (2 mg/kg) through a disposable plastic gavage tube (Instech Laboratories) using PBS as a vehicle. Telmisartan (3 mg kg⁻¹ day⁻¹) was mixed in drinking water 5 days before the operation. The pH of the drinking water was adjusted to 7.4 using 1% hydrochloric acid. Animals were divided into the following treatment groups (was adjusted to 7.4 using 1% hydrochloric acid. Animals were divided into the following treatment groups (n = 7/group): 1) Sham operated, 2) UOO, 3) UOO+SSAOI, 4) UOO+telmisartan, and 5) UOO+SSAOI+telmisartan. Mice were housed singly in filter-top cages in a pathogen-free facility and had free access to standard chow and drinking water for 7 days. All animal experiments were performed with approval from the Royal North Shore Animal Ethics Committee and adhered to the Australian Code of Practice for the Care and Use of Animals for Scientific Purposes.

Measurement of Physiological Parameters

Body weight was obtained at the time of the operation and animal euthanasia. Blood pressure was measured using a noninvasive tail vein cuff methodology (CODA Blood Pressure apparatus, Kent Scientific) before initiation of treatment and preterminally. Animals were euthanized by pericardectomy under 2% isoflurane,庙纹ime, and examined using a light microscope (Leica photomicroscope linked to a DFC 480 digital camera). Ten nonoverlapping fields were captured, and tubulointerstitial fibrosis was graded on a scale of zero to four in a blinded manner by two independent pathologists. (0 = normal; 0.5 = small focal areas of damage; 1 = involvement of <10% of the cortex; 2 = involvement of 10–25% of the cortex; 3 = involvement of 25–75% of the cortex; and 4 = extensive damage involving >75% of the cortex) (18). The total score was the calculated average of all tubular scores.

RNA Isolation and Quantitative Real-Time RT-PCR

The other half of the ligated kidney was cut in half, snap-frozen with liquid nitrogen, and stored at -80°C for RNA analysis. Real-time quantitative (q) RT-PCR was performed to compare mRNA expression of inflammatory and profibrotic cytokines known to be relevant to progressive renal disease. In brief, total RNA was extracted from the kidney cortexes using an RNA isolation kit (Qiagen, RNeasy Mini Kit). To ensure samples were without genomic DNA contamination, total RNA was treated with DNase (Qiagen, RNase-Free DNase Set), and cDNA was synthesized using a Synthesis Kit (Bio-Rad). Predrimered primers for fibronectin, collagen IV, transforming growth factor (TGF)-β1, monocyte chemoattractant protein-1 (MCP-1), and actin are listed in Table 1. Twenty-five microliters of real-time PCR included Brilliant SYBR Green QRT-PCR Master Mix according to the manufacturer’s instructions (Stratagene, La Jolla, CA). Quantitative real-time PCR was performed using the ABI Prism 7,900 HT Sequence Detection System (Applied Biosystems, Mulgrave, Victoria, Australia). Reactions were performed in at least triplicate and analyzed by relative quantitation using RQ Manager software, version 1.2 (Applied Biosystems). All data are presented as fold-change compared with control after normalization to the housekeeping gene β-actin.

Immunohistochemistry

Paraffin-embedded sections (3 μm) were dewaxed in xylene and rehydrated in graded concentrations of ethanol. Epitope retrieval was performed in 0.01 mol/l citrate buffer, pH 6.0. The sections were immersed in 3% hydrogen peroxide to inhibit endogenous peroxidase activity and then incubated with nonserum protein block (Dako, Glostrup, Denmark) to block nonspecific protein binding. They were then incubated with primary antibodies against fibronectin (dilution 1:1,000, Sigma-Aldrich, Dublin, Ireland) and collagen IV (dilution 1:1,000, Abcam, Cambridge, UK), the pan leukocyte common antigen (Dako) at 4°C overnight. The primary antibodies were subsequently localized using biotinylated secondary anti-rabbit IgG antibodies. Horseradish peroxidase (HRP)-conjugated streptavidin was subsequently used to visualize the tissue immune complexes using the LSAB + detection system (Dako). The slides were counterstained with Mayer’s hematoxylin (Fronine, Taren Point, NSW, Australia). Control sections were also prepared in which the authentic primary antibodies were replaced with an irrelevant isotype-matched IgG. The tissue specimens were examined by brightfield microscopy using a Leica photomicroscope linked to a DFC 480 digital camera. For fibronectin, collagen IV, and nitrotyrosine, 20 consecutive nonoverlapping fields from each section of renal cortex were photographed under high magnification (×400). Areas indicated by brown staining were quantified using a computer-aided manipulator, Image J (Java-based software program, National Institutes of Health). The percentage of the stained area relative to the whole area in each vision field was determined. For CD45, the number of positively stained cells in each section was counted in each replicate, and the total was averaged and expressed.

Table 1. Real-time qPCR primers

<table>
<thead>
<tr>
<th>Gene Name</th>
<th>Forward Primer (5’-3’)</th>
<th>Reverse Primer (5’-3’)</th>
</tr>
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<tbody>
<tr>
<td>Fibronectin</td>
<td>TCGAGAGGGCCACATTACTCAT</td>
<td>CTTCAGGCGGAAATGCTGTTAA</td>
</tr>
<tr>
<td>TGF-β1</td>
<td>TCAAGATTTGGGAGCACTCTT</td>
<td>ACGGCGAAGTTGTCTGTTAA</td>
</tr>
<tr>
<td>Collagen IV</td>
<td>TCAGAATTTGCCGAAGCACTT</td>
<td>ACGGCGAAGTTGTCTGTTAA</td>
</tr>
<tr>
<td>MCP-1</td>
<td>GGGTCGCTTGGTCCAGATGTCG</td>
<td>CGGATGGTGGAACCGTGTTAA</td>
</tr>
<tr>
<td>β-Actin</td>
<td>CGGATGCGGAGGAAAGATCTG</td>
<td>CGGATGCGGAGGAAAGATCTG</td>
</tr>
</tbody>
</table>

Primers sequence for mice fibronectin, transforming growth factor (TGF)-β1, collagen IV, monocyte chemoattractant protein (MCP-1), and actin genes are shown.
visualized with the chromogen diaminobenzidine, and counterstaining was performed using Mayer’s hematoxylin (Fronine). Semiquantitative analysis of the stained area was performed as described above.

**Radiometric \( ^{14} \text{C} \)Benzylamine SSAO Activity**

SSAO activity was determined radiometrically as previously described, with minor modification (7). Homogenized kidney lysates were preincubated for 30 min at 37°C with 0.5 mM parglyline (MAO-A and MAO/B inhibitor, background control) or 0.5 mM parglyline with PX4728A (an SSAO inhibitor, as SSAO-specific activity control). \( ^{14} \text{C} \)Benzylamine (200 μM, PerkinElmer, Meriden, CT) was used as a substrate. The reaction was stopped using 2 M citric acid. Radiolabeled products were extracted using toluene with a MicroScint-20 cocktail before counting in a scintillator counter (PerkinElmer). SSAO activity was corrected for the protein concentration and expressed as picomoles per minute per milligram protein.

**Statistical Analysis**

Statistical analysis was done using Graph Prism 6 software. Six to eight animals were used in each group unless otherwise stated. Statistical significance was assessed using a paired t-test, unpaired t-test, and one way ANOVA. Statistical significance was considered only if the probability value was <0.05.

**RESULTS**

**Animal Characteristics and Physiological Parameters**

All animals survived until the end of the study and did not demonstrate any signs of drug toxicity. Body weight was similar between the groups. Mean blood pressure (MBP) was similar among all groups. In particular, this dose of telmisartan (3 mg·kg\(^{-1}\)·day\(^{-1}\)) did not significantly lower MBP. The groups receiving SSAOi, telmisartan, or both agents showed no statistically difference in the urinary albumin/creatinine ratio at the termination of the study (Table 2).

**Effects of SSAO Inhibition on Tubulointerstitial Fibrosis in the Mouse UUO Model**

The sham-operated kidney showed that tubules were back to back and epithelial cells were cuboidal in shape. In all UUO groups, there were simplification of the tubular epithelium, diminishment of the total thickness of the cortex and medulla, atrophic tubules, thickened basement membrane, a widened tubulointerstitial space, and increased cellular infiltrate compared with the sham-operated group (Fig. 1). Kidneys treated with SSAO and telmisartan exhibited reduced epithelial cell flattening and less tubular damage. The tubulointerstitial fibrosis index was lower in mice treated with the SSAO inhibitor compared with untreated UUO mice (2.1 ± 0.2 vs. 3.7 ± 0.2, respectively; \( P < 0.0001 \)). Telmisartan-treated obstructed kidneys also showed decreased fibrosis in the cortex compared with the untreated UUO kidneys (2.7 ± 0.6 vs. 3.7 ± 0.2, respectively; \( P = 0.005 \)). There was no significant difference between the treatment effects of the SSAO inhibitor and telmisartan (Fig. 1).

**SSAO Activity in Unilateral Ureteral-Ligated Kidneys**

SSAO activity in the UUO kidneys was significantly higher compared with the sham-operated animals (582.2 ± 181.7 vs. 51.5 ± 32.9, \( P < 0.01 \)). PX4728A effectively suppressed kidney SSAO activity (\( P < 0.01 \) vs. UUO) (Fig. 2). Since the kidneys are perfused with ice-cold PBS at the time of harvest, the SSAO activity detected reflects the intrarenal SSAO activity.

**SSAO Inhibition Suppresses ECM Deposition**

Collagen IV is a major component of the tubular basement membrane (TBM). Untreated obstructive injury clearly caused a disruption of TBM integrity compared with the sham-operated group, which showed a normal renal cortex. This was notably reduced with the SSAO treatment. Immunostaining of fibronectin showed a normal localization in tissues from sham-operated groups. A marked increase in interstitial fibronectin was seen in tissues from the untreated obstructed kidneys. In contrast, mice treated with SSAO inhibition showed only moderate, and statistically less, histologically identifiable renal injury and minimal fibrosis. Semiquantitative analysis showed a significant reduction in the expression of collagen IV and fibronectin in mice treated with SSAOi compared with the untreated UUO animals (2.3 ± 1.6 vs. 9.6 ± 5.3; \( P < 0.001 \) and 3.2 ± 2.7 vs. 12.0 ± 8.5; \( P < 0.05 \), respectively) (Fig. 3).

**SSAO Inhibition Prevents Inflammatory Cell Infiltrate**

Increased interstitial inflammatory infiltrate was observed, predominantly in the perivascular areas and interstitial space, which was not seen in the sham-operated animals. F4/80 is a macrophage marker, and CD45 is a panleukocyte marker. There was a significant reduction in the expression of F4/80 in SSAOi-treated kidneys (5.4 ± 0.5 vs. 11.3 ± 0.8; \( P < 0.01 \)) as well as telmisartan-treated kidneys (8.3 ± 0.8 vs. 11.3 ± 0.8, \( P < 0.05 \)) vs. control, respectively. Importantly, the combination therapy of SSAOi and telmisartan showed a greater effect in limiting inflammatory cell accumulation against the untreated UUO kidneys (3.0 ± 1.6 vs. 11.3 ± 0.8; \( P < 0.001 \)). There was also a significant reduction in the expression of CD45 in SSAOi-treated kidneys (1.3 ± 0.7 vs. 6.7 ± 2.3; \( P < 0.05 \)). Telmisartan-treated kidneys did not reduce CD45 expression (5.3 ± 2.8 vs. 6.7 ± 2.3; \( P = 0.56 \)) vs. the untreated UUO kidneys. Interestingly, the combination therapy showed

**Table 2. Metabolic and physiological parameters of studied animals**

<table>
<thead>
<tr>
<th></th>
<th>SHAM</th>
<th>UUO</th>
<th>UUO+SSAOi</th>
<th>UUO+Telmisartan</th>
<th>UUO+Telmisartan+SSAOi</th>
</tr>
</thead>
<tbody>
<tr>
<td>Weight, g</td>
<td>21.3 ± 0.8</td>
<td>19.5 ± 0.6</td>
<td>20.9 ± 0.6</td>
<td>21.5 ± 0.9</td>
<td>21.4 ± 21.8</td>
</tr>
<tr>
<td>Initial mean blood pressure, mmHg</td>
<td>85.5 ± 2.3</td>
<td>83.6 ± 2.9</td>
<td>89.0 ± 3.0</td>
<td>86.7 ± 2.8</td>
<td>80.2 ± 3.1</td>
</tr>
<tr>
<td>Final mean blood pressure, mmHg</td>
<td>81.4 ± 5.5</td>
<td>83.0 ± 2.5</td>
<td>86.9 ± 4.3</td>
<td>80.8 ± 3.5</td>
<td>79.8 ± 2.0</td>
</tr>
<tr>
<td>Albumin/creatinine, mg·mg(^{-1})·24 h(^{-1})</td>
<td>0.4 ± 0.1</td>
<td>1.4 ± 0.4</td>
<td>0.7 ± 0.1</td>
<td>0.9 ± 0.3</td>
<td>0.9 ± 0.2</td>
</tr>
</tbody>
</table>

Values are means ± SE; \( n = 12 \). SHAM, sham operated; UUO, unilateral ureteric obstruction, SSAOi, semicarbazide-sensitive amine oxidase inhibitor.
the greatest inhibitory effect (0.09 ± 0.02 vs. 6.7 ± 2.3; P = 0.02) compared with UUO-untreated kidneys (Fig. 4).

Effect of SSAO Inhibition on Renal Proinflammatory Cytokines and Nitrotyrosine Expression

The mRNA expression of TGF-β1 was suppressed by SSAO inhibition (2.6 ± 1.9 vs. 7.4 ± 2.6; P < 0.01) compared with the untreated UUO mice. Similarly, MCP-1 expression was suppressed in mice treated with SSAO inhibition (2.7 ± 0.7 vs. 7.1 ± 1.1; P < 0.01) compared with untreated UUO mice (Fig. 5). Nitrotyrosine was minimally expressed in sham-operated animals but was markedly elevated in the UUO kidneys (0.2 ± 0.1 vs. 5.4 ± 1.4, P < 0.001). SSAOi, telmisartan, or SSAOi and telmisartan combined effectively suppressed nitrotyrosine expression (0.5 ± 0.2, 0.2 ± 0.1, and 0.2 ± 0.1, P < 0.001 vs. UUO, respectively) (Fig. 6).

DISCUSSION

A cascade of events takes place during the progression of chronic kidney disease, including the release of cytokines, expression of adhesion molecules, inflammatory infiltrate, renal epithelial cell damage, and finally fibrosis. In this study, we uniquely demonstrate that SSAO plays a crucial role in this cascade. We have shown that SSAO activity is markedly increased in the ligated kidney, which is effectively suppressed by the SSAOi PXS4728A.

PXS4728A is a selective mechanism-based SSAOi. It is very similar to the previously published PXS4681A compound (5), with an improved selectivity profile. PXS4728A blocks SSAO enzymatic activity between 2 and 7 nM in various species (human, rat, dog, mouse, and rabbit) and has >500-fold selectivity over any other amine oxidase or macromolec-
ular target (Ricerca-Eurofins Lead Profiling Screen). A single oral dose of PXS4728A at 2 mg/kg provides >80% inhibition of SSAO in various tissues (lung, liver, fat, heart) in healthy rats or mice. The recovery of enzyme activity occurred with a half-life of 2–3 days as previously published (16). PXS4728A is very well tolerated with no overt adverse effects in a 28-day rat study using 10 mg/kg. In this study, we used 2 mg/kg, based on existing pharmacokinetic and pharmacodynamic studies, to examine the effect of SSAO inhibition in kidney fibrosis. None of the animals died during the study, and we found no toxicity or side effects with the dose used.

The ECM proteins fibronectin and collagen are matrix proteins classically upregulated by the profibrotic cytokine TGF-β that contribute to the expanded cortical interstitium characteristic of CKD. As expected, TGF-β1 was upregulated in the UUO model. As reported by others (10), we confirmed that telmisartan reduced the expanded renal cortical interstitium and blunted the increase in TGF-β1 mRNA and collagen IV mRNA. The upregulated expression of TGF-β1 was similarly attenuated by SSAO inhibition, suggesting that SSAO is at least in part responsible for increased TGF-β1 expression, either directly or secondarily to the inflammatory response observed.

Infiltration of circulating leukocytes is triggered by locally secreted chemokines (29), which contributes to the initiation and progression of renal disease (1). The role of macrophages in inducing tissue injury by releasing reactive oxygen species, nitric oxide, complement factors, and proinflammatory cytokines, or even an opposite role in resolution of inflammation or assisting regeneration, is increasingly recognized (22). Monocytes are attracted to tissue expression of MCP-1, which in the kidneys are the proximal tubular cells (19). Nitrotyrosine is a product of tyrosine nitration mediated by reactive nitrogen species and is an indicator or marker of cell damage, inflammation, and NO (nitric oxide) production. MCP-1 and nitrotyrosine expression were reduced by SSAO inhibition. Telmisartan inhibited MCP-1 and nitrotyrosine to a similar extent to that observed with SSAO inhibition. Angiotensin II is a well-recognized pro-oxidant. Hence it is unsurprising that telmisartan reduces oxidative stress. The specific pathways via which SSAO increases oxidative stress requires further delineation. Recently, Wolf et al. (26) have described a regulatory loop...
between MCP-1 and TGF-β1 in renal injury independent of macrophage recruitment. In the present study, TGF-β1 mRNA and MCP-1 mRNA were reduced in parallel. Whether macrophages are a key source of TGF-β1 in the obstructed kidney with UUO remains to be determined. This suggests that both drugs have the potential to further limit macrophage accumulation in the UUO model and limit the increase in active and total TGF-β1, which should limit production of ECM.

Mechanistic studies performed in vitro and in vivo are consistent with the observed effect of SSAO in stimulating leukocyte migration from the circulation to sites of inflammation (8). The current hypothesis suggests that leukocytes initially bind to endothelial VAP-1 using receptors for the anti-VAP-1 antibody-defined surface epitopes of VAP-1. This interaction and activities of other adhesion molecule pairs bring leukocytes and endothelial cells into close contact. This allows penetration of SSAO substrates present on the surface of leukocytes into the enzymatic change of VAP-1.

In the present study, we demonstrate that SSAOi increases UUO-induced increase in F4/80-positive macrophage infiltration in the kidney. Decreased interstitial macrophage infiltration was correlated with reduced tubulointerstitial fibrosis in the UUO model (10). The reduction of inflammatory cell infiltration with SSAO inhibition is most likely a consequence of blocking SSAO-dependent leukocyte adhesion and transmigration. The VAP-1 receptor is expressed on circulating endothelial cells in the peripheral blood of mice and humans. Chemokine ligand binding to VAP-1 is required for film leukocyte adhesion to activated endothelium during the rolling phase. SSAO mediates transmigration through the endothelium basement membrane barrier into the interstitial space. However, in addition to its effects on leukocyte infiltration, SSAO may have additional roles in leukocyte homeostasis such as regulating leukocyte apoptosis within the kidney or emigration of leukocytes out of the kidney into the blood or lymph nodes, an issue that needs further clarification.

In this study, pretreatment of mice before UUO with the angiotensin receptor-1 blocker telmisartan led to a significant decrease in fibrosis and macrophage infiltration. These effects were seen with a relatively low dose of telmisartan, which did not affect the mice MBP. However, despite a marked effect of telmisartan on markers of renal damage, residual injury was present, suggesting that additional agents may be necessary to prevent progressive renal pathology. The rationale for the combination therapy of telmisartan and SSAO inhibition is based on the different mechanisms of actions of these two drug classes. We found that each drug individually provided protection against several parameters of renal damage, including...
macrophage infiltration, and fibrosis and fibroblast expression. The combination of the two drugs did not, for the most part, enhance their activities nor did it provide complete protection against renal damage in UUO. The data does clearly show that the SSAOi effect is through inhibiting leukocyte migration as well as suppressing oxidative stress. This suggests that there may be underlying differences in the mechanisms which result in the renal damage in UUO compared with other renal diseases.

It should be noted that this study has some limitations. One potential drawback is related to the UUO model itself, as it is an extremely aggressive model of nephropathy characterized

Fig. 5. SSAOi suppressed the expression of proinflammatory and profibrotic markers in acute kidney fibrosis. Real-time PCR analysis showed mRNA expression of fibrotic markers CIV (A), FN (B), and TGF-β1 (C), and inflammatory marker monocyte chemoattractant protein-1 (MCP-1; D) was lower in kidney tissue of animals treated with SSAOi. Results are means ± SE. *P < 0.05, **P < 0.01 vs. UUO.

Fig. 6. SSAOi suppressed nitrotyrosine expression. Nitrotyrosine is a marker of cell damage, inflammation, and nitric oxide (NO) production. It is minimally expressed in the kidneys of sham-operated animals but is markedly induced in UUO kidneys. Both telmisartan and SSAOi significantly suppressed nitrotyrosine expression, similarly seen with the combination of SSAOi and telmisartan. Results are means ± SE. ***P < 0.001 vs. Sham. ### P < 0.001 vs. UUO.
by high inflammation and acute fibrosis, features that are contrary to the slow progression of many forms of CKD relevant to a clinical setting. Since UUO begins as a mechanical insult to the kidney, compared with the metabolic or immunological changes in diabetes or in glomerulonephritis, and the outlet obstruction remains in place during treatment, this could contribute to the differences between the response of UUO and that of other renal diseases. In addition, UUO is characterized predominantly by interstitial changes, whereas in more common forms of CKD such as diabetes and glomerulonephritis, there is significant glomerular involvement, which could also contribute to the differences noted. Furthermore, renal functional assessment and the long-term efficacy of combination therapy were not examined in this model. Nevertheless, our study offers significant insights into designing a rational therapeutic regimen for the use of an SSAOi in the treatment of CKD.

Conclusion

This study establishes that SSAO inhibition is equally as effective as standard RAAS inhibition in suppressing matrix gene expression, interstitial inflammation, oxidative stress, and total collagen accumulation in obstructive nephropathy. Furthermore, a combination of both may lead to additive efficacy in ameliorating renal inflammation, suggesting that multitargeted therapy using different classes of drugs is a rational strategy in designing future treatment regimens for patients with CKD. In conclusion, we have shown that SSAO inhibition can significantly suppress profibrotic and proinflammatory cytokine secretion, limit inflammatory cell accumulation, extracellular matrix expression, and oxidative stress in an acute model of renal fibrosis. Given the reduction in inflammatory cell accumulation, we hypothesize that this will have longer term benefits in a more chronic model of renal fibrosis. This study, together with previous reports, illustrate that by dissecting key pathways and their mediators in renal fibrogenesis, it is plausible to develop a novel class of therapeutic modalities to limit progressive structural and functional decline in CKD.

GRANTS

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DISCLOSURES

C. Pollock has received an Australian Research Council grant from Pharmaxis to explore the renal effects of a proprietary SSAO inhibitor.

AUTHOR CONTRIBUTIONS

Author contributions: M.Y.W., J.Z., S.G., H.C.S., A.J.G., and M.G.W. performed experiments; M.Y.W., S.S., W.J., J.A.C., and M.G.W. analyzed data; M.Y.W. prepared figures; M.Y.W. drafted manuscript; M.Y.W., S.S., W.J., J.A.C., A.J.G., C.A.P., and M.G.W. approved final version of manuscript; S.S., C.A.P., and M.G.W. interpreted results of experiments; C.A.P. and M.G.W. provided conception and design of research.

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