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ORIGINAL ARTICLE

WILEY

Enteropeptidase inhibition improves kidney function in a rat model of diabetic kidney disease

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Abstract

Aim: To examine the effects of an enteropeptidase inhibitor, SCO-792, on kidney function in rats.

Materials and Methods: The pharmacological effects of SCO-792 were evaluated in Wistar fatty (WF) rats, a rat model of diabetic kidney disease (DKD).

Results: Oral administration of SCO-792 increased faecal protein content and improved glycaemic control in WF rats. SCO-792 elicited a rapid decrease in urine albumin-to-creatinine ratio (UACR). SCO-792 also normalized glomerular hyperfiltration and decreased fibrosis, inflammation and tubular injury markers in the kidneys. However, pioglitazone-induced glycaemic improvement had no effect on kidney variables. Dietary supplementation of amino acids (AAs), which bypass the action of enteropeptidase inhibition, mitigated the effect of SCO-792 on UACR reduction, suggesting a pivotal role for enteropeptidase. Furthermore, autophagy activity in the glomerulus, which is impaired in DKD, was elevated in SCO-792-treated rats. Finally, a therapeutically additive effect on UACR reduction was observed with a combination of SCO-792 with irbesartan, an angiotensin II receptor blocker.

Conclusions: This study is the first to demonstrate that enteropeptidase inhibition is effective in improving disease conditions in DKD. SCO-792-induced therapeutic efficacy is likely to be independent of glycaemic control and mediated by the regulation of AAs and autophagy. Taken together with a combination effect of irbesartan, SCO-792 may be a novel therapeutic option for patients with DKD.

KEYWORDS

anti-diabetic drug, diabetic nephropathy, drug development

1 | INTRODUCTION

Type 2 diabetes mellitus is a chronic metabolic disorder characterized by hyperglycaemia, insulin resistance and relative insulin insufficiency. It is a global clinical problem that leads to microvascular and macrovascular diabetic complications.[1] Diabetic kidney disease (DKD) is a major microvascular diabetic complication that results in chronic kidney disease (CKD) and end-stage renal disease. In the

United States, more than 40% of patients with type 2 diabetes have DKD.[2] DKD is defined by microalbuminuria followed by proteinuria, reflecting glomerular damage caused by hyperglycaemia-induced hyperfiltration, hypertension and podocyte injury.[3] In proteinuria, an excessive protein load to proximal tubular cells causes renal inflammation and interstitial fibrosis, key pathways in renal failure progression.[4] Blockade of the renin-angiotensin system (RAS) with either angiotensin-converting enzyme inhibitors or angiotensin II receptor blockers (ARBs) is clinically used as a standard treatment for patients with DKD.[5] Recently, sodium-glucose co-transporter-2 inhibitors have been introduced as a new class of renoprotective drugs.[6,7] However, regardless of these drug interventions, patients with gradually deteriorating kidney function remain, and novel drugs with new mechanisms of action are required to improve their renal pathological state.

Excess dietary protein intake is considered to accelerate the progression of kidney failure by inducing glomerular hyperfiltration, an accumulation of uraemic toxins, and an inhibition of glomerular autophagy.[8] It has been reported that increased dietary protein intake is positively correlated with an increased risk of progression to end-stage renal failure in patients with CKD;[9] however, the therapeutic significance of lowering dietary protein intake for kidney function remains controversial,[10] and the effectiveness of pharmacological interventions in regulating protein digestive cascades is unclear.

Enteropeptidase is a type II transmembrane serine protease, highly expressed on the luminal side of enterocytes on the brush border of the duodenum. It converts inactive trypsinogen into active trypsin through cleavage at the trypsinogen-activation site, and the activated trypsin subsequently activates other digestive zymogens secreted from the pancreas, including chymotrypsinogen, proelastase and procarboxypeptidases.[11–13] These activated enzymes regulate protein breakdown into amino acids (AAs). Thus, enteropeptidase plays a key role in protein digestion and AA absorption. SCO-792 is a novel, orally available enteropeptidase inhibitor with a half maximal inhibitory concentration of <10 nM against rat and human enteropeptidase *in vitro*.[14] In mouse models, enteropeptidase inhibition improves obesity and diabetes.[15] However, the pharmacological effects of enteropeptidase inhibition on diabetic kidney complications are largely unknown. Considering that enteropeptidase is the furthest upstream of the enzymes involved in protein digestion and the absorption process, an investigation of the therapeutic effect of enteropeptidase inhibition on the DKD condition may be worthwhile.

In the present study, we examined the effects of an enteropeptidase inhibitor, SCO-792, on kidney variables in Wistar fatty (WF) rats, an animal model with DKD.[16] First, we evaluated the metabolic and renoprotective effects of SCO-792 in WF rats. Next, the contribution of the changes in AA absorption to the efficacy of SCO-792 was evaluated. Additional experiments were performed to reveal the effect of SCO-792 on glomerular autophagy activity, and to examine a combined effect of SCO-792 with an ARB.

2 | MATERIALS AND METHODS

2.1 | Materials

All reagents were purchased from FUJIFILM Wako Pure Chemical Corporation (Osaka, Japan), unless otherwise indicated. SCO-792 was synthesized by Takeda Pharmaceutical Co. Ltd (Tokyo, Japan). Pioglitazone hydrochloride was purchased from Tokyo Chemical Industry (Tokyo, Japan). Irbesartan was purchased from LKT Laboratories, Inc. (St Paul, Minnesota).

2.2 | Animals

Male WF rats and the corresponding untyped Wistar lean (WL) rats were obtained from RABICS Ltd (Kanagawa, Japan). All animals were housed in a room with controlled temperature (23°C), humidity (55%), and lighting (lights were on between 7:00 AM and 7:00 PM), with free access to standard laboratory chow diet (CE-2; CLEA Japan, Inc., Tokyo, Japan) and tap water. The animal care procedures and animal experimental protocols used in the present studies were approved by the Institutional Animal Care and Use Committee (Shonan Health Innovation Park), which is accredited by the American Association for Accreditation of Laboratory Animal Care.

2.3 | Two-week repeated dosing study in WF rats

Male WF rats, 31 weeks of age, with a baseline body weight of 693.5 ± 22.5 g and glycated haemoglobin level of 7.4 ± 0.4%, were randomized into five groups (vehicle, 6, 20 and 60 mg/kg SCO-792, and 1 mg/kg pioglitazone hydrochloride; n = 7), considering the initial urine albumin-to-creatinine ratio (UACR), glycated haemoglobin, plasma glucose, plasma insulin and body weight. WL rats were used as controls (n = 5). Rats were orally administered either vehicle (0.5% [w/v] methylcellulose), SCO-792, or pioglitazone hydrochloride once daily for 15 days. The first treatment day was designated as day 0. Body weight and food intake were monitored every 1 to 4 days, and faeces were collected on day 7. Blood samples were collected before randomization and on day 14 to assess blood variables. Urine samples were collected before randomization and on days 3, 7 and 13 to measure urine variables. On day 15, kidney tissues were isolated from the anaesthetized rats and stored at −80°C until use.

2.4 | Measurement of glomerular filtration rate

Male WF rats, 30 weeks of age, were randomized into two groups (n = 7) based on their initial glomerular filtration rate (GFR), UACR, glycated haemoglobin and body weight and were then orally administered vehicle (0.5% methylcellulose) or 60 mg/kg SCO-792 for 4 days. WL rats were used as controls (n = 5). GFR was measured using

fluorescein isothiocyanate (FITC)-inulin (F3272; Sigma-Aldrich, St Louis, Missouri). FITC-inulin dissolved in saline was injected into the rat tail veins at 36 mg/kg, and blood was collected at 20, 40, 60 and 80 minutes after injection to measure FITC-inulin concentration. GFR was calculated from FITC-inulin clearance.[17]

2.5 | Evaluation of AA supplementation in WF rats

Male WF rats, 24 weeks of age, were randomized into four groups (n = 5) based on their initial UACR, glycated haemoglobin, plasma glucose and body weight. Rats received either a powder CE-2 diet or diet including a 51.8% increase in AAs (at the same ratio of CE-2) following supplementation with 18 L-AAs (aspartic acid, threonine, serine, glutamic acid, glycine, alanine, valine, isoleucine, leucine, tyrosine, phenylalanine, lysine, histidine, arginine, proline, cysteine, methionine and tryptophan). Either vehicle (0.5% methylcellulose) or 60 mg/kg SCO-792 was orally administered to the rats once daily for 11 days. To evaluate changes in the plasma AA levels, blood samples were collected before and at 3, 8 and 13 hours after SCO-792 administration on day 9. Urine samples were collected on day 11 to measure UACR.

2.6 | Measurement of glomerular autophagy markers

Male WF rats, 29 weeks of age, were randomized into two groups based on their UACR, glycated haemoglobin level, plasma glucose level and body weight (n = 7) and were then orally administered vehicle (0.5% methylcellulose) or 60 mg/kg SCO-792. After 3 hours, kidney tissues were isolated from the anaesthetized rats, and then the glomeruli were isolated by a sieving method.[18] The levels of target proteins, including LC3A/B, p62, p-S6, S6, Wilms tumour 1 (WT1) and β-actin, in the glomeruli were detected by Western blotting.

2.7 | Four-week combination treatment of SCO-792 with irbesartan in WF rats

Male WF rats, 32 weeks of age, with a baseline body weight of 675.7 ± 31.2 g and glycated haemoglobin of 7.0% ± 0.3% were randomized into six groups (vehicle, 0.02% SCO-792, 0.05% SCO-792, 15 mg/kg irbesartan, 0.02% SCO-792 plus 15 mg/kg irbesartan, and 0.05% SCO-792 plus 15 mg/kg irbesartan; n = 7) based on their UACR, glycated haemoglobin, plasma glucose and body weight. WL rats were used as normal controls (n = 5). Rats had free access to the CE-2 powder diet containing each compound (w/w: 0.02 and 0.05% SCO-792) or CE-2 powder diet alone. Either vehicle (0.5% methylcellulose) or irbesartan (15 mg/kg) was orally administered once daily. Blood samples were collected on day 28 to determine blood variables. Urine samples were collected before randomization, and on days 7 and 30 to determine urine variables. Kidney tissues were isolated from the anaesthetized rats on day 33 and stored at −80°C until use.

2.8 | Statistical analyses

Statistical significance was first analysed using Bartlett's test for homogeneity of variances, followed by Williams' test ($P > 0.05$) and Shirley-Williams test ($P \leq 0.05$) for dose-dependent studies, Dunnett's test ($P > 0.05$) and Steel test ($P \leq 0.05$) for multiple comparisons versus a control, or Tukey's test ($P > 0.05$) for all pairwise comparisons. Alternatively, statistical significance was analysed using the F test for homogeneity of variances, followed by Student's *t*-test ($P > 0.2$) or an Aspin-Welch test ($P \leq 0.2$). Williams' and Shirley-Williams tests were conducted using a one-tailed significance level of 2.5% (0.025). Other tests were conducted using two-tailed significance levels of 1% (0.01) and 5% (0.05). To evaluate if combination treatment with SCO-792 and irbesartan had significant additive or synergistic effects, two-way ANOVA was performed, which provided the main effects and interaction effect of SCO-792 and irbesartan. All data are presented as mean ± SD.

Methods for quantifying faecal protein content, measuring blood and urine variables, analysing gene expression, isolating the glomerulus, conducting Western blotting, and measuring renal collagen content are described in Appendix S1.

3 | RESULTS

3.1 | SCO-792 improved metabolic variables in WF rats

To evaluate the anti-DKD potential of enteropeptidase inhibition, SCO-792 was orally administered to WF rats once daily for 2 weeks. The WF rats were generated by crossbreeding Wistar Kyoto rats and Zucker fatty rats[19] and are ideal for use as a DKD model because of their representative metabolic phenotypes (hyperglycaemia, insulin resistance with severe obesity, progressive proteinuria from the age of 12 weeks[16]). Faecal protein levels, which are a pharmacodynamic marker of diminished protein digestion following enteropeptidase inhibition in the gut, were dose-dependently increased in SCO-792-treated rats (Figure 1A). Food intake reduction was observed during the study and cumulative food intake was significantly decreased by SCO-792 (Figure 1B). Consistently, body weight was lower in SCO-792-treated rats (Figure 1C). Diabetic variables such as plasma glucose, glycated haemoglobin, plasma insulin and plasma glucagon levels were decreased after the 2-week repeated dosing of SCO-792 (Figure 1D–G). Plasma lipid levels were also decreased by SCO-792 (Figure 1H,I).

3.2 | SCO-792 improved renal variables in WF rats

In addition to the improvement in diabetic variables, SCO-792 reduced UACR promptly and this effect was maintained during the repeated dosing (Figure 2A). In contrast, UACR remained unchanged in rats administered pioglitazone, a peroxisome proliferator-activated receptor γ agonist, despite a potent reduction in plasma glucose level

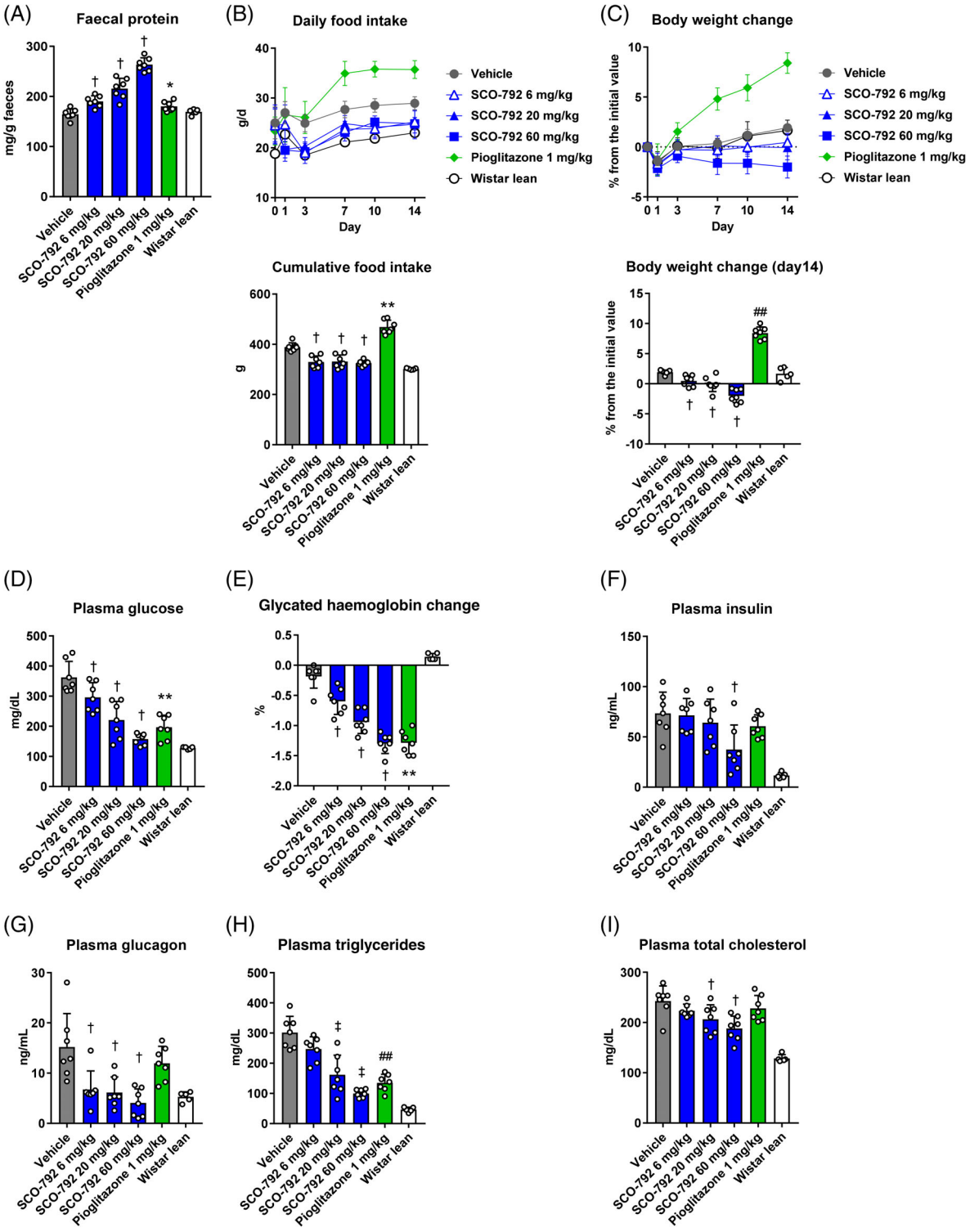

FIGURE 1 Effects of repeated administration of SCO-792 on metabolic variables in Wistar fatty (WF) rats. SCO-792 was orally administered to rats once daily for 2 weeks. **A**, Faeces were collected on day 7 and faecal protein contents were measured. **B**, Daily and cumulative food intake during the study. **C**, Percent changes in body weight from the initial values. Plasma glucose level (**D**), the changes in glycated haemoglobin level from the initial values (**E**), plasma level of insulin (**F**), glucagon (**G**), triglycerides (**H**), and total cholesterol (**I**) were measured on day 14. Data are expressed as mean ± SD (n = 7 and 5 for WF and Wistar lean rats, respectively). $^{\dagger}P$ <0.025 vs. vehicle by one-tailed Williams' test. $^{*}P$ <0.05 and $^{**}P$ <0.01 vs. vehicle by Student's *t*-test. $^{\#\#}P$ <0.01 vs. vehicle by Aspin-Welch test

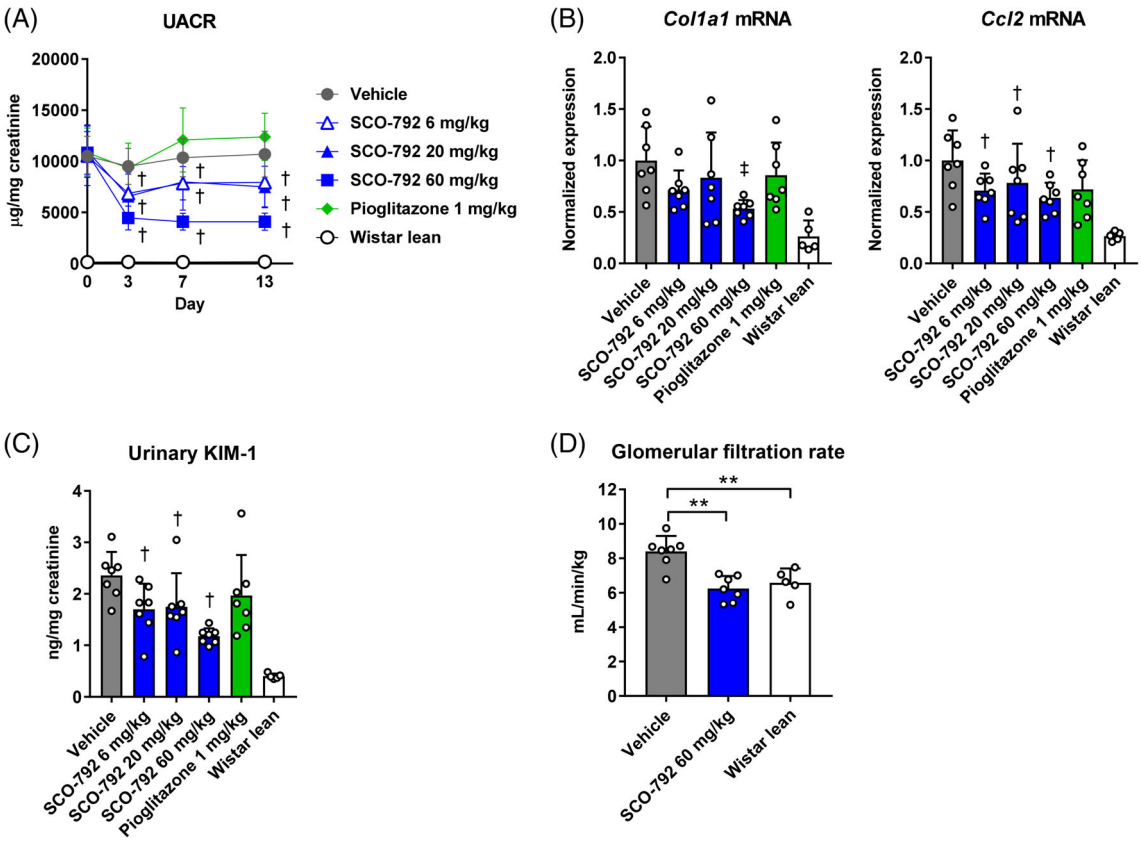

FIGURE 2 Effects of repeated administration of SCO-792 on kidney variables in Wistar fatty (WF) rats. SCO-792 was orally administered to rats once daily for 2 weeks, and urine albumin-to-creatinine ratio (UACR) was measured before administration and on days 3, 7 and 14 (**A**). *Cola1a* and *Ccl2* mRNA levels in the kidney tissues (**B**) and urinary kidney injury molecule-1 (KIM-1) protein levels (**C**) were evaluated after 2 weeks of drug administration. Glomerular filtration rate was measured using fluorescein isothiocyanate-inulin in WF rats dosed with SCO-792 for 4 days (**D**). Data are expressed as mean ± SD (n = 7 and 5 for WF and Wistar lean rats, respectively). $^{\dagger}P$ <0.025 vs. vehicle by one-tailed Williams' test. $^{\ddagger}P$ <0.025 vs. vehicle by one-tailed Shirley-Williams test. $^{**}P$ <0.01 by Tukey test

equivalent to that of SCO-792 (Figures 1D, 2A). At the end of the study, renal mRNA levels of *Col1a1*, a fibrosis marker,[20] and *Ccl2*, an inflammation marker,[21] were decreased in rats administered SCO-792 (Figure 2B). Urinary kidney injury molecule-1 (KIM-1), which is a biomarker of kidney tubular injury,[22] was also reduced in SCO-792-treated rats (Figure 2C). Measurement of GFR using FITC-inulin revealed a glomerular hyperfiltration state in WF rats compared to WL rats. However, after 4-day administration of SCO-792, the glomerular overload in WF rats was completely normalized (Figure 2D).

3.3 | AA supplementation of diet reversed UACR reduction by SCO-792 in WF rats

The observed increase in faecal protein level following SCO-792 treatment confirmed that enteropeptidase inhibition prevented protein digestion (Figure 1A). Thus, SCO-792 attenuated the generation of AAs from proteins in the gut lumen. To investigate the role of AA intake in the renoprotective effects of SCO-792, rats dosed with vehicle or SCO-792 were housed with free access to a normal chow or an AA-supplemented chow. As expected, in normal chow-fed rats, SCO-792 lowered plasma total AA level (Figure 3A,C). However, AA supplementation to the diet negated the enteropeptidase-induced change (Figure 3B,C). While plasma levels of branched-chain amino acids (BCAAs) such as valine, leucine and isoleucine were especially decreased by SCO-792 in normal chow-fed rats, again, these changes were negated by AA supplementation (Figure 3D–F). Consistent with the observed changes in plasma AA levels, SCO-792-induced UACR reduction was largely attenuated in AA-supplemented chow-fed rats (Figure 3G).

3.4 | SCO-792 activated autophagy signalling in the glomerulus of WF rats

Amino acids act as a key regulator of intracellular protein homeostasis, thus modulating the balance between anabolic (protein synthesis) and catabolic (autophagy) processes.[23] This is achieved through AA-sensing proteins, such as mammalian target of rapamycin (mTOR), and general control nonderepressible kinase 2. To examine whether SCO-792 affects an autophagy pathway in the glomerulus, expression of LC3A/B-I and LC3A/B-II proteins in the glomerulus of WF rats was

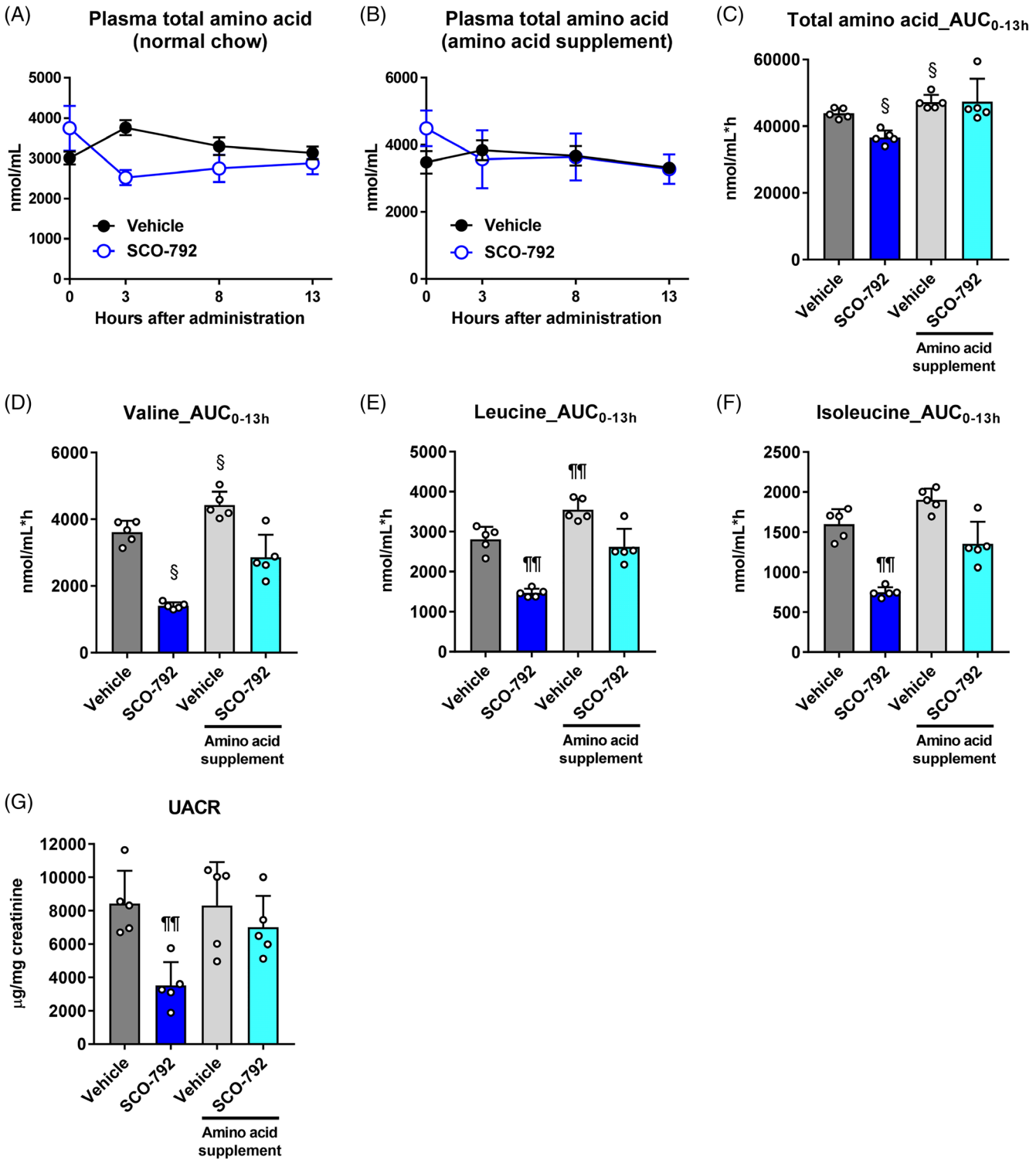

FIGURE 3 Effects of amino acid (AA) supplementation on SCO-792-induced urine albumin-to-creatinine ratio (UACR) reduction in Wistar fatty rats. SCO-792 was orally administered to rats fed a normal chow or an AA-supplemented chow. Plasma total AA concentrations were measured after the dosing of SCO-792 to normal chow-fed rats (**A**) and AA-supplemented chow-fed rats (**B**) on day 9. The area under the plasma AA concentration-time curves from 0 to 13 hours after dosing ($AUC_{0\text{-}13\ h}$) was calculated for total AAs (**C**), valine (**D**), leucine (**E**) and isoleucine (**F**). UACR was measured on day 11 (**G**). Data are expressed as mean ± SD (n = 5). $^{¶¶}P$ <0.01 vs. vehicle with normal chow by Dunnett's test. $^{§}P$ <0.05 vs. vehicle with normal chow by steel test

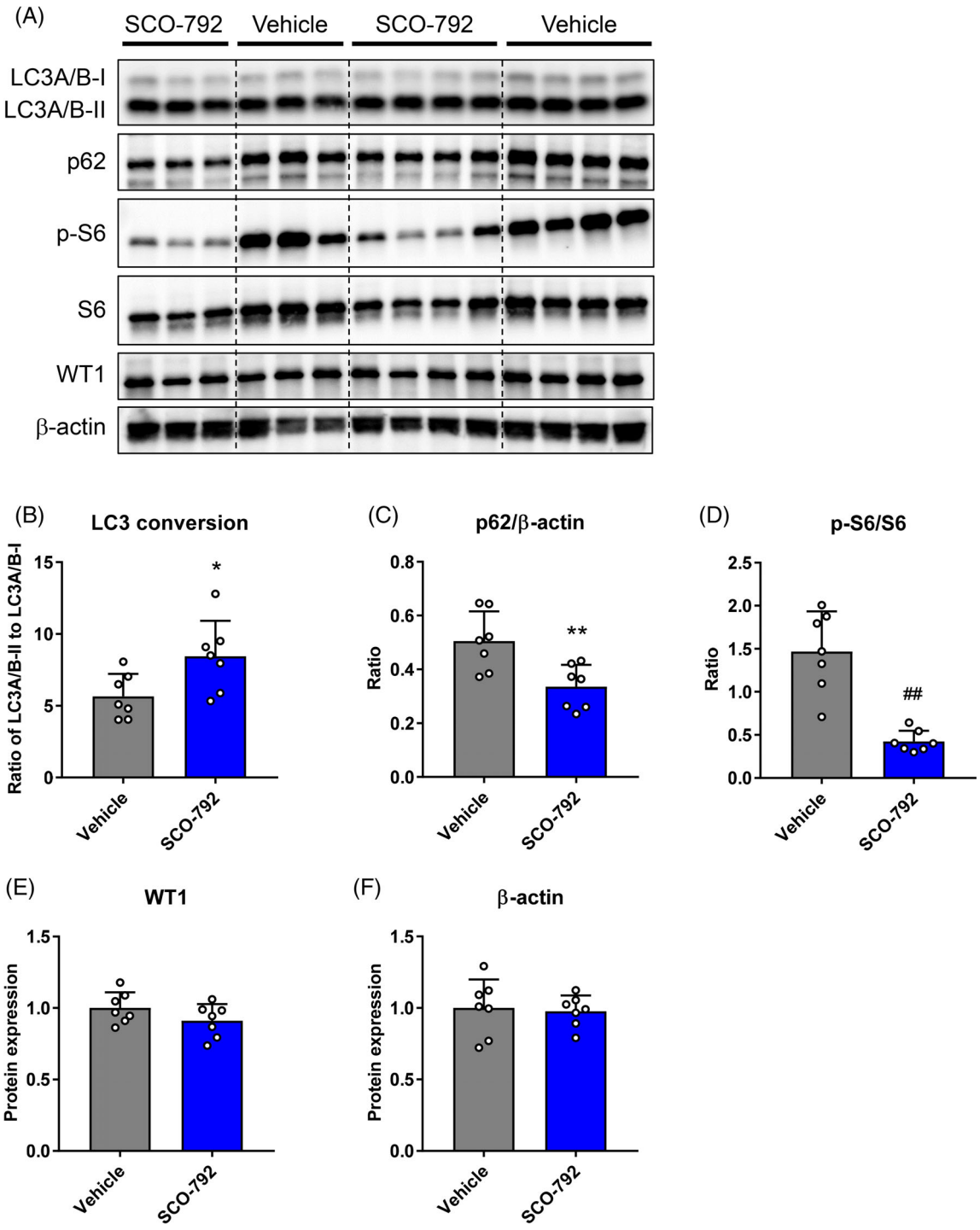

FIGURE 4 Effects of SCO-792 on glomerular autophagy activity in Wistar fatty rats. **A**, Protein expression levels in the glomeruli of rats were detected by immunoblotting, and the blots were quantified by a luminescent image analyser. **B**, LC3A/B expression was expressed as the ratio of LC3A/B-II to LC3A/B-I. **C**, The expression level of p62 was normalized to that of β-actin. **D**, Phosphorylated S6 level was normalized by the total S6 protein level. Wilms tumour 1 (WT1) (**E**) and β-actin (**F**) levels were expressed as the ratio to the vehicle. Data are expressed as mean ± SD (n = 7). $^{*}P$ <0.05 and $^{**}P$ <0.01 vs. vehicle by Student's *t*-test. $^{\#\#}P$ <0.01 vs. vehicle by Aspin-Welch test

measured by Western blotting. LC3A/B is an autophagosome marker, while LC3A/B-I is converted to LC3A/B-II by C-terminal lipidation during autophagy. Therefore, the protein expression ratio of LC3A/B-II to LC3A/B-I is used as an indicator of autophagy.[24] Here, the Western blotting analysis revealed that SCO-792 significantly increased the ratio of LC3A/B-II to LC3A/B-I in the glomerulus of WF rats (Figure 4A,B). In contrast, the protein level of p62, an alternative autophagy marker that delivers autophagic substrates to autophagosomes and is degraded by autophagy,[25] was reduced by SCO-792 (Figure 4A,C). Additionally, SCO-792 significantly decreased

the phosphorylation level of S6 protein; this indicated the inactivation of mTOR signalling (Figure 4A,D). By contrast, the expression levels of WT1, a podocyte marker, and β-actin, a loading control for Western blotting, were unchanged between the groups (Figure 4A,E,F).

3.5 | Combination of SCO-792 with irbesartan additively decreased UACR in WF rats

As RAS inhibitors are widely used as standard treatments for patients with DKD, an add-on effect to RAS inhibitors is required for novel anti-DKD drugs. To evaluate the combined effect of SCO-792 with an ARB, SCO-792 (0.02% and 0.05% in the diet) and irbesartan (15 mg/kg once daily oral dose) alone or in combination were administered to WF rats for 4 weeks. It was of interest to evaluate the efficacy of sustained enteropeptidase inhibition on disease variables; hence diet-admix administration of SCO-792 was conducted in this study. The calculated SCO-792 doses (mg/kg/d) for each group were 6.81 for the 0.02% SCO-792 group, 13.10 for the 0.05% SCO-792 group, 6.59 for the 0.02% SCO-792 plus irbesartan group, and 11.44 for the 0.05% SCO-792 plus irbesartan group. While SCO-792 alone and irbesartan alone decreased UACR, a combination of SCO-792 and irbesartan further decreased UACR. A significant additive effect on UACR reduction was observed following the administration of SCO-792 in combination with irbesartan (Figure 5A,B). In contrast, irbesartan did not affect the anti-diabetic or anti-obese effect achieved by SCO-792 (Figure 5C-F). Notably, SCO-792 significantly reduced the renal collagen content (Figure 5G).

4 | DISCUSSION

In the present study, we evaluated the effects of a novel enteropeptidase inhibitor, SCO-792, on disease state in a preclinical rat model of DKD. Oral administration of SCO-792 to WF rats promptly alleviated albuminuria after only 3 days of treatment, and this effect was maintained throughout the drug administration period. Moreover, this effect was probably glucose control-independent. Additionally, a decrease in fibrosis, inflammation and tubular injury markers in the kidney was observed in SCO-792-treated rats. SCO-792 dosing induced additional kidney protective effects, including relief of glomerular hyperfiltration and activation of autophagy in the glomerulus. An SCO-792-induced reduction of UACR was largely attenuated by AA supplementation of the diet. Finally, combining SCO-792 and irbesartan effectively decreased UACR.

Amino acids are a known inducer of hyperfiltration.[26] In humans, a single protein-rich meal or an AA infusion transiently increases GFR.[27,28] The hyperfiltration state caused by a protein-rich diet is presumed to accelerate the progressive decline in renal function in patients with CKD.[29] In the present study, WF rats showed elevated GFR, indicating glomerular hyperfiltration that likely elevates UACR. While SCO-792 treatment reduced plasma AA levels and decreased UACR in WF rats, this effect was negated after AA supplementation to the diet. In addition, SCO-792 treatment normalized GFR in WF rats. Taken together, the SCO-792-induced decrease in circulating AAs may play a role in ameliorating glomerular hyperfiltration and reducing UACR in WF rats. Furthermore, SCO-792 improved fibrosis, inflammation and tubular injury markers in the kidney. Excess protein in the glomerular filtrate may induce renal inflammation and interstitial fibrosis in proximal tubular cells.[4] Mitigation of albuminuria might therefore protect glomerular and renal tubular cells in SCO-792-treated rats from the progression of renal impairment.

The changes in LC3AB-II to LC3AB-I ratio and p62 protein level demonstrated SCO-792-induced autophagy activation in the glomerular fraction of the kidney tissues in WF rats. Interestingly, SCO-792 acutely decreased plasma BCAA levels in WF rats. BCAAs, especially leucine, are key regulators of the autophagy pathway with mTOR signalling, and inhibit the formation of autophagosomes.[23,30] In fact, the phosphorylation of S6 protein, which is a downstream signalling molecule of mTOR, was potently inhibited by SCO-792, indicating that the decreased plasma BCAA levels by enteropeptidase inhibition may be involved in glomerular autophagy activation via the inactivation of the mTOR pathway. Autophagy is generally a response to nutrient deprivation, but it is also involved in various physiological processes and disease development, including kidney diseases.[31] In the glomerulus, autophagy is necessary to protect podocytes from endoplasmic reticulum stress and to maintain podocyte function, and thus, there is a high basal level of autophagy activity in podocytes.[32] An impairment of podocyte-specific autophagy results in proteinuria, indicating the pivotal role of autophagy in the glomerular filtration machinery.[32,33] Hence, the activation of glomerular autophagy might be one of the AA-related mechanisms by which SCO-792 improved albuminuria in WF rats.

Chronic hyperglycaemia is reported to be involved in the pathogenesis of DKD by inducing the activation of the polyol pathway, generation of advanced glycation endproducts, oxidative stress, and inflammation.[34] In the present study, pioglitazone, which improved hyperglycaemia, failed to decrease UACR in WF rats. This suggests that improving hyperglycaemia is not a main factor in the prompt UACR reduction by SCO-792 in this model. RAS is a crucial pathway for the progression of renal failure, and the beneficial effect of its inhibition with ARBs in patients with DKD is well established.[5] A combination study revealed that time to onset of UACR-lowering is different between SCO-792 and irbesartan, and that these compounds acted additively to improve UACR in WF rats. These results indicate that the mechanism underlying the anti-albuminuria effect of SCO-792 is RAS-independent. WF rats are normotensive and SCO-792 had no effect on blood pressure in WF rats; hence, the observed effect of SCO-792 may not depend on blood pressure control (Figure S1). Therefore, SCO-792 may be used as an add-on therapy to RAS inhibitors in any future clinical setting. Furthermore, sustained inhibition of enteropeptidase has been suggested to be more effective in treating disease variables because diet-admix administration of SCO-792 showed more potent therapeutic efficacy at lower daily dose than once-daily oral dosing.

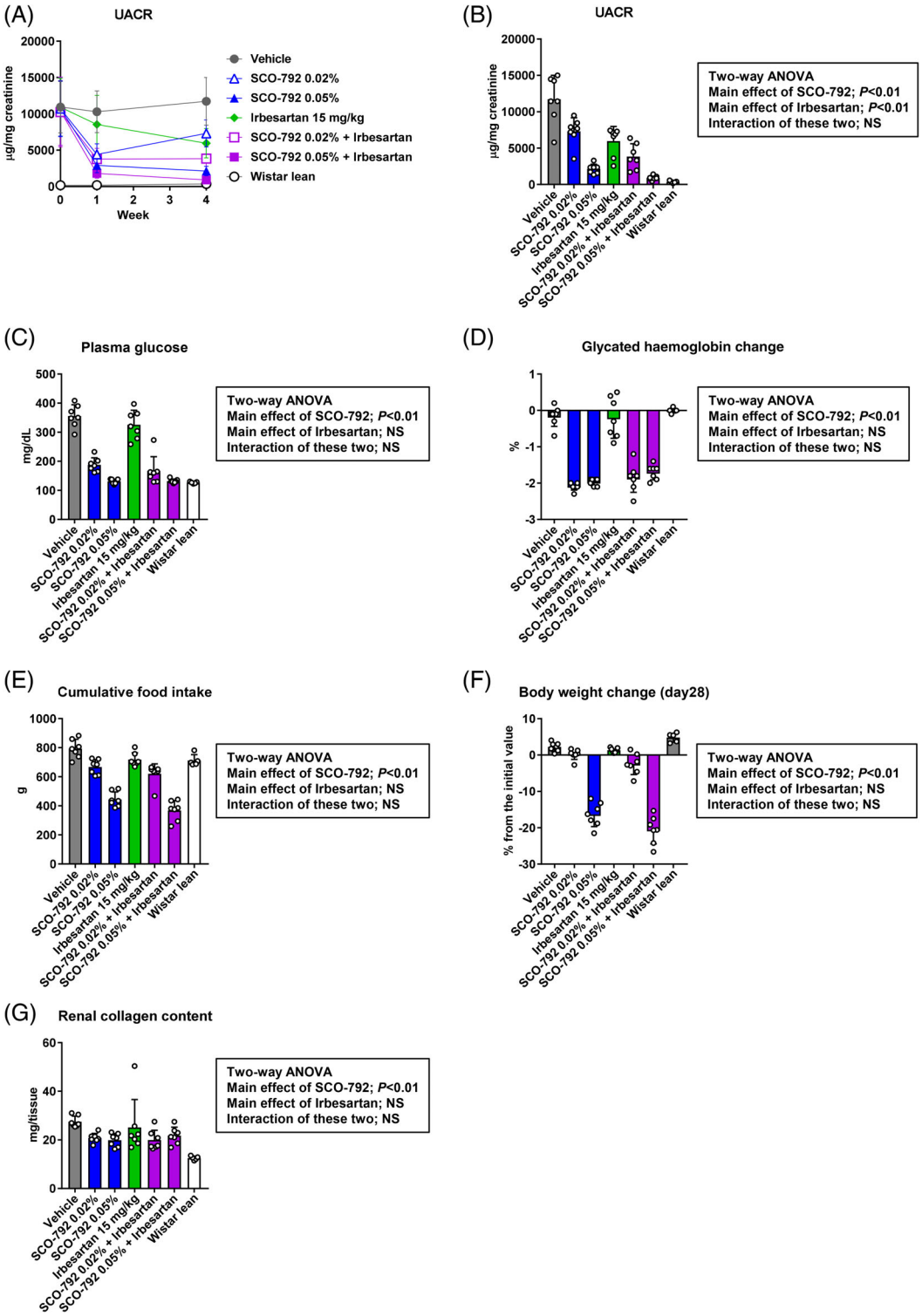

FIGURE 5 Effects of 4-week combination treatment of SCO-792 and irbesartan on kidney and diabetic variables in Wistar fatty (WF) rats. Urine albumin-to-creatinine ratio (UACR) during the study (**A**) and on day 30 (**B**). Plasma glucose level (**C**) and the changes in glycated haemoglobin level from the initial values (**D**) were measured on day 28. Cumulative food intake during the study (**E**). Percent changes in body weight from the initial values (**F**). Renal collagen content on day 33 (**G**). The results of a two-way ANOVA are presented in the figure inserts. Data are expressed as mean ± SD (n = 7 and 5 for WF and Wistar lean rats, respectively)

As mentioned above, plasma BCAA levels were particularly decreased among AAs by SCO-792 in WF rats. The relationships between metabolic diseases and BCAAs have been extensively investigated. For instance, a relationship between BCAAs and insulin sensitivity has been suggested; thus, obese people showed higher plasma BCAA levels than healthy subjects, and the levels were

correlated with insulin resistance.[35] Additionally, protein ingestion induces muscular insulin resistance in humans.[36] Therefore, plasma BCAA reduction caused by the administration of SCO-792 may improve insulin resistance and ameliorate the diabetic phenotype in WF rats. This is consistent with previous study results in diabetic mice which indicate that SCO-792 increased insulin sensitivity (mainly in the muscle) in a hyperinsulinaemic-euglycaemic clamp study.[15] Moreover, the decreased AA levels by SCO-792 may be involved in plasma glucagon reduction via a mutual feedback cycle between glucagon and AAs.[37] Interestingly, the potent improvement in diabetic variables was observed even at the dose that had only minor effects on body weight in the 2-week repeated dosing study of SCO-792. This indicates AA-dependent mechanisms including the improvement in insulin resistance and the reduction in plasma glucagon levels are likely to be involved in the anti-diabetic effect of SCO-792 besides the body weight reduction-dependent action.

The main limitation of the present study is that the effect of SCO-792 on the late phase of DKD was not evaluated. WF rats showed albuminuria with elevated GFR, a disease condition representing a relatively early phase of DKD. To address this limitation, other CKD models presenting a progressive decline in GFR would be required for the evaluation of long-term renal outcome following SCO-792 treatment. Recently, the gut–kidney axis has become one of the topics of interest, and the association of gut microbiota and its metabolites with kidney diseases has been investigated.[38] Considering the physiological role of enteropeptidase, its inhibition may modulate gut microbiota composition. Therefore, additional analyses focusing on SCO-792-induced changes in the microbiota are also needed.

In conclusion, this is the first study to demonstrate that the enteropeptidase inhibitor SCO-792 effectively improves kidney variables in rat models of DKD. The inhibition of protein breakdown in the gut, followed by decreased AA absorption, is likely to be a major pathway for the renoprotective effects of SCO-792, effecting relief of glomerular hyperfiltration and activation of autophagy in the glomerulus. Taken together, the results suggest that SCO-792 has potential as a novel therapeutic option for patients with DKD.

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AUTHOR CONTRIBUTIONS

The research was designed by all authors. Experiments were conducted by J.S. and Y.K. Data were analysed and interpreted by all authors. The manuscript was written by J.S. and Y.M., and important intellectual contents of the manuscript were reviewed and revised by all authors. All authors have agreed to be accountable for all aspects of the work, and ensuring that questions related to the accuracy or integrity of any part of the work were appropriately investigated and resolved.

CONFLICTS OF INTEREST

J.S., Y.K., Y.M. and M.W. are employees of SCOHIA PHARMA, Inc.

PEER REVIEW

The peer review history for this article is available at https://publons.com/publon/10.1111/dom.14190.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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REFERENCES

1. Khalil H. Diabetes microvascular complications-A clinical update. *Diabetes Metab Syndr*. 2017;11(Suppl 1):S133-S139.
2. Doshi SM, Friedman AN. Diagnosis and Management of Type 2 diabetic kidney disease. *Clin J Am Soc Nephrol*. 2017;12(8):1366-1373.
3. Bhattacharjee N, Barma S, Konwar N, Dewanjee S, Manna P. Mechanistic insight of diabetic nephropathy and its pharmacotherapeutic targets: an update. *Eur J Pharmacol*. 2016;791:8-24.
4. Vallon V. The proximal tubule in the pathophysiology of the diabetic kidney. *Am J Physiol Regul Integr Comp Physiol*. 2011;300(5):R1009-R1022.
5. Perico N, Ruggenenti P, Remuzzi G. ACE and SGLT2 inhibitors: the future for non-diabetic and diabetic proteinuric renal disease. *Curr Opin Pharmacol*. 2017;33:34-40.
6. Perkovic V, Jardine MJ, Neal B, et al. Canagliflozin and renal outcomes in type 2 diabetes and nephropathy. *N Engl J Med*. 2019;380(24):2295-2306.
7. Zelniker TA, Wiviott SD, Raz I, et al. SGLT2 inhibitors for primary and secondary prevention of cardiovascular and renal outcomes in type 2 diabetes: a systematic review and meta-analysis of cardiovascular outcome trials. *Lancet*. 2019;393(10166):31-39.
8. Kitada M, Ogura Y, Monno I, Koya DA. Low-protein diet for diabetic kidney disease: its effect and molecular mechanism, an approach from animal studies. *Nutrients*. 2018;10(5). https://doi.org/10.3390/nu10050544.
9. Metzger M, Yuan WL, Haymann JP, et al. Association of a low-Protein Diet with Slower Progression of CKD. *Kidney Int Rep*. 2018;3(1):105-114.
10. Fouque D, Mitch WE. Low-protein diets in chronic kidney disease: are we finally reaching a consensus? *Nephrol Dial Transplant*. 2015;30(1):6-8.
11. Light A, Janska H. Enterokinase (enteropeptidase): comparative aspects. *Trends Biochem Sci*. 1989;14(3):110-112.
12. Mann NS, Mann SK. Enterokinase. *Proc Soc Exp Biol Med*. 1994;206(2):114-118.
13. Zheng XL, Kitamoto Y, Sadler JE. Enteropeptidase, a type II transmembrane serine protease. *Front Biosci*. 2009;1:242-249.
14. Sasaki M, Miyahisa I, Itono S, et al. Discovery and characterization of a small-molecule enteropeptidase inhibitor, SCO-792. *Pharmacol Res Perspect*. 2019;7(5):e00517.
15. Yashiro H, Hamagami K, Hiyoshi H, et al. SCO-792, an enteropeptidase inhibitor, improves disease status of diabetes and obesity in mice. *Diabetes Obes Metab*. 2019;21(10):2228-2239.

16. Imai G, Satoh T, Kumai T, et al. Hypertension accelerates diabetic nephropathy in Wistar fatty rats, a model of type 2 diabetes mellitus, via mitogen-activated protein kinase cascades and transforming growth factor-beta1. *Hypertens Res*. 2003;26(4):339-347.
17. Isaka Y, Fujiwara Y, Yamamoto S, et al. Modified plasma clearance technique using nonradioactive iothalamate for measuring GFR. *Kidney Int*. 1992;42(4):1006-1011.
18. Rush BM, Small SA, Stolz DB, Tan RJ. An efficient sieving method to isolate intact glomeruli from adult rat kidney. *J Vis Exp*. 2018;141. https://doi.org/10.3791/58162.
19. Ikeda H, Shino A, Matsuo T, Iwatsuka H, Suzuoki Z. A new genetically obese-hyperglycemic rat (Wistar fatty). *Diabetes*. 1981;30(12):1045-1050.
20. Bisgaard LS, Bosteen MH, Fink LN, et al. Liraglutide reduces both atherosclerosis and kidney inflammation in moderately uremic LDLr−/− mice. *PLoS One*. 2016;11(12):e0168396.
21. Tesch GH. MCP-1/CCL2: a new diagnostic marker and therapeutic target for progressive renal injury in diabetic nephropathy. *Am J Physiol Renal Physiol*. 2008;294(4):F697-F701.
22. Zhao X, Zhang Y, Li L, et al. Glomerular expression of kidney injury molecule-1 and podocytopenia in diabetic glomerulopathy. *Am J Nephrol*. 2011;34(3):268-280.
23. Carroll B, Korolchuk VI, Sarkar S. Amino acids and autophagy: cross-talk and co-operation to control cellular homeostasis. *Amino Acids*. 2015;47(10):2065-2088.
24. Kabeya Y, Mizushima N, Yamamoto A, Oshitani-Okamoto S, Ohsumi Y, Yoshimori T. LC3, GABARAP and GATE16 localize to autophagosomal membrane depending on form-II formation. *J Cell Sci*. 2004;117(Pt 13):2805-2812.
25. Liu WJ, Ye L, Huang WF, et al. p62 links the autophagy pathway and the ubiqutin-proteasome system upon ubiquitinated protein degradation. *Cell Mol Biol Lett*. 2016;21:29.
26. Bankir L, Roussel R, Bouby N. Protein- and diabetes-induced glomerular hyperfiltration: role of glucagon, vasopressin, and urea. *Am J Physiol Renal Physiol*. 2015;309(1):F2-F23.
27. Hadj-Aissa A, Bankir L, Fraysse M, et al. Influence of the level of hydration on the renal response to a protein meal. *Kidney Int*. 1992;42(5):1207-1216.
28. Giordano M, Castellino P, McConnell EL, DeFronzo RA. Effect of amino acid infusion on renal hemodynamics in humans: a dose-response study. *Am J Physiol*. 1994;267(5 Pt 2):F703-F708.
29. Ko GJ, Obi Y, Tortorici AR, Kalantar-Zadeh K. Dietary protein intake and chronic kidney disease. *Curr Opin Clin Nutr Metab Care*. 2017;20(1):77-85.
30. Son SM, Park SJ, Stamatakou E, Vicinanza M, Menzies FM, Rubinsztein DC. Leucine regulates autophagy via acetylation of the mTORC1 component raptor. *Nat Commun*. 2020;11(1):3148.
31. Lenoir O, Tharaux PL, Huber TB. Autophagy in kidney disease and aging: lessons from rodent models. *Kidney Int*. 2016;90(5):950-964.
32. Hartleben B, Godel M, Meyer-Schwesinger C, et al. Autophagy influences glomerular disease susceptibility and maintains podocyte homeostasis in aging mice. *J Clin Invest*. 2010;120(4):1084-1096.
33. Chen J, Chen MX, Fogo AB, Harris RC, Chen JK. mVps34 deletion in podocytes causes glomerulosclerosis by disrupting intracellular vesicle trafficking. *J Am Soc Nephrol*. 2013;24(2):198-207.
34. Magee C, Grieve DJ, Watson CJ, Brazil DP. Diabetic nephropathy: a tangled web to unweave. *Cardiovasc Drugs Ther*. 2017;31(5–6):579-592.
35. Badoud F, Lam KP, DiBattista A, et al. Serum and adipose tissue amino acid homeostasis in the metabolically healthy obese. *J Proteome Res*. 2014;13(7):3455-3466.
36. Smith GI, Yoshino J, Stromsdorfer KL, et al. Protein ingestion induces muscle insulin resistance independent of leucine-mediated mTOR activation. *Diabetes*. 2015;64(5):1555-1563.
37. Holst JJ, Wewer Albrechtsen NJ, Pedersen J, Knop FK. Glucagon and amino acids are linked in a mutual feedback cycle: the liver-alpha-cell Axis. *Diabetes*. 2017;66(2):235-240.
38. Yang T, Richards EM, Pepine CJ, Raizada MK. The gut microbiota and the brain-gut-kidney axis in hypertension and chronic kidney disease. *Nat Rev Nephrol*. 2018;14(7):442-456.

SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of this article.

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