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The dipeptidyl peptidase-4 inhibitor teneligliptin reduces kidney damage from hypercholesterolemia in apolipoprotein E-deficient mice

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Hypercholesterolemia is a well-established risk factor for kidney injury that can lead to chronic kidney disease (CKD). Many clinic data show that dipeptidyl peptidase-4 (DPP-4) inhibitor is protective against kidney damage in diabetes patients. The goal of this study was to investigate the possible protective effects of teneligliptin against kidney injury in apolipoprotein E knockout ($ApoE^{-/-}$) mice. Eight-week-old male $ApoE^{-/-}$ mice were randomly divided into the following 3 groups: a control group fed a normal diet (ND group), a group fed a high cholesterol diet (HD group) or a group fed HD mixed with teneligliptin (HD + Tene group). All groups received different treatments for 6 weeks. The metabolic characteristics of total cholesterol (TC), low-density lipoprotein-cholesterol (LDL-c) and creatinine (Cre) were lower in $ApoE^{-/-}$ HD + Tene mice than $ApoE^{-/-}$ HD mice. Oil-red O staining revealed excessive lipid deposition in the kidneys of $ApoE^{-/-}$ HD mice; however, it was significantly suppressed in the $ApoE^{-/-}$ HD + Tene mice. Lectin-like oxidized low-density lipoprotein receptor-1 (LOX-1) gene and protein expression was lower in the kidney tissue of $ApoE^{-/-}$ HD + Tene mice than $ApoE^{-/-}$ HD mice. These results indicate that teneligliptin may provide a potential therapeutic target for kidney damage from hypercholesterolemia.

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Introduction

$ApoE^{-/-}$ mice are considered a well-accepted model of hypercholesterolemia, and they have been used extensively to study the effects of this disease on atherosclerosis and renal injury.[1] In $ApoE^{-/-}$ mice, dyslipidaemia-related kidney injury is associated with marked pathological alterations, including lipid deposition in the glomerulus, mesangial expansion, and an increased extracellular matrix (ECM) area.[2,3] Increasing evidence has shown that lipid accumulation in the kidney contributes to the progression of chronic kidney disease (CKD).[4,5] However, the underlying pathophysiological mechanisms of the relationship between hypercholesterolemia and renal injury are not yet fully understood.

Dipeptidyl peptidase-4 (DPP-4), also known as lymphocyte cell surface marker CD26, exists as a smaller soluble form in blood plasma. DPP-4 is widely expressed on T and B cells, subsets of macrophages, haematopoietic stem cells, and haematopoietic progenitor cells as well as on epithelial, endothelial, and acinar cells in a variety of tissues.[6,7] The complex biological roles of DPP-4 include cell membrane-associated activation of intracellular signal transduction pathways, cell-to-cell interaction, and enzymatic activity.[8] DPP-4 inhibitors have recently emerged as a new class of anti-diabetic drug that have favourable results in improving glycaemic control with a minimal risk of hypoglycaemia and weight gain. Teneligliptin is a novel DPP-4 inhibitor for use in managing type 2 diabetes mellitus.[9] Many clinical data suggest that teneligliptin protects against diabetic nephropathy injury.[10] Meta-analyses have suggested a potential beneficial effect of DPP-4 inhibitors on cholesterol, which could reduce the cardiovascular risk.[11,12] However, the function of teneligliptin in hypercholesterolemia-induced renal injury is unclear.

Materials and methods

Animals and experimental protocols

All animal studies were approved by the Animal Studies Committee of the first affiliated hospital of Dalian Medical

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University. ApoE$^{-/-}$ mice were purchased from Shanghai Slac Laboratory Animal Co., Ltd. (Shanghai, China). All mice were housed in a room with a 12 : 12 h light–dark cycle with a temperature maintained at 24 °C. At 8 weeks old, the male mice were randomly divided into the following three groups: ApoE$^{-/-}$ mice fed a normal diet ($n = 7$) or a high-cholesterol diet ($n = 6$) and ApoE$^{-/-}$ mice fed teneligliptin (20 mg kg^{-1} d^{-1}; Mitsubishi Tanabe Pharma, Osaka, Japan) + a high-cholesterol diet ($n = 7$). The high-cholesterol diet contained 1.5% cholesterol and 15% fat. The experimental diet was purchased from the Shanghai Slac Laboratory Animal Co., Ltd. (Shanghai, China). Each group was fed their diet for 6 weeks. Blood samples were obtained from the inferior vena cava and collected in serum tubes; they were then stored at −80 °C until use. Coronal sections of the kidneys were fixed in 10% formalin and then embedded in paraffin for histological evaluation or embedded in OCT compound (Torrance, CA, USA) and stored at −80 °C for oil-red O staining. The remainder of the kidney was snap-frozen in liquid nitrogen for mRNA or immunohistochemical analysis. All animal experiments were performed in accordance with the Guide for the Care and Use of Laboratory Animals. The study was approved by the ethical committee of the first affiliated hospital of Dalian Medical University.

Biochemical measurements

TC, LDL-c and Cre were measured using an automatic analyser (Dimension, Wilmington, DE, USA).

Morphologic analysis and immunohistochemistry

Kidney samples were collected and either fixed in 4% paraformaldehyde or snap frozen in liquid nitrogen. Samples were embedded in paraffin or OCT and were cut into slices using a microtome (Leica RM 2235 or Leica CM1850UV; Leica, Solms, Germany). Slices were then mounted onto glass slides and histological examinations were performed. Frozen sections were used to evaluate lipid deposition using oil-red O staining (Sigma, Santa Clara, CA, USA).

Immunohistochemistry was performed using Histone Simple stain kits (Nichirei, Tokyo, Japan) according to the manufacturer's instructions. Briefly, paraffin-embedded sections were deparaffinised with xylene and then rehydrated in a descending series of ethanol washes. The sections were treated for 15 min with 3% H_2O_2 in methanol to inactivate endogenous peroxidases and were then incubated at room temperature for 1 hour with primary antibodies for collagen IV (rabbit anti-collagen IV antibody, 1 : 500; Abcam, England) or LOX-1 (rabbit anti-LOX-1 antibody, 1 : 250; Abcam). All sections were observed under an Olympus B ×40 upright light microscope (Olympus, Tokyo, Japan).

RNA isolation and real-time RT-PCR

Total RNA was isolated from renal cortical tissues using the ISOGEN (Nippon gene, Tokyo, Japan) according to the manufacturer's protocol. Complementary DNA (cDNA) was synthesized from total RNA using a first-strand cDNA synthesis kit (SuperScript VILO cDNA Synthesis Kit; Life Technologies Carlsbad, CA, USA) according to the manufacturer's protocol. Gene expression was quantitatively analysed by real-time RT-PCR using fluorescent SYBR Green technology (Light Cycler; Roche Molecular Biochemicals). β-Actin cDNA was amplified and quantitated in each cDNA to normalize the relative amounts of the target genes. Primer sequences are listed in Table 1.

Western blot for kidney tissue

Proteins were extracted from renal cortical tissues using radioimmunoprecipitation assay buffer (P0013B; Beyotime, Shanghai, China). Samples were electrophoresed on 10% SDS-PAGE gel and proteins were transferred to polyvinylidene fluoride membrane (Immobilon, Millipore, Billerica, MA, USA). Membranes were blocked in Tris-buffered saline with 0.1% Tween-20 (TBS-T) containing 5% skim milk and then were incubated in primary antibody diluents (P0023A; Beyotime) and gently shaken overnight at 4 °C. Primary antibodies against LOX-1 (rabbit anti-LOX-1 antibody, 1 : 250; Abcam), phospho-PKC (rabbit anti-phospho-PKC, 1 : 1000; Cell Signaling Technology), and anti-β-actin (1 : 1000; Cell Signaling Technology). Membranes were then incubated with secondary antibody (anti-rabbit Ig-G, 1 : 1000; Cell Signaling Technology for 1 hour). This analysis was performed independently three times. Protein levels are expressed as the protein/β-actin ratios to minimize loading differences. The relative signal intensity was quantified using NIH ImageJ software.

Statistical analysis

All data were presented as the mean ± SEM. Statistical analysis was performed using SPSS software version 23.0 (SPSS Inc., Chicago, IL, USA). Inter-group variation was measured by one-way ANOVA and subsequent Tukey's test. The minimal level for statistical significance was $P < 0.05$.

Results

Metabolic characteristics

The metabolic characteristics of ApoE$^{-/-}$ mice after 6 weeks of dietary treatment are summarized in Table 2. The HD group

Table 1 Primer oligonucleotide sequences[a]

Gene	Primers
LOX-1	F: 5′-CAAAGTCTCCCAACCAACCTGCAA-3′
	R: 5′-ACATCCTGTCTTTCATGCGGCAAC-3′
β-Actin	F: 5′-CGATGCCCTGAGGGTCTTT-3′
	R: 5′-TGGATGCCACAGGATTCCAT-3′
SR–A	F: 5′-GTTAAAGGTGATGGGGGACA-3
	R: 5′-TCCCCTTCTCTCCCTTTTGT-3′
CD36	F: 5′-CCTTAAAGGAATCCCCGTGT-3′
	R: 5′-TGCATTTGCCAATGTCTAGC-3″
ABCA1	F: 5′-AGCCAGAAGGGAGTGTCAGA-3′
	R: 5′-CATGCCATCTGGGTAAACCT-3′

[a] Abbreviations: LOX-1, lectin-like oxidized low-density lipoprotein receptor-1; SR–A, scavenger receptor-A; ABCA1, ATP-binding cassette transporter A1.

Table 2 Metabolic data from the three groups after 6 weeks of dietary treatment[a]

	$ApoE^{-/-}$ ND	$ApoE^{-/-}$ HD	$ApoE^{-/-}$ HD + Tene
	$n = 7$	$n = 6$	$n = 7$
Body weight (g)	24.29 ± 0.51	23.65 ± 0.42	24.63 ± 0.86
Kidney weight (mg)	178.65 ± 7.21	158.32 ± 8.16	176.1 ± 7.03
T-cholesterol (mg dl^{-1})	595.33 ± 72.07*	2505 ± 386.72	830.63 ± 64.29*
LDL-c (mg dl^{-1})	147.17 ± 36.78*	677.5 ± 105.16	284 ± 13.86*
CRE (mg dl^{-1})	126.37 ± 17.32*	352.8 ± 37.26	167.15 ± 19.35*

[a] Abbreviations: TC, total cholesterol; LDL-c, low-density lipoprotein cholesterol; CRE, creatinine. Data are means ± SEM; $n = 6$–7 per group. *$P <$ 0.01 *vs.* $ApoE^{-/-}$ HD.

showed a marked increased in total cholesterol and low-density lipoprotein levels in $ApoE^{-/-}$ mice, but these were significantly decreased in the HD + Tene group. There was no difference between the HD + Tene and ND groups. Body weights and kidney weights did not differ among the three groups. Creatinine was significantly decreased in the HD + Tene group compared with the HD group.

Teneligliptin reduced renal lipid accumulation in $ApoE^{-/-}$ HD group mice

We used oil-red O staining to evaluate renal lipid accumulation. We detected increased lipid retention in the kidneys of $ApoE^{-/-}$ HD group mice. Interestingly, the HD + Tene group mice showed markedly reduced renal lipid deposition compared with $ApoE^{-/-}$ mice despite the consumption of an HD (Fig. 1).

Teneligliptin reduced glomerulosclerosis in the $ApoE^{-/-}$ HD group mice

To evaluate glomerulosclerosis, collagen type IV immunostaining was performed (Fig. 2). The HD + Tene group mice showed markedly reduced collagen type IV accumulation in kidney tissue compared with $ApoE^{-/-}$ HD mice. This result indicates that teneligliptin reduced glomerulosclerosis in $ApoE^{-/-}$ HD mice.

Teneligliptin reduced LOX-1 gene expression in the kidneys of $ApoE^{-/-}$ mice with HD

To investigate the mechanism of lipid accumulation in the kidney, kidney tissue gene expression of relevant receptors and the ATP-binding cassette transporter A1 (ABCA1) were examined by RT-PCR. LOX-1 gene expression was significantly increased in the kidney tissue of the $ApoE^{-/-}$ HD group compared with the ND group. The increased expression of LOX-1 was suppressed in the $ApoE^{-/-}$ HD + Tene group. Expression of scavenger receptor-class A (SR-A) and CD36 were increased in the $ApoE^{-/-}$ HD group compared with ND group; however, the levels were similar to those for $ApoE^{-/-}$ HD + Tene mice. Expression of ABCA1 did not differ among the three groups (Fig. 3). These results suggest that LOX-1, SR-A, and CD36 influence lipid accumulation in the kidney tissue of $ApoE^{-/-}$ HD mice. LOX-1, in particular, appears to be a critical factor for mitigating of lipid accumulation in the kidney tissue of $ApoE^{-/-}$ HD + Tene mice compared to $ApoE^{-/-}$ HD mice.

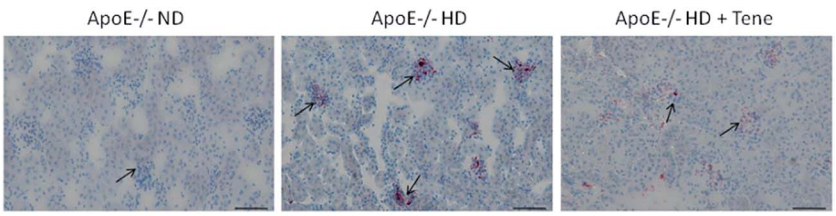

Fig. 1 Renal lipid accumulation in the three groups after 6 weeks with different treatments. Representative oil-red O staining in the kidney tissue. Red: oil-red O-positive cells and blue: haematoxylin counterstaining. Scale bar = 200 μm.

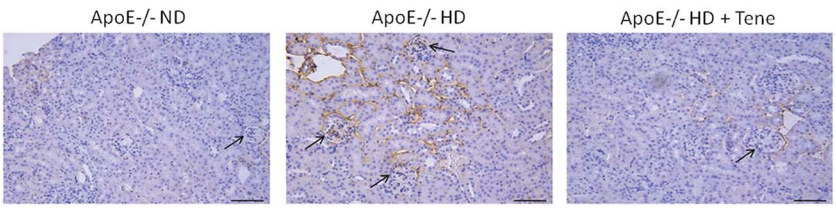

Fig. 2 Collagen type IV expression in the kidney tissue of the three groups after 6 weeks with different treatments. Representative immunohistochemistry for collagen type IV in kidney tissue. Scale bar = 200 μm. Arrows indicate positively stained cells.

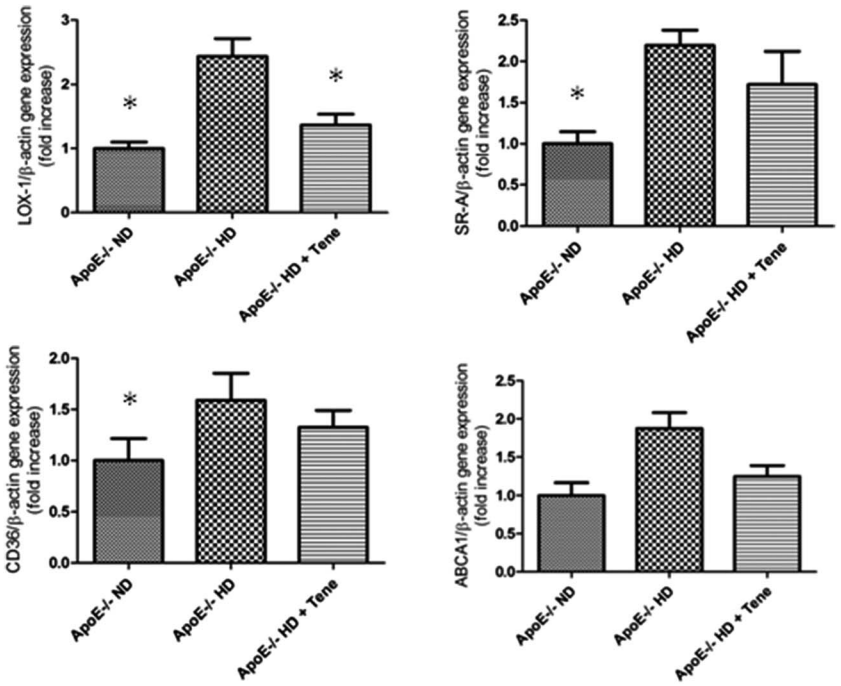

Fig. 3 Scavenger receptors gene expression in the kidney tissue of the three groups after 6 weeks with different treatments. Relative mRNA expression of LOX-1, SR–A, CD36 and ABCA1 in the kidney tissue of each group after 6 weeks with different treatments. Data are given as the means ± SEM; $n = 6–7$ in each group. *$P < 0.05$.

Teneligliptin reduced LOX-1 expression in the kidney tissue with immunohistochemistry

To evaluate LOX-1 expression in the kidney tissue, LOX-1 immunostaining was performed (Fig. 4). The ApoE$^{-/-}$ HD + Tene group had markedly reduced LOX-1 expression in kidney tissue compared to the ApoE$^{-/-}$ HD group. This result indicates that teneligliptin reduced LOX-1 expression in ApoE$^{-/-}$ HD mice.

Teneligliptin reduced LOX-1 protein expression in the kidney tissue of ApoE$^{-/-}$ HD mice

To evaluate LOX-1 protein expression in the kidney tissue, LOX-1 protein immunoblotting was performed (Fig. 5A). We found the ApoE$^{-/-}$ HD + Tene group was significantly suppressed compared with the ApoE$^{-/-}$ HD group (Fig. 5B).

Teneligliptin reduced the phosphor-PKC expression in the kidney tissue of ApoE$^{-/-}$ HD mice

Protein kinases play a role in foam cell formation and lipid deposition, and phosphor-PKC protein immunoblotting was performed (Fig. 6A). We found that phosphor-PKC in the ApoE$^{-/-}$HD + Tene group was significantly suppressed compared to the ApoE$^{-/-}$ HD group (Fig. 6B).

Discussion

This study demonstrates that teneligliptin has a protective effect against progressive lipid deposition and glomerulosclerosis elicited by hypercholesterolemia.

According to the metabolic characteristics, we found that TC and LDL-c were increased in the ApoE$^{-/-}$ HD group compared with the ApoE$^{-/-}$ ND group mice. Those results were agreement with reports by Daniel Kolbus.[13] Interestingly, TC and LDL-c in the ApoE$^{-/-}$ HD + Tene group were significantly suppressed compared with the ApoE$^{-/-}$ HD group. These results indicated that teneligliptin influences cholesterol metabolism, however, further studies are needed to clarify the mechanisms.

Hypercholesterolemia, is a major independent risk factor for kidney disease.[14] Hyperlipidaemia promotes glomerular lipid deposition and glomerulosclerosis.[15,16] Cellular lipid

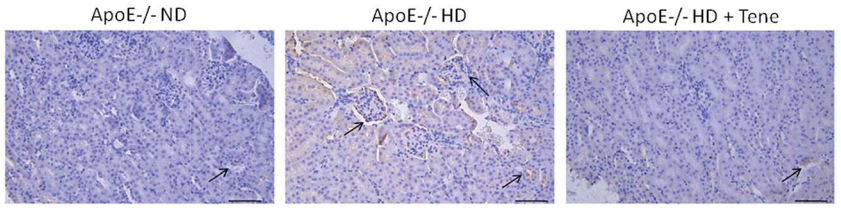

Fig. 4 LOX-1 expression in the kidney tissue of the three groups after 6 weeks with different treatments. Representative immunohistochemistry for LOX-1 in kidney tissue. Scale bar = 200 μm. Arrows indicate positively stained cells.

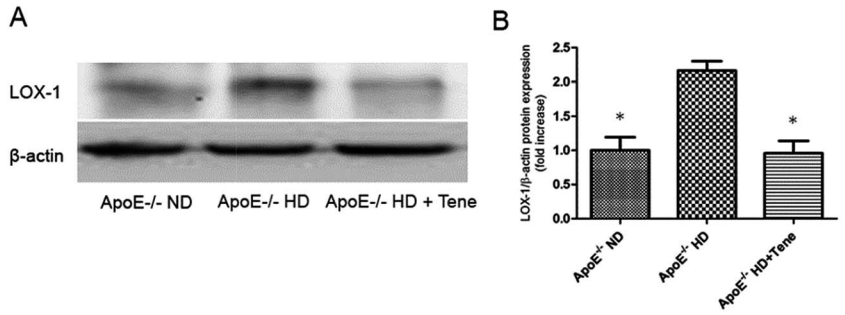

Fig. 5 LOX-1 protein expression in the kidney tissue of the three groups after 6 weeks with different treatments. (A) Immunoblotting for LOX-1 protein expression in kidney tissue. (B) Bar graph showing quantification of LOX-1 protein expression. Data are given as the means ± SEM; $n = 3$ in each group. *$P < 0.05$ *vs.* ApoE$^{-/-}$ HD.

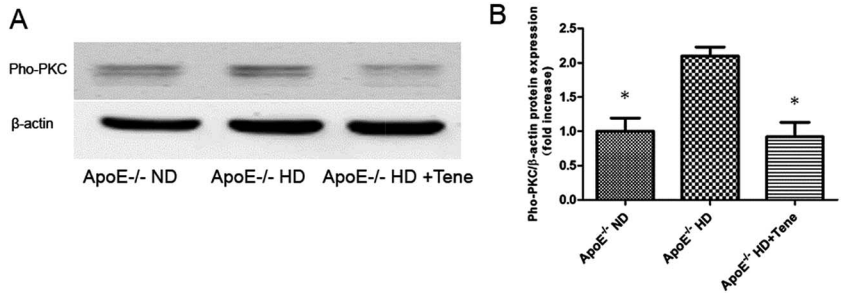

Fig. 6 Phosphor-PKC protein expression in the kidney tissue of the three groups after 6 weeks with different treatments. (A) Immunoblotting for phosphor-PKC protein expression in kidney tissue. (B) Bar graph showing quantification of phosphor-PKC protein expression. Data are given as the means ± SEM; $n = 3$ in each group. *$P < 0.05$ *vs.* ApoE$^{-/-}$ HD.

homeostasis involves regulation of the influx, synthesis, catabolism, and efflux of lipids. An imbalance in these processes can result in conversion of macrophages, mesangial cells, and vascular smooth muscle cells to foam cells. This process is mediated by several independent pathways, including SR-A, class B (CD36), and LOX-1, and it regulates the expression of its target gene ABCA1.[17–19]

DPP-4 is a ubiquitous, type II cell surface glycoprotein that is widely expressed in all tissues.[20] Treatment with DPP-4 inhibitors, which increase the GLP-1 levels, has been shown to exert numerous renoprotective effects. These effects include a reduction in the blood glucose and lipid levels, inhibition of inflammation and oxidative stress, amelioration of mesangial expansion, and an elevation of the glomerular filtration rate (GFR), among other effects.[21] Previous studies have suggested that therapeutic intervention with a DPP-4 inhibitor is effective in postponing the development of neuropathy, cardiovascular disease, and diabetic nephropathy.[22–25]

In the present study, we analysed the gene expression of scavenger receptors including SR-A, CD36, and LOX-1. We found that LOX-1 gene expression was suppressed in the ApoE$^{-/-}$ HD + Tene group. LOX-1 was originally identified in endothelial cells, and it is a 50 kDa type II membrane glycoprotein that contains a short N-terminal cytoplasmic domain, a single transmembrane domain, a short neck or stalk region, and an ox-LDL-binding C-terminal extracellular C-type lectin-like domain. On the cell surface, LOX-1 consists of 3 homodimers that are bound to ox-LDL, and it plays a leading role in ox-LDL uptake and foam cell formation.[26,27] In contrast, deletion of LOX-1 reduced the uptake of oxidized LDL and inhibited atherosclerosis in mice fed a high-cholesterol diet.[28] Therefore, suppression of LOX-1 expression in ApoE$^{-/-}$ HD + Tene mice may reduce foam cell formation. Teneligliptin also reduced LOX-1 protein expression in the kidney tissue of ApoE$^{-/-}$ HD mice. Protein kinases play a role in foam cell formation and lipid deposition. As shown earlier, enhanced LOX-1 expression was attenuated by inhibitors of PKC, ERK, and NF-κB inhibitors, indicating that increased production of intracellular ROS and activation of the PKC/MAPK pathways are initial signalling events in LOX-1 gene regulation.[29] Our results show that phosphor-PKC expression in the ApoE$^{-/-}$ HD + Tene group was significantly suppressed compared with the ApoE$^{-/-}$ HD group. It is speculated that teneligliptin regulated LOX-1 *via* the phosphor-PKC pathway.

Hyperlipidaemia has been shown to accelerate the induction and progression of renal injury leading to glomerulosclerosis.[30,31] Glomerulosclerosis can be evaluated by measuring the accumulation of collagen IV as detected by immunohistochemistry.[32,33] In our study, collagen type IV staining was significantly suppressed in the ApoE$^{-/-}$ HD + Tene group mice compared with the ApoE$^{-/-}$ HD group mice, indicating that teneligliptin contributes to reduced glomerulosclerosis and lipid accumulation.

In conclusion, our data establish that teneligliptin contributes to mitigation of hypercholesteraemic kidney injury as shown by downregulation of LOX-1, as well as suppression of foam cell formation, lipid deposition, and glomerulosclerosis. These findings provide new insights into the role of DPP-4 inhibitor teneligliptin in hypercholesterolemia kidney injury and raise the possibility of a novel therapeutic intervention for treating chronic kidney disease progression.

Author contributions

Hongyang Liu designed this study; Jing Xing, Mengye Li and Ying Liu helped perform the experiments; Hui Liu, Jinping Liu and Nan Li analysed the data and interpreted the experimental results; Hui Liu and Huiling Gao prepared the figures; Hui Liu drafted the manuscript; and Hongyang Liu, Lu Yan and Shuai Feng helped to revise the manuscript. All authors read and approved the final manuscript.

Conflicts of interest

The authors declare no conflict of interest.

Acknowledgements

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