Results: HFHS diet led to vacuolization and thyroidisation of the renal tubules. Glucose caused mitochondrial fragmentation and cellular apoptosis in HK2 cells. Autophagy was activated in glucose-treated cells as evidenced by the enhanced LC3B-II expression and autophagosome formation. When autophagy was inhibited, either by the inhibitor, 3-MA, or by siATG5, tubular cells were more susceptible to glucose-induced mitochondrial fragmentation and cellular apoptosis. When MFN1 or MFN2 was knockdown, mitochondria became more fragmented and autophagy was activated. When FIS1 was silenced, mitochondria were not fragmented and the expressions of LC3B-II, p62 and BECN1 remained unchanged.

Conclusion: Our results characterized the pathology of diabetic tubulopathy and suggested that glucose leads to mitochondrial fragmentation and cellular apoptosis in renal tubules. We also offered evidence that autophagy protects mitochondrial from fragmentation in renal tubules.

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0090
Protective Effect of Tangshen Formula on Renal Inflammation in Type 2 Diabetes
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Objective: Tangshen Formula (TSF), a traditional Chinese medicine, had been reported to have beneficial effects on diabetic nephropathy (DN), but its action mechanism is still unclear. The study was designed to elucidate the potential mechanism of TSF in treating DN.

Methods: Type 2 DN rat models were established by high-fat diet-fed and low-dose-streptozotocin injection. The rats were treated with or without TSF by gavage for 20 weeks and examined by 24h-albuminuria, histology, immunohistochemistry, molecular analyses.

Results: The results showed that TSF treatment significantly inhibited urinary excretion of albumin, and attenuated renal histological injuries in the rats. TSF treatment also inhibited renal inflammation which was associated with inactivation of NF-κB signaling. In addition, TSF treatment also suppressed expressions of fibronectin, collagen I, and collagen IV.

Conclusion: The present study revealed that TSF might attenuate renal inflammation of type 2 DN by inhibiting NF-κB signaling pathway.

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0091
The Expression of Electron Transfer Flavoprotein β Mediating Apoptosis of Renal Tubular Epithelial Cells in Diabetic Nephropathy
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Objective: In diabetic nephropathy, it has been proven lipotoxicity is an important pathological mechanism of progressive tubulointerstitial injury. Electron transport flavoprotein (ETF) is an electron acceptor in fatty acid β oxidation, which is composed by α and β subunits. Our previous studies have found ETFβ point mutations in conserved sequences by renal cortex proteome in diabetic nephropathy. In this study, the expression and distribution of ETFβ and its role in diabetic kidney damage were explored to provide new ideas for further insight into the pathogenesis of diabetic nephropathy.

Methods: Expression of ETFβ in kidney was detected by Western blot and immunohistochemistry in a model of spontaneous type 2 diabetic OLETF rats. The effects of ETFβ at low expression on fatty acid-induced apoptosis by knocking down the gene were detected in NRK 52E cells. Apoptosis and related apoptosis pathways induced by fatty acid or knock down of ETFβ combined with fatty acids stimulate were compared to analyze the influence of ETFβ on lipotoxic apoptosis.

Results: Decreased ETFβ expression occurred only in 56-week-old rats which was correlated with massive proteinuria and severe tubular damage. We found increased ROS generation, decreased mitochondrial membrane potential, increased expression of BAX, and decreased Bcl-2, increased Cleaved caspase-3, and a lot of apoptotic cells in NRK 52E cells induced by palmitate. After ETFβ gene was knocked out, the above change by PA stimulation in mitochondrial apoptosis pathway was furtherly aggravated.

Conclusion: The expression of ETFβ in kidney cortex was significantly decreased with the progression of renal injury in diabetic nephropathy rats. Knock down of ETFβ under palmitate stimulation can significantly increase apoptosis rate. Thus, we speculate decreased ETFβ expression may be involved in renal tubular epithelial cellul apoptosis and the progression of diabetic nephropathy.

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0092
Haematuria and Outcomes in Patients with Diabetic Chronic Kidney Disease
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Objective: Microscopic haematuria (denoted as haematuria) is considered a risk factor for end-stage renal disease (ESRD) in glomerulonephritis. Haematuria is not infrequent in patients with diabetic chronic kidney disease (CKD) and is associated with albuminuria and the duration of diabetes. However, the clinical significance of haematuria in patients with diabetic CKD has been rarely studied.

Methods: We included 1958 patients with type 2 diabetes and CKD stages 1–5 from nephrology outpatient department. They were divided into 3 groups according to haematuria in 3 consecutive urinalyses: no haematuria (0 e red blood cells/C21 RBCs/hpf), < 5 e red blood cells/C21 RBCs/hpf, ≥ 5 e red blood cells/C21 RBCs/hpf. In the subgroup analysis, we observed that the association between haematuria and ESRD was more prominent in patients with CKD stages 1–3 or UPCR < 500 mg/g.

Results: The average estimated glomerular filtration rate was 24.5 (12.0–40.0) ml/min/1.73 m2 and the average urine protein-to-creatinine ratio (UPCR) was 1448 (471–3622) mg/g. Among 102 patients received biopsy, 42 patients (41.2%) had biopsy-proven diabetic nephropathy. We observed that haematuria was associated with age, UPCR, duration of diabetes, mean blood pressure, albumin, glycated haemoglobin, and C-reactive protein. The hazard ratio (HR) of moderate haematuria for ESRD was 1.39 (95% confidence interval [CI]: 1.10–1.76, P < 0.001). The odds ratios (ORs) of moderate haematuria for rapid renal progression was 1.81 (95% CI: 1.29–2.53, P < 0.001). Moreover, the HRs of mild haematuria and moderate haematuria for mortality were 1.43 (95% CI: 1.12–1.84, P = 0.005) and 1.73 (95% CI: 1.27–2.36, P < 0.001), respectively. In the subgroup analysis, we observed that the association between haematuria and ESRD was more prominent in patients with CKD stages 1–3 or UPCR < 500 mg/g.

Conclusion: Microscopic haematuria in patients with diabetic CKD is associated with risks for ESRD and mortality.

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0093
The Study of Syndrome Differentiation Based on Renal Biopsy Pathology of Diabetic Kidney Disease
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Objective: Research the distribution of syndrome differentiation in Traditional Chinese Medicine (TCM) on diabetic kidney disease (DKD) and its relationship with pathological stage of DKD.
Methods: Bring into the DKD patients through pathological diagnosis which have more than one year disease course, to classify by the Tervaet criterion of pathological stage of DKD, to differentiate the syndrome differentiation in TCM by collecting clinical data, and have a statistical processing at last.

Results: We collected 353 DKD patients meeting the criterion in total, classified by the pathological stage criterion of DKD, among them there were 52 patients with phase I, 90 patients with phase IIa, 29 patients with phase IIb, 141 patients with phase III, 41 patients with phase IV. About syndrome differentiation, there were 13 patients with Yin-Xu-Zao-Re type, there were 151 patients with Qi-Yin-Liang-Xu type, there were 140 patients with Pi-Shen-Qi-Xu type, the glomerular segmental sclerosis, nodular changes, Pi-Shen-Qi-Xu type, the glomerular segmental sclerosis, nodular changes, the glomerular segmental sclerosis, nodular changes, the glomerular segmental sclerosis, nodular changes, the renal interstitial fibrosis, hyaline degeneration, angiosclerosis, and fibrinoid exudation were more serious than other types significantly.

Conclusion: Among the syndrome differentiation of DKD, Qi-Yin-Liang-Xu type and Yu-Xue type were more frequently than other types. The result showed that syndrome differentiation was correlated significantly with pathological stage of DKD, to differentiate the syndrome differentiation were independent risk factors for renal prognosis.

Objective: Diabetic nephropathy (DN) is one of the most severe microvascular complications of diabetes. Recently, Berberine (BBR) has the beneficial effects to diabetes. The aim of this study is to observe the renoprotective effect and mechanisms of berberine in a type 2 diabetes nephropathy on rat.

Methods: Twenty seven male Wistar rats (8-wk old, 200–250 g) randomly divided into 9 rats in normal control and 18 rats in diabetic groups. Diabetic rats were fed with a high-fat diet (consisting of 50% carbohydrate, 12% protein, and 38% fat) for 5 wk, followed by intraperitoneal injection with a single low-dose of streptozotocin (STZ, 25 mg/kg) after 12-h fasting. The normal control rats were fed with a standard chow diet. Diabetic rats were randomly divided into two subgroups: vehicle treated and BBR treated (n = 9 each). Rats were housed individually in metabolic cages for 24-h urinary collection at 4-wk intervals. Rats were sacrificed 20 weeks later. We detected the kidney weight index, 24 h urine protein, morphology study, inflammation and fibrosis markers, such as Iba1, TGFβ1, TGFβ2, and Smad7 by realtime-PCR and Western Blot.

Results: (1) Compared with normal rats, the diabetic rats showed higher kidney weight index and 24h urine protein, berberine treatment decreased kidney weight index and 24 h urine protein. (2) BBR treatment inhibited histological injury including deposition of extracellular matrix, thickening of glomerular basement membrane, and tubular atrophy of DN rats. (3) DN rats showed decreased Smad7 mRNA and protein expression (P < 0.01), increased TGFβ1 mRNA (P < 0.05) and TGFβ1 protein expression (P < 0.05); however, BBR treatment down-regulated TGFβ1, TGFβ2 level (P < 0.05), up-regulated Smad7, Iba1 level (P < 0.05).

Conclusion: Berberine has renoprotective effect on a type 2 diabetic rat model. The mechanism might suppress inflammation and fibrosis via upregulating expression of Smad7.

Effects of Tangshen Formula on Dihydropteridine Reductase Transcription

Objectives: Tangshen Formula (TSF) is a Chinese herbal medicine used for DN treatment. In the current study, we aimed to identify the core regulatory region of QDPR gene promoter. We also studied whether the Tangshen Formula could up-regulate QDPR promoter activity in vitro study and to investigate the Tangshen Formula’s mechanism at molecular level.

Results: Identification of core regulatory region in QDPR gene promoter: as compared with −2092 ~ −1 fragment, the relative fluorescence intensity of the deleted fragment −529 ~ −116 reduced to 1% (P < 0.01), the fragment which contained −529 ~ −1 kept 64% (P < 0.05). The relative fluorescence intensity of those deleted fragments −529 ~ −429, −428 ~ −250 and −141 ~ −116 were increased (P < 0.01), and the deleted fragment −249 ~ −142 was decreased (P < 0.05). QDPR gene promoter could be recognized as Tangshen Formula’s drug targets. LH4R experimental data showed that: the suitable Tangshen Formula’s concentration in cell experiment were 800 g/ml or less. Results showed that: Tangshen Formula can improve QDPR gene promoter activity. Tangshen Formula can improve D5’/pGL3, D3’/pGL3, D4’/pGL3 three recombinant plasmids activity. But comparing with no drug treated group, drug treated group is no effect on D1-1/pGL3, D1’/pGL3, D2’/pGL3’s activity (P > 0.01).

Conclusion: Rat QDPR core regulatory region might locate between −529 and −116 where existing putative negative and positive regulatory elements, especially, existing several Sp1 binding sites. There may be a potential Sp1 binding sites at −141 ~ −116 which could inhibit the transcriptional activity of the promoter.