Original Paper

Effect of Chenodeoxycholic Acid on Fibrosis, Inflammation and Oxidative Stress in Kidney in High-Fructose-Fed Wistar Rats

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Key Words

Farnesoid X Receptor • Chenodeoxycholic acid • Lipid accumulation • Chronic kidney disease

Abstract

Background: Recent studies indicate farnesoid X receptor (FXR) plays an important role in regulating lipid metabolism in kidney disease. The purpose of the present study is to investigate the effect of chenodeoxycholic acid (CDCA), a FXR agonist, on fibrosis, inflammation and oxidative stress in kidney in rats fed on high fructose. **Methods:** Twenty-four healthy male Wistar rats were randomly divided into three groups (n=8): normal control group, high fructose group and chenodeoxycholic acid group. Rats were sacrificed by the end of 16 weeks after feeding. Blood urea nitrogen, serum creatinine, fast glucose, lipid concentration were observed, spot urine samples were obtained to measure the albumin and creatinine levels. Triglyceride of renal cortices was detected. The mRNA level and protein contents of the fibrosis-inducing growth factor transforming growth factor β 1 (TGF- β 1) and plasminogen activator inhibitor (PAI-I), inflammatory cytokines tumor necrosis factor α (TNF- α) and interleukin 6 (IL-6), oxidative stress index NADPH oxidase 2 (Nox2) and p22phox in kidney were examined. The pathological changes of kidney were examined by PAS staining and immunohistochemical staining. Electron microscope sections were made to measure glomerular basement membrane (GBM) width. **Results:** Renal injuries including mesangial expansion, GBM thickness and podocyte foot process effacement were found in fructose-fed Wistar rats, FXR agonist CDCA modulates renal lipid metabolism, decreases proteinuria and improves renal fibrosis, inflammation and oxidation stress. High-fructose-feeding may cause lipid nephrotoxicity through down-regulated farnesoid X receptor and increases expression of profibrotic growth factors, proinflammatory cytokines, and oxidative stress in Wistar rats. **Conclusion:** FXR activation by chenodeoxycholic acid can prevent the injury in kidney induced by high fructose feeding.

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Introduction

In 1858, Virchow described in Bright's disease the successive progression of fatty metamorphosis and fatty detritus in renal epithelium and first suggested the association between lipids and renal disease [1]. Then, in 1936 Kimmelstiel and Wilson described the pathological sign of nodular sclerosis and demonstrated the presence of lipid deposition in the kidney of diabetic patients [2]. After that there is growing evidence based on human and animal studies indicating that abnormal lipid metabolism and lipid accumulation in kidney play an important role in the pathogenesis of kidney disease [3-7].

In the past several decades, the consumption of fructose, which is a major component in high-fructose corn syrup (HFCS), has increased considerably. Fructose consumption has been shown to contribute to the current epidemic of the metabolic syndrome, which is pathophysiologically based on insulin resistance and can manifest as obesity, hypertension, hyperglycemia, hypertriglyceridemia, hyperuricemia, non-alcoholic fatty liver disease etc. [8-11]. Rats fed on high fructose diets for a long term showed insulin resistance [12, 13], dyslipidemia [14-19], hypertension [13, 20, 21], hyperuricemia [22, 23] and proteinuria [24]. The metabolic syndrome is a well-established risk factor for diabetes, cardiovascular disease, mortality and also contributes to the development of chronic kidney disease (CKD) [25-28].

Farnesoid X receptor is a ligand-regulated transcription factor belonging to the nuclear receptor super-family and can be activated by structurally different ligands including several primary and secondary bile acid (BA) species [29-32]. The most potent natural activator appears to be chenodeoxycholic acid. FXR has been shown to be largely expressed in liver, intestine, and kidney. FXR plays an important role in regulating bile acid metabolism [29, 33], lipid metabolism and carbohydrate metabolism [34-37]. Moreover, activation of FXR prevents liver fibrosis [38] and atherosclerotic lesions [39, 40]. It has been shown that FXR control lipid metabolism by a mechanism involving repression of sterol regulatory element-binding protein 1c (SREBP-1c) expression in liver [41] so to inhibit triglyceride (TG) synthetic pathway [42].

In the present study, we first observe the kidney injuries in rats fed on long-term high fructose diet. Furthermore, since the kidney has a high expression level of FXR and some research have shown FXR and SREBPs coexist in the glomeruli and proximal tubule cells of mouse [43], we investigated whether CDCA administration could attenuate renal injury in high-fructose-fed rats.

Materials and Methods

Animals and treatment

Twenty-four male Wistar rats weighed ~200g were obtained from Hebei medical university animal laboratory (No. 1003188). Rats were maintained on a 12-h light/12-h dark cycle. The rats were randomly divided into three groups with 8 rats in each group and were fed either a control chow diet, a high fructose diet, or a 35% fructose diet with CDCA (Sigama) 100mg/kg body wt. via gavage once a day. The controls were also gavaged with equivalence physiological saline. The control diet was obtained from Hebei medical university animal laboratory, containing 24.2% protein, 65.5% carbohydrate, and 10.3% fat. High-fructose diet contains 35% calories from fructose, 35% calories from starch, 9% calories from fat and 21% calories from protein which was based on a recipe described in a study by Thorburn AW [44]. Body weight and food intake were measured per week. Animal studies and relative protocols were approved by the Animal Care and Use Committee at the Hebei medical university. At the end of the 16 weeks, spot urine samples were obtained so that albumin and creatinine levels could be measured. The rats were fasted for 10 hours before harvesting blood, after anesthesia (intraperitoneal injection of 3% pentobarbital, 0.2ml/100g wt.), blood was drawn from right carotid artery and then the kidneys were removed and processed for lipid extraction and lipid measurements, real-time quantitative PCR and Western blotting. The kidneys were fixed by in vivo

Table 1. Primers used in quantitative real-time PCR

Gene	Sense	Antisense		
GAPDH	TGA ACG GGA AGC TCA CTG G	GCT TCA CCA CCT TCT TGA TGT C	120	NM_017008
FXR	GAG AGA TGG GAA TGT TGG CTG	CTT GGT CGT GGA GGT CACT T	149	NM_021745.1
SHP	GGA GTC TTT CTG GAG CCT TGA G	CTG GCA CAT CTG GGT TGA AG	83	NM_057133.1
SREBP1c	ACC GTT TCT TCG TGG ATG GG	CGA TAC AGT TCA ATG CTC GCT C	147	XM_213329
SCD-1	ACA AGA ACA TTC AAT CTC GGG AG	CTC ACT GGC AGA GTA GTC GT	107	NM_139192.2
TGF- β 1	GGA CCG CAA CAA CGC AAT CT	TGG CAC TGC TTC CCG AAT GT	94	NM_021578
PAI-1	TTC AGC CCT CAC TTG CCT CA	GGA GAG AAG ACC ACA TTT CGG T	165	NM_012620.1
TNF- α	GCT CCC TCT CAT CAG TTC CA	CTC CTC TGC TTG GTG GTT TG	108	NM_012675.2
IL-6	TTG CCT TCT TGG GAC TGA TGT	TGT TGT GGG TGG TAT CCT CTG	93	NM_012589.1
Nox2	AAA CTT CTT GGG TCA GCA CTG	AGA TTC CGA CAC ACT GGC AG	95	NM_023965.1
p22phox	GAG CGG TGT GGA CAG AAG TA	AGG CAC GGA CAG CAG TAA GT	105	NM_024160.1

perfusion fixation and processed for periodicacid Schiff (PAS) stain for renal histopathology, oil red O stain for neutral lipids, immunohistochemistry and electron microscopy [45].

Blood chemistry

Blood glucose levels were measured by hexokinase method. Urea nitrogen levels were measured by urease method. Creatinine levels were measured by picric acid method. Uric acid levels were measured by uricase method. All above kits were purchased from Wako Pure Chemical Industries, Ltd. Plasma lipid levels were measured by enzymatic method with kits from KHB (Shanghai, PRC).

Measurement of urine albumin and creatinine

Urine albumin concentration was determined by competitive enzyme-linked immunosorbent assay using an r Alubumin kit (Exocell, Philadelphia, PA). Urine creatinine concentration was determined by Jaffe's reaction of alkaline picrate with creatinine using a Creatinine Companion kit (Exocell, Philadelphia, PA). Results were expressed as the urine albumin-to-creatinine ratio (micrograms per milligram) [45].

Lipid extraction and measurement of triglyceride in Kidney

Lipids from the renal cortex were extracted by the method of Bligh and Dyer [46]. Triglyceride content was measured using kits from Sigma according to instructions of the manufacturer.

Total RNA extraction and real-time

PCR 50mg of each sample were subjected to total RNA isolation according to Trizol protocol (Invitrogen, USA). Total RNA (2 μ g) of was subject to DNase digestion. Following DNase I treatment, reverse transcription was performed using the First-Strand cDNA Synthesis system (Promega, USA). Real-time PCR were performed with SYBR Green I mix (Promega, USA) using primer sets listed in Table 1. PCR was performed using a thermocycler for 40 cycles according to the following programme: 5 min at 94°C, 45s at 94°C, and 1 min at 60°C. Relative gene expression levels were normalized to the gene of GAPDH. All data were calculated from duplicate reactions. Primers used are listed in Table 1.

Nuclei and microsome isolation

Kidneys were homogenized at 4°C in a buffer (20 mmol/l Tris-Cl, pH 7.4, 75 mmol/l NaCl, 2 mmol/l EGTA, 2 mmol/l EDTA, 1 mmol/l Na₃VO₄, and 1 mmol/l dithiothreitol), supplemented with a protease inhibitor cocktail that consisted of 104 mmol/l AEBSF, 0.08 mmol/l aprotinin, 2 mmol/l leupeptin, 4 mmol/l bestatin, 1.5 mmol/l pepstatin A, and 1.4 mmol/l E-64 (Sigma-Aldrich, St. Louis, MO)[44]. Nuclear extracts were prepared according to the method of Morooka et al. [47]. The protein concentration was determined by the method of Lowry et al. [48]. The nuclear extracts were stored at -80°C.

Protein electrophoresis and Western blotting

Protein samples were prepared using RIPA buffer, and 60 µg of each sample were subjected to 10% SDS-PAGE, then transferred to PVDF membranes. After blockage with 5% fat-free milk powder with 1% Triton X-100 in Tris-buffered saline (20 mmol/l Tris-Cl, 150 mmol/l NaCl, pH 7.4), the films were incubated with one of the antibodies against FXR (Santa Cruz), SHP (Santa Cruz), SREBP-1 (Santa Cruz), SCD-1 (Santa Cruz), TGF-β1 (Bioworld), PAI-I (Bioworld), TNF-α (Bioworld), IL-6 (Santa Cruz), Nox2 (Bioworld), p22phox (Santa Cruz). Corresponding secondary antibodies (1:5000 dilution) were visualized using enhanced chemiluminescence (Pierce, Bradford, IL). The bands were detected on an X-ray film using Pierce ECL Western Blotting Substrate.

Histology staining and electron microscopy

Frozen sections were used for oil red O staining of neutral lipid deposits. Sections of 4-µm thickness cut from 10% formalin-fixed, paraffin embedded kidney samples were used for periodic acid-Schiff (PAS) staining. The stained kidney sections were imaged with an Olympus microscope. Immunohistochemical was performed by Elivison two-step process. Antibodies were rabbit anti-type IV collagen and anti-α-SMA (Santa Cruz). Electron microscopy (EM) was conducted in Hebei medical university. Perfusion-fixed tissue was immediately postfixed in 1% buffered osmium tetroxide. The sample was dehydrated in a graded series of ethanol and embedded in an epoxy resin. Tissue was surveyed with a series of 1-µm sections for a representative sample. The selected specimens were thin sectioned, viewed, and photographed with an electron microscope (HITACHI H7500, Japan). The sections were read for determination of basement membrane thickness and podocyte morphology. The histological analyses were performed with the pathologist being blinded as to the study group.

Statistical analysis

The results were expressed as the means ± SD. SPSS 11.0 for Windows was used for statistical analysis. The statistical significance of differences was assessed by ANOVA and Student-Newman-Keuls tests for multiple comparisons or by Student's test for unpaired data between two groups. Statistical significance was accepted at the $P < 0.05$ level.

Results

Activation of FXR by CDCA improves serum lipid

The kidney weight/body weight and their ratio in high-fructose-fed rats were significantly higher than those in the control group. Serum triglyceride and very low density lipoprotein (VLDL) levels increased markedly, as well as uric acid levels ($P < 0.01$). Intervention by CDCA reduced kidney weight and body weight ratio, decreased triglyceride, VLDL and uric acid levels remarkably ($P < 0.01$). While blood glucose, blood urea nitrogen and creatinine were not much more affected ($P > 0.05$) (Table 2). The use of picric acid to determine plasma creatinine concentrations can overestimate the results and limits the interpretation of the data [49].

Intervention of fructose-fed rats with CDCA improves proteinuria

Rats on high fructose diet developed severe proteinuria, which was significantly decreased and was almost normalized by CDCA intervention ($P < 0.01$) (Table 2).

FXR activation by CDCA prevents renal triglyceride accumulation

Although the oil red staining in the kidney of high-fructose-fed rats is minimal, the triglyceride contents in renal cortex is much more abundant than that in control group ($P < 0.01$), and CDCA treatment decreased the level of triglyceride in the renal cortex ($P < 0.01$). (Table 2, Fig. 1).

Table 2. Renal function and metabolic data

	Control	High-fructose-fed	High-fructose-fed+CDCA
Kidney weight(g)	1.07±0.07	1.45±0.10**	1.24±0.13***
Body weight(g)	332.63±17.94	363.38±23.16**	349.00±11.34##
Kidney/Body weight ratio(x10 ⁻³)	3.24±0.36	4.00±0.42**	3.56±0.39#
Serum urea nitrogen(mmol/L)	6.59±1.05	6.69±0.85	6.58±0.90
Serum creatinine(μmol/L)	41.39±6.39	45.57±3.67	44.67±6.45
Serum uric acid(μmol/L)	61.76±9.34	92.65±12.21**	70.20±10.09##
Serum chlesterol(mmol/L)	1.66±0.14	1.82±0.29	1.59±0.25
Serum triglyceride(mmol/L)	1.17±0.19	2.13±0.35**	1.43±0.23##
Serum very low density lipoprotein (mmol/L)	0.52±0.07	0.97±0.14**	0.64±0.09***
Serum glucose (mmol/L)	5.60±0.46	6.01±0.37	5.80±0.45
Urin albumin-to creatinine ratio(μg/mg)	27.32±2.45	162.91±17.26**	89.14±12.33***
Kidney triglyceride content(mg/g protein)	5.16±0.86	7.99±0.86**	6.18±0.61***

Values are means±SD (n=8 rat/group); *P<0.05 vs control. **P<0.01 vs control. #P<0.05 vs high-fructose-fed.

##P<0.01 vs high-fructose-fed.

Fig. 1. Total triglyceride contents in the kidney cortex of control, high-fructose-fed and CDCA intervention rats. There is a significant increase in high-fructose-fed rats, CDCA intervention decreases triglyceride contents. **P<0.01 vs control. ## P<0.01 vs high-fructose-fed. (n=8 rats/group).

FXR activation by CDCA ameliorates renal structural changes

By the end of 16 weeks, compared with control rats, the kidney tissue of high-fructose-fed rats clearly showed mesangial expansion by Periodic acid Schiff staining, increased matrix protein accumulation as shown by type IV collagen and tubulointerstitial fibrosis by α -smooth muscle actin (α -SMA) by immunohistochemical staining, podocyte foot processes effacement and irregular basement membrane thickening by electron microscopy. CDCA intervention led to significant alterations of renal structural changes as shown by decreased mesangial expansion, attenuated type IV collagen and α -SMA expression. Podocyte foot effacement and basement membrane thickening was not that severe (Fig. 2, Fig. 3, Fig. 4).

FXR activation by CDCA upregulates FXR, SHP and downregulates SREBP1 and regulates renal lipid metabolism

The mRNA and protein expression of FXR in the kidney of high-fructose-fed rats were decreased by 71% and 62% respectively ($P<0.01$). The mRNA and protein expression of small heterodimer partner (SHP), a well-characterized target gene of FXR, were also decreased by 98% and 68% in the kidney of high-fructose-fed rats ($P<0.01$). Meanwhile, SREBP-1 mRNA and protein which is a master regulator of lipogenic gene, were increased by 5.30 and 4.78 fold respectively in kidney ($P<0.01$). Our study also found marked increases in the mRNA and protein expression of stearoyl-CoA desaturase 1 (SCD-1) (increased by 4.19 and

Fig. 2. CDCA intervention ameliorates renal structural changes in high-fructose-fed rats. PAS staining of kidney sections($\times 200$), showing increased mesangial expansion in high-fructose-fed rats. The depicted pictures are representative.

Fig. 3. CDCA intervention ameliorate renal structural changes in high-fructose-fed rats. Immunohistochemical detection of collagen IV in glomeruli. Type IV collagen staining indicated increased matrix protein accumulation($\times 200$). Immunohistochemical detection of α -SMA in tubule and interstitium. α -SMA staining indicated tubulointerstitial fibrosis($\times 200$). The depicted pictures are representative.

4.85 fold respectively, $P < 0.01$), which is the target enzyme of SREBP-1c and mediated fatty acid synthesis. Intervention of CDCA upregulated the FXR and SHP levels in the kidney of high-fructose-fed rats (FXR mRNA and protein expression were increased by 0.48 and 0.71 fold respectively, SHP mRNA and protein expression were increased by 2.83 and 0.84 fold respectively ($P < 0.01$) (Fig. 5, Fig. 6).

FXR activation by CDCA decreases profibrotic growth factors

The proteinuria, mesangial expansion, increased matrix protein accumulation, and tubulointerstitial fibrosis in high-fructose-fed rats are associated with significant increases mRNA and protein levels of fibrosis-inducing factors TGF- $\beta 1$ (increased by 4.21 and 5.02 fold respectively, $P < 0.01$) and PAI-I (increased by 5.88 and 5.49 fold respectively, $P < 0.01$) in

Fig. 4. CDCA intervention ameliorate renal structural changes in high-fructose-fed rats. Electron micrographs of glomeruli showing podocyte foot processes effacement and irregular basement membrane thickening in high-fructose-fed rats ($\times 20,000$). The depicted pictures are representative.

renal cortex. Intervention with CDCA downregulate these fibrosis-inducing factors (TGF- β 1 mRNA and protein expression were decreased by 27% and 42% respectively, PAI-I mRNA and protein expression were decreased by 51% and 45% respectively, $P < 0.01$), indicating that CDCA plays an anti-fibrotic role in this process (Fig. 5, Fig. 6).

FXR activation by CDCA improved renal inflammation

Inflammation is a major factor to promote renal injury, involving glomerulosclerosis, tubulointerstitial fibrosis and proteinuria. TNF- α and IL-6 are important inflammatory cytokines. In high-fructose-fed rats, we found marked increases in the mRNA and protein expression of TNF- α (increased by 3.21 and 4.17 fold respectively, $P < 0.01$) and IL-6 (increased by 5.41 and 5.29 fold respectively, $P < 0.01$). CDCA inhibited the mRNA and protein expression of TNF- α (by 23% and 32% respectively, $P < 0.01$), IL-6 (by 53% and 37% respectively, $P < 0.01$). (Fig. 5, Fig. 6).

CDCA protects against oxidative stress in kidney

Renal oxidative stress is a characteristic finding of many diseases, such as obesity, metabolic syndrome and diabetic mellitus. Our study showed an activated oxidative stress in kidney in the high-fructose-fed rats, manifested by upregulated gene and protein of Nox2 (increased by 5.55 and 4.89 fold respectively, $P < 0.01$) and p22phox (increased by 5.25 and 3.76 fold respectively, $P < 0.01$). FXR activation by CDCA was associated with antioxidative effects, as shown by decreased gene and protein of Nox-2 expression (decreased by 51% and 28% respectively, $P < 0.01$) and p22phox expression (decreased by 53% and 33% respectively, $P < 0.01$) in kidneys from CDCA-intervented high-fructose-fed rats (Fig. 5, Fig. 6).

Discussion

The purpose of our study was to examine whether FXR activation can protect the renal injury induced by high fructose feeding in Wistar rats. In our study, rats fed on high fructose diet develop hypertriglyceridemia, hyperuricemia, hyperhyperproteinuria, kidney triglyceride accumulation, glomerulosclerosis and tubulointerstitial fibrosis in kidney, characterized by proteinuria, mesangial expansion, accumulation of extracellular matrix proteins, irregular glomerular basement membrane thickness, and podocyte foot process effacement. Intervention of FXR agonist CDCA significantly improves renal injury by modulating renal lipid metabolism and showed the effect of antifibrosis, antiinflammation and antioxidation.

Fig. 5. FXR, SHP, SREBP-1c, SCD-1, TGF-β1, PAI-I, TNF-α, IL-6, Nox 2 and p22phox mRNA expression by real-time quantitative PCR. CDCA intervention regulates renal lipid metabolism, promotes antifibrotic, antiinflammatory and antioxidative effects. **P < 0.01 vs control. ##P < 0.01 vs high-fructose-fed (n = 8 rats/group).

Fig. 6. FXR, SHP, SREBP-1, SCD-1, TGF-β1, PAI-I, TNF-α, IL-6, Nox 2 and p22phox protein expression by western blotting. CDCA intervention regulates renal lipid metabolism, promotes antifibrotic, antiinflammatory and antioxidative effects. **P < 0.01 vs control. ##P < 0.01 vs high-fructose-fed (n = 8 rats/group).

Studies on diabetic nephropathy have suggested that hyperlipidemia contributes to its progression. Several investigations have shown the presence of lipid deposition in the kidney of experimental diabetic animals and diabetic individuals [50, 51]. The increased serum lipid levels were first thought to lead to lipid deposition in kidney. However, an increase in renal lipid synthesis could be an even more important factor contributing to renal lipid accumulation. From this point of view, we investigated the lipid-metabolism-related markers in kidney in high-fructose-induced rats to investigate the mechanisms of renal injury.

Our study found that in rats fed on high fructose diets for 16 weeks, accompanying with hypertriglyceridemia, there is high-level triglyceride in renal cortex. The mRNA and protein expression of SREBP-1 were both upregulated. There is also an increased mRNA and protein abundance of SCD-1, the key enzyme mediating fatty acid synthesis, which results in increased triglyceride synthesis and accumulation [52, 53]. The increases in renal triglyceride content are most likely mediated by increased nuclear protein levels

and transcriptional activities of SREBP-1. Previous studies in cultured renal mesangial and tubular cells have shown that lipids induce upregulation of profibrotic factors, including TGF- β 1 [54], PAI-I [55], and accumulation of matrix proteins [56], suggesting a direct role of lipids in mediating glomerulosclerosis. In SREBP-1a transgenic mice, when serum glucose, triglyceride, cholesterol level is normal, there is triglyceride accumulation in the renal resulting in increased TGF- β , VEGF, type IV collagen and fibronectin abundance and renal injury [6].

The nuclear hormone receptor FXR which is also highly expressed in kidney, plays an important role in maintaining glucose, lipid, and bile acid homeostasis [57, 58], despite its antifibrosis effect in liver [59, 60]. CDCA is one of the highest affinity endogenous activating ligands for FXR. We intervened high-fructose-fed Wistar rats with CDCA for 16 weeks to observe the effect of FXR activation in regulating renal metabolism and preventing renal injury.

In our study, we found decreased expression of FXR and its immediate target SHP in the kidneys of high-fructose-fed Wistar rats, which is consist with the studies in Akita and OVE26 mice which develop diabetic nephropathy [61]. Our results indicate that the decreased expression of FXR and its target SHP could mediate the increases in SREBP-1c, which result in increase in triglyceride synthesis and accumulation in the kidney.

There are studies showing that FXR agonist modulate fibrosis. In the models of liver cirrhosis and fibrosis, 6-ethy chenodeoxycholic acid decreases the expression of TGF- β 1, collagen, and α smooth muscle actin and relieve hepatic fibrosis [60]. Our study indicate that the decreased expression of FXR and its immediate target SHP mediate the increased expression of TGF- β 1 and PAI-I in kidneys of high-fructose-fed Wistar rats, also manifested by high expression of type IV collagen and α -SMA in immunohistochemical staining. This finding is consistent with the diabetic mice model in previous study [44, 61]. CDCA intervention increase FXR, SHP expression, decrease SREBP-1c expression, downregulate profibrotic factors and ameliorate renal fibrosis.

Inflammation and oxidative stress play an important role in the development of many kidney diseases. Studies show that anti-inflammatory agents including mycophenolate mofetil can prevent the progress of renal injury in diabetic rats [62]. In our study, we find the two important factors involved in the inflammation process, TNF- α and IL-6, were upregulated in the kidney of high-fructose-fed rats. Intervention with CDCA induces downregulation of these proinflammatory cytokines. Proinflammatory cytokines can also induce downregulation of FXR in diabetic kidney [63]. FXR activation is also associated with antioxidative effects, as shown by decreased NAPDH oxidase2 (Nox 2) and NADPH oxidase p22phox subunit expression from CDCA-intervented high-fructose-fed Wistar rats. However, the mechanism by which FXR activation regulate inflammation, oxidative stress and their interaction is not well understood. A better understanding of the mechanism can help us find better therapeutic targets for some common disorders occurring in obesity, diabetes, and metabolic syndrome characterized by abnormal lipid metabolic, oxidative stress, and microinflammation.

Wang et al [64] and Jiang et al. [43] found mice fed a high cholesterol and (or) a high fat diet result in proteinuria, renal lipid accumulation, renal injury and increased expression of proinflammatory factors, oxidative stress and profibrotic growth factors. Treatment of these mice with the highly selective and potent FXR-activating ligand INT-747 or GW4064 ameliorates triglyceride accumulation by modulating fatty acid synthesis and oxidation, improves renal injury, and modulates inflammation, oxidative stress and fibrosis. SREBP-1 plays a critical role in renal lipid accumulation and increases the activity of proinflammatory cytokines and profibrotic growth factors. FXR modulates renal SREBP-1 activity. This provides a new therapeutic avenue for treatment of kidney pathology in patients with obesity and diabetes. Our result is consisting with theirs.

In summary, our study show that high fructose feeding induced hypertriglyceridemia, hyperuricemia, renal triglyceride accumulation, glomerular mesangial matrix expansion, tubulointerstitial fibrosis, podocyte foot processes effacement and irregular basement

membrane thickening in Wistar rats. The intervention of FXR agonist CDCA prevented renal injury in rats induced by high-fructose-feeding. The potential mechanism might be due to the effect of CDCA in decreasing renal lipid accumulation and the antifibrosis, antiinflammation, and anti-oxidative-stress effect of CDCA by FXR activation. This knowledge may lead to important new therapeutic target for the treatment of kidney disease associated with lipid metabolism disorders. Whether CDCA induces beneficial effects on the kidney in the absence of high fructose and the role of the lipid-lowering effect of CDCA versus potential direct effects on the kidney has not been tested in this study.

Abbreviations

FXR (farnesoid X receptor); CDCA (chenodeoxycholic acid); HFCS (high-fructose corn syrup); GBM (glomerular basement membrane); CKD (chronic kidney disease); BA (bile acid); SREBP-1c (sterol regulatory element-binding protein 1c); VLDL (very low density lipoprotein); α -SMA (α -smooth muscle actin); SHP (small heterodimer partner); SCD-1 (stearoyl-CoA desaturase 1); TGF- β 1 (transforming growth factor β 1); PAI-I (plasminogen activator inhibitor I); TNF- α (tumor necrosis factor α); IL-6 (interleukin 6); Nox2 (NADPH oxidase 2)

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